

**School of psychology
Division of Health Sciences**

**Australian Twin and Molecular Genetic Study on Attention Deficit
Hyperactivity Disorder and its Co-morbidity with Reading
Disability**

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**This thesis is presented for the degree of
Doctor of Philosophy
of
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DECLARATION

This is a declaration certifying that this thesis is my own work, and to the best of my knowledge it does not include any materials previously published by any other person except where due recognition has been acknowledged, and it has not been submitted in any form for another degree or diploma at any university or other institute of tertiary education.

In addition, information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Abdullah Ramadan Sheikhi
November, 2007

LIST OF CONTENTS

LIST OF CONTENTS	i
ACKNOWLEDGEMENTS	vi
DEDICATION	viii
LIST OF FIGURES	ix
LIST OF TABLES	x
ABSTRACT	xv
CHAPTER 1: INTRODUCTION	1
1.1 <i>Dissertation Content</i>	5
CHAPTER 2: LITERATURE REVIEW	7
2.1 <i>Attention Deficit Hyperactivity Disorder (ADHD)</i>	7
2.1.1 <i>What is ADHD?</i>	7
2.1.2 <i>Diagnostic Criteria</i>	7
2.1.3 <i>Prevalence and Persistence</i>	8
2.1.4 <i>The Importance of ADHD Studies</i>	10
2.1.5 <i>Role of Environmental Factors</i>	10
2.1.6 <i>Medication</i>	11
2.2 <i>Behavioural Genetic Studies</i>	13
2.2.1 <i>Family Studies</i>	13
2.2.2 <i>Adoption Studies</i>	14
2.2.3 <i>Twin Studies</i>	15
2.2.3.1 <i>Twin identification methods</i>	16
2.2.3.2 <i>Ascertainment of disorder</i>	17
2.2.3.3 <i>Twin concordance measurement</i>	18
2.2.3.4 <i>ADHD twin studies</i>	18
2.3 <i>ADHD Candidate Genes</i>	24
2.3.1 <i>DAT1 or SLC6A3 (Sodium-Dependent Dopamine Transporter) Gene</i>	25
2.3.2 <i>DRD4 (Dopamine Receptor D4) Gene</i>	28
2.3.3 <i>DRD5 (Dopamine Receptor D5) Gene</i>	31
2.3.4 <i>Serotonin Receptors (HTR1B and HTR2A) and Transporter (5-HTT or SLC6A4) Genes</i> 34	
2.3.5 <i>COMT (Catechol-O-Methyltransferase) Gene</i>	37
2.4 <i>Neuropsychology</i>	43
2.5 <i>Endophenotypes of ADHD</i>	47
2.6 <i>Reading Disability (RD)</i>	51
2.6.1 <i>Prevalence and Heritability of RD</i>	51
2.6.2 <i>Phenotype Definition of Reading Disability</i>	54
2.7 <i>RD Twin Studies</i>	56
2.7.1 <i>The International Longitudinal Twin Study (ILTS)</i>	58
2.7.1.1 <i>The Colorado Learning Disabilities Research Center (CLDRC)</i>	59
2.7.1.2 <i>Other RD twin studies</i>	61
2.8 <i>RD Candidate Genes</i>	62

2.8.1	<i>DYX1C1 on Chromosome 15q21</i>	62
2.8.2	<i>DYX5 (ROBO1 genes) on Chromosome 3p12</i>	63
2.8.3	<i>DYX2 (KIAA0319 and DCDC2 genes) on Chromosome 6p22</i>	63
2.9	<i>The Comorbidity of ADHD with RD</i>	66
2.9.1	<i>Genetic Studies of ADHD-RD Comorbidity</i>	70
2.9.2	<i>Endophenotypes of ADHD-RD Comorbidity</i>	72
2.10	<i>Phenotype Definitions of Complex Disorders by Latent Class Analysis (LCA)</i>	74
2.11	<i>Molecular Genetic Approaches</i>	80
2.11.1	<i>QTL Association Procedure</i>	81
2.11.2	<i>Significance of Single Nucleotide Polymorphisms (SNPs)</i>	82
2.11.2.1	<i>How SNPs are used to find genes contributing to disorder</i>	84
2.12	<i>Linkage Disequilibrium and Haplotype Block Analysis</i>	84
2.12.1	<i>What is Linkage Disequilibrium?</i>	84
2.12.2	<i>Patterns of LD</i>	85
2.12.3	<i>LD and defining haplotypes in the human genome</i>	86
2.12.4	<i>Measures of Linkage Disequilibrium</i>	86
2.12.5	<i>The Concept of Haplotype Blocks</i>	88
2.12.6	<i>Haplotype-Tagging SNPs (htSNPs)</i>	89
2.12.7	<i>The International HapMap Project</i>	90
2.13	<i>Rationale, Aims, and Design</i>	91
2.13.1	<i>The Study Rationale</i>	91
2.13.2	<i>The study aims and design</i>	95
2.13.2.1	<i>Selection of ADHD and RD candidate genes and their SNPs for this study</i>	96
CHAPTER 3: IDENTIFICATION OF PARTICIPANTS		99
3.1	<i>Introduction</i>	99
3.2	<i>Australian Twin ADHD Project</i>	99
3.3	<i>Participant Recruitment</i>	101
3.4	<i>Measures</i>	102
3.4.1	<i>The Australian Twin Behaviour Rating Scale (ATRBS)</i>	102
3.4.2	<i>Measurement of Zygosity</i>	103
3.4.3	<i>DSM-IV ADHD items measure</i>	105
3.4.4	<i>Reading Disability Measure</i>	108
3.5	<i>Procedure</i>	111
CHAPTER 4: PREVALENCE AND TWIN-SIBLING DIFFERENCES FOR ADHD AND READING DISABILITY		113
4.1	<i>DSM-IV ADHD subtypes</i>	113
4.1.1	<i>Prevalence</i>	113
4.1.2	<i>Symptom overlap</i>	113
4.1.3	<i>Gender</i>	115
4.1.4	<i>Age</i>	116
4.2	<i>Reading Disability</i>	118
4.2.1	<i>Prevalence</i>	118
4.2.2	<i>Gender</i>	118
4.2.3	<i>Age</i>	119
4.3	<i>Twin-sibling differences</i>	119
4.3.1	<i>ADHD Twin-sibling differences</i>	120
4.3.1.1	<i>Prevalence</i>	120
4.3.1.2	<i>Gender</i>	123
4.3.1.3	<i>Age</i>	126
4.3.2	<i>RD twin-sibling differences</i>	127
4.3.2.1	<i>Prevalence</i>	127
4.3.2.2	<i>Gender</i>	128

4.3.2.3 Age.....	130
4.3.3 The Prevalence of DSM-IV ADHD-RD comorbidity.....	131
4.4 Conclusion.....	132
CHAPTER 5: LATENT CLASS ANALYSIS.....	136
5.1 Introduction.....	136
5.2 Methodology.....	137
5.2.1 Implementation of Latent Class Analysis.....	137
5.3 Results.....	138
5.3.1 Best-Fitting Model.....	138
5.3.2 Characteristics of the Nine ADHD/RD Latent Classes.....	140
5.3.2.1 The prevalence of the nine ADHD/RD latent classes.....	140
5.3.2.2 Sex differences among the nine ADHD-RD latent classes.....	141
5.3.2.3 Age differences among the nine ADHD-RD latent classes.....	141
5.3.2.4 Endorsement of the 18 DSM-IV ADHD items among the ADHD latent classes.....	142
5.3.2.4.1 Few symptoms.....	142
5.3.2.4.2 Predominantly Hyperactive-Impulsive.....	143
5.3.2.4.3 Predominantly Inattentive.....	144
5.3.2.4.4 Predominantly Inattentive with Reading Disability.....	144
5.3.2.4.5 Combined Latent Class.....	144
5.3.2.4.6 Combined with Reading Disability.....	145
5.3.2.5 Endorsements of the 18 DSM-IV ADHD items among the DSM-IV ADHD subtypes.....	145
5.3.3 The overlap between ADHD/RD Latent Class-9 and DSM-IV ADHD subtypes.....	146
5.3.3.1 Male and female overlaps.....	147
5.3.3.2 Endorsement of RD items among ‘No RD’ and ‘Yes RD’ groups.....	149
5.3.3.3 Endorsement of RD items among the RD Latent Classes.....	149
5.3.3.4 Endorsement of RD items among the ADHD latent classes.....	151
5.3.3.5 Endorsement of RD items among the DSM-IV ADHD subtypes.....	153
5.3.4 Characteristics of ADHD/ Reading Disability Comorbidity.....	153
5.3.4.1 Sex differences among the nine ADHD-RD latent classes and RD diagnosis.....	154
5.3.5 Characterisation of the nine ADHD-RD latent classes with Zygoty.....	155
5.4 Discussion.....	158
5.4.1 Examining the 18 DSM-IV ADHD and seven RD item endorsements.....	159
5.4.2 Zygoty.....	163
5.4.3 Conclusion.....	163
CHAPTER 6: GENETIC MODEL FITTING.....	166
6.1 Introduction.....	166
6.2 Methodology.....	167
6.2.1 Participants.....	167
6.2.2 Analyses.....	167
6.2.2.1 Data transformation, standardisation and assumption testing.....	167
6.2.2.1.1 Assumption testing for zygoty.....	167
6.2.2.1.2 Assumption testing for age.....	168
6.2.2.1.3 Assumption testing for sex.....	168
6.2.2.3 Univariate model fitting (Univariate twin analyses).....	168
6.2.2.3 Bivariate model fitting.....	173
6.3.1 Data transformation and standardisation.....	175
6.3.2 Assumption testing for age limitation.....	177
6.3.3 Assumption testing for sex limitation.....	177
6.3.3 Univariate model fitting.....	184
6.3.4 Bivariate Mx modelling.....	186
6.4 Discussion.....	196

CHAPTER 7: ASSOCIATION AND HAPLOTYPE BLOCK ANALYSES	201
7.1 Introduction.....	201
7.2 Methodology.....	204
7.2.1. Participants.....	204
7.2.2. Measures	204
7.2.2.1 Zygosity	204
7.2.2.2. Ascertainment of DSM-IV ADHD subtypes	205
7.2.2.3 Reading Disability measures	205
7.2.2.4 ADHD-RD latent class measures.....	206
7.2.3. Participants' recruitment for Genotyping Analysis.....	209
7.2.3.1 Genotyping analysis.....	210
7.2.4 Statistical Analysis	211
7.2.4.1 QTDT	212
7.2.4.2 Haploview.....	214
7.2.4.2.1 Determining pairwise statistics LD by Haploview	214
7.3 Results	215
7.3.1 Estimation of heterozygosity rates for the 21 SNPs.....	215
7.3.2. Single Locus Association Analyses	217
7.3.2.1. QTDT.....	217
7.3.2.1.1 DSM-IV ADHD and RD subtypes.....	217
7.3.2.2. Haploview.....	218
7.3.2.2.1 The nine latent ADHD-RD classes.....	218
7.3.3 Haplotype Mapping Analyses.....	222
7.3.3.1. Pair-wise linkage disequilibrium analysis and Construction of SNP Blocks.....	222
7.3.3.2 Haplotype-based association analysis.....	223
7.4 Discussion	225
7.4.1 Family-based association analysis on the DSM-IV ADHD subtypes and RD continuous data	225
7.4.2 Family-based association analysis on the nine ADHD-RD latent classes.....	227
7.4.2.1 DRD4 gene	227
7.4.2.2 DAT1 gene.....	227
7.4.2.3 SNAP-25 gene.....	228
7.4.2.4 COMT gene.....	229
7.4.2.5 KIAA0319 and TTRAP genes	231
7.4.2.6 HTR1B gene.....	232
7.4.3. Haplotype Block Analysis	233
7.4.3. Conclusion	234
CHAPTER 8: GENERAL DISCUSSION	236
8.1 <i>Significant findings of the research</i>	236
8.1.1 Significant findings for ADHD alone	237
8.1.2 Significant findings for RD alone.....	241
8.1.3 Significant findings of ADHD-RD comorbidity	244
8.2 <i>Study limitations</i>	248
8.2.1 Limitations with DSM-IV ADHD items	248
8.2.2 Limitations with the seven RD items	249
8.2.3 Limitations of genetic fit modelling.....	249
8.2.4 Limitations of genotyping analysis	250
8.3 <i>Implications, recommendations and future directions</i>	250
REFERENCES.....	254
APPENDIXES	286
<i>Appendix 1: Twin and Sibling Questionnaire</i>	286
<i>Appendix 2: Assumption Testing for Age Limitation</i>	292

2.1 Monozygotic and Dizygotic univariate correlations for ADHD subtypes and Reading Disability by age variable (Younger age).....	292
2.2 Monozygotic and Dizygotic univariate correlations for ADHD subtypes and Reading Disability by age variable (Older age).....	294
<i>Appendix 3</i>	296
A3.1 DNA collections and extractions protocol by Oragene Saliva Kit DNA.....	296
A3.2 Genotyping Analysis.....	299
<i>Appendix 4</i>	301
4.1 ATR Family Approach Letter.....	301
4.2 Study Information Sheet.....	302
4.3 Parent’s DNA Consent Form.....	305
4.4 Child’s DNA Consent Form.....	307
4.5 Reminder Letter.....	308
4.6 Saliva Kit Information Sheet.....	309
<i>Appendix 5: ATR Ethics Application</i>	311

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DEDICATION

TO MY WIFE 'ASMAA' ... PRECIOUS GIFT

LIST OF FIGURES

Figure 2.1	Estimated heritability of attention-deficit/hyperactivity disorder, based on pooled results from 20 studies	20
Figure 2.2	Candidate Endophenotypic Measures	49
Figure 2.3	Location of RD candidate genes on chromosome 6p	65
Figure 3.1	Waves of ATAP study	100
Figure 5.1	The Eight Latent Classes	139
Figure 5.2	The nine latent classes	139
Figure 6.1	Univariate model for Mx modeling	172
Figure 6.2	Bivariate model for Mx modeling	174
Figure 6.3	Distribution of Untransformed and Square-Root Transformed Score Variable	176
Figure 7.1	Haplotype Block Analyses for <i>DSM-IV</i> ADHD Subtypes	222

LIST OF TABLES

Table 2.1	Genetic linkage and association studies for ADHD	39
Table 2.2	Genetic association studies for Reading Disability	66
Table 3.1	Zygoty measures	104
Table 3.2	The Nine Inattention Items	105
Table 3.3	The Six Hyperactive Items	106
Table 3.4	The Three Impulsive Items	106
Table 3.5	The Reliability Test for the Nine Inattention Items	107
Table 3.6	The Reliability Test for the Nine Hyperactive-Impulsive Items	107
Table 3.7	The Four-Point Scale used by the Twin and Sibling Questionnaire	108
Table 3.8	The Reading Disability Items	109
Table 3.9	The Reliability Test for the Seven RD Items	111
Table 3.10	The Reliability Test for the Seven RD Items	111
Table 4.1	The Frequencies and Prevalence of DSM-IV ADHD Subtypes	113
Table 4.2	The distribution of Hyperactive-Impulsive symptoms in Inattentive individuals	114
Table 4.3	The distribution of Inattentive symptoms in Hyperactive-Impulsive individuals	115
Table 4.4	Prevalence of Males and Females among DSM-IV ADHD subtypes	115
Table 4.5	Total numbers of Males and Females among DSM-IV ADHD categories	116
Table 4.6	Descriptive of Age among DSM-IV ADHD subtypes	116
Table 4.7	Scheffe homogeneity test between age groups between ADHD subtypes	117
Table 4.8	The frequencies and prevalence of Reading Disability	118
Table 4.9	Prevalence of males and females among Reading Disability	118
Table 4.10	The score mean of Reading Disability among male and females	119
Table 4.11	Age differences among unaffected and affected Reading Disability individuals	119
Table 4.12	The prevalence of gender among twins and siblings	120
Table 4.13	The means of ages of twins and siblings	120
Table 4.14	Prevalence of MZ, DZ twins and siblings among DSM-IV ADHD subtypes	121
Table 4.15	The distribution of DSM-IV ADHD subtypes among twins and siblings	121

Table 4.16	The mean scores for Inattentive and Hyperactive-Impulsive symptoms for all twins and siblings	122
Table 4.17	The results of Mann-Whitney U-test on MZ versus DZ twins and sibling 1 versus sibling 2 individuals on Inattentive and Hyperactive-Impulsive scores	122
Table 4.18	The results of a Mann-Whitney U-test on twins versus siblings on Inattentive and Hyperactive-Impulsive scores	123
Table 4.19	The means of the Inattentive and Hyperactive-Impulsivity scores between gender with twin-sibling individuals	124
Table 4.20	Results of Kruskal Wallis Test on the means of the Inattention and Hyperactive-Impulsive scores between gender with twin-sibling individuals	125
Table 4.21	The post hoc multiple comparison tests on gender with twin-sibling groups (Games-Howell) by Inattentive and Hyperactive-Impulsive scores	126
Table 4.22	Non-parametric correlation tests between age and Inattentive scores and age and Hyperactive-Impulsive scores for MZ versus DZ twins and sibling1 versus sibling 2 individuals	127
Table 4.23	Prevalence of a RD among MZ, DZ twins and their siblings	127
Table 4.24	The distribution of RD among twins and siblings	128
Table 4.25	The results of Mann-Whitney U test on twins versus siblings for RD scores	128
Table 4.26	The score means of Reading Disability among gender with twin-sibling individuals	129
Table 4.27	Results of Kruskal Wallis Test on the means score of Reading Disability between gender with twin-sibling individuals	129
Table 4.28	The post hoc multiple comparison test on gender with twin-sibling groups (Games-Howell) by Reading Disability scores	130
Table 4.29	Non-parametric correlation tests between age and Reading Disability scores for MZ versus DZ twins and sibling1 versus sibling 2 individuals	131
Table 4.30	The prevalence of the comorbidity between DSM-IV ADHD and Reading Disability	131
Table 4.31	The prevalence of comorbidity between DSM-IV ADHD and Reading Disability among twins and siblings	132
Table 5.1	The Frequencies and Percentages for each of Latent Class-9	140
Table 5.2	Sex Differences among the Nine ADHD/RD Latent Classes	141
Table 5.3	Age Significant Test with LC-9	142
Table 5.4	The Frequency of Latent Class Subtype Endorsements and Subtype Prevalence	143
Table 5.5	The Frequency of DSM-IV ADHD Subtypes Endorsement and Subtype Prevalence	146
Table 5.6	Cross-tabulation between Total Latent Class-9 and the DSM-IV ADHD Subtypes	147
Table 5.7	The Male Overlap between the Nine ADHD/RD Latent Classes and the DSM-IV ADHD Subtypes	148

Table 5.8	The Female Overlap between the Nine ADHD/RD Latent Classes and the DSM-IV ADHD Subtypes	149
Table 5.9	The Prevalence of Reading Disability Items Endorsements	149
Table 5.10	The Prevalence and the Frequency of Reading Disability Latent Class Endorsement with RD Items	151
Table 5.11	The Endorsement of the Seven RD Items among the ADHD Latent Classes	152
Table 5.12	The Prevalence and the Frequency of DSM-IV ADHD Endorsements with Reading Disability Items	153
Table 5.13	The Prevalence of the Comorbidity between the Nine ADHD/RD Latent Classes and RD	154
Table 5.14	Sex Prevalence among the Nine ADHD/RD Latent Classes and Reading Disability Comorbidity	155
Table 5.15	The Cross-tabulation of Twin 1 Latent Class-9 with Twin 2 Latent Class-9 by Zygosity	157
Table 5.16	Chi-Square Test of the Proportion of Concordant Versus Discordant MZ and DZ Twin Pairs for Each Latent Class	158
Table 5.17	The Correspondence between the Willcutt's Seven RD Items and the RD Components	161
Table 5.18	Matching up the Predominant Phenotypic RD Item(s) with each RD Latent Class	162
Table 5.19	The Overlapped and Non-overlapped Cases between the Nine ADHD/RD Latent Classes and the DSM-IV ADHD Subtypes by 'No RD' and 'Yes RD' Categories	164
Table 6.1	The Four Genetic Model Fitting Assumptions	169
Table 6.2	Monozygotic and Dizygotic Univariate Sex Correlations for Inattention Subtype	178
Table 6.3	Male and Female Model of Inattentive Subtype	179
Table 6.4	Male and Female Model of Hyperactive-Impulsive Subtype	180
Table 6.5	Male and Female Model of Combined Subtype	181
Table 6.6	Male and Female Model of Reading Disability	182
Table 6.7	MZ and DZ males model on Reading Disability	183
Table 6.8	MZ and DZ females model on Reading Disability	184
Table 6.9	Monozygotic and Dizygotic Univariate Correlations for ADHD Subtypes and Reading Disability	184
Table 6.10	Univariate Model-fitting on ADHD Subtypes and Reading Disability	186
Table 6.11	The MZ and DZ Bivariate Correlations between Inattentive Subtype and Reading Disability	187

Table 6.12	The MZ and DZ Bivariate Correlations between Hyperactive-Impulsive Subtype and Reading Disability	187
Table 6.13	The MZ and DZ Bivariate Correlations between Combined Subtype and Reading Disability	188
Table 6.14	Bivariate Model-fitting on Inattentive Subtype and Reading Disability of All Sample	191
Table 6.15	Bivariate Model-fitting on Hyperactive-Impulsive Subtype and Reading Disability of All Sample	192
Table 6.16	Bivariate Model-fitting on Combined Subtype and Reading Disability of All Sample	194
Table 7.1	Zygoty and Sex Frequencies of the Genotyping Sample	204
Table 7.2	The Frequencies of RD and DSM-IV ADHD Subtypes Recruited for the Genotyping Analysis	206
Table 7.3	The Frequencies of the Nine Latent Classes	207
Table 7.4	The Assignment of the Selected Latent ADHD RD Classes with DSM-IV ADHD Subtypes by Reading Disability	208
Table 7.5	Family Structure of the Recruited Families in the Genotyping Analysis	209
Table 7.6	ADHD RD Designed SNPs Assays	211
Table 7.7	List of Selected SNPs with their Estimated Heterozygosity Rates	217
Table 7.8	Total Evidence for Association for DSM-IV ADHD Subtypes & Reading Disability	218
Table 7.9	Association- Single Marker Table for the Nine Latent ADHD RD Classes	220
Table 7.10	Association- Single Marker Table for the 'Combined RD' Latent Class	221
Table 7.11	Haplotype Block Analyses for DSM-IV ADHD Subtypes	224
Table 7.12	Haplotype Block Analysis for the Nine Latent Classes	225
Table A2.1	Monozygotic and Dizygotic Univariate Correlations for ADHD Subtypes and Reading Disability for 13 years old and younger	292
Table A2.2	The MZ and DZ Bivariate Correlations between Inattentive Subtype and Reading Disability for 13 years old and younger	292
Table A2.3	The MZ and DZ Bivariate Correlations between Hyperactive-Impulsive Subtype and Reading Disability for 13 years old and younger	293
Table A2.4	The MZ and DZ Bivariate Correlations between Combined Subtype and Reading Disability for 13 years old and younger	293
Table A2.5	Monozygotic and Dizygotic Univariate Correlations for ADHD Subtypes and Reading Disability for 13 years old and older	294

Table A2.6	The MZ and DZ Bivariate Correlations between Inattentive Subtype and Reading Disability for 13 years old and older	294
Table A2.7	The MZ and DZ Bivariate Correlations between Hyperactive-Impulsive Subtype and Reading Disability for 13 years old and older	295
Table A2.8	The MZ and DZ Bivariate Correlations between Combined Subtype and Reading for 13 years old and older	295
Table A3.1	The apparatus and consumables used in DNA extraction	296
Table A3.2	PCR and Genotyping Conditions for the ADHD-RD Candidate Genes	300

ABSTRACT

Aim: This study aims to investigate the genetic components of Attention Deficit Hyperactivity Disorder (ADHD), Reading Disability (RD), and their comorbidity.

Methods: Three approaches were applied to data from 2610 Australian twin families. This data was obtained by parental completion of the ‘Twin and Sibling Questionnaire’. 1) Latent Class Analysis (LCA) was applied to generate genetically independent classes that defined ADHD subtypes and RD based on related cluster symptoms. 2) Genetic modelling was used to study the particular genetic and environmental effects of each ADHD subtype and of RD, and to examine whether children identified with comorbid ADHD-RD are a genetically distinct group from those who have only ADHD without RD. 3) A family-based genetic association, including haplotype block analysis, was applied to compare the efficacy of *DSM-IV* diagnostic criteria and LCA in the genotyping analysis, to test the genetic overlap of ADHD candidate genes on RD phenotypes and vice versa, and to detect some of the risk alleles of ADHD alone, RD alone, and comorbid ADHD-RD. This analysis was performed on a data set that included 190 individuals from the original sample; it tested twenty-one Single Nucleotide Polymorphisms (SNPs) from five ADHD candidate genes (DRD4, DAT1, SNAP25, COMT, and HTR1B), and four RD candidate genes (MRS2L, KIAA0319, TTRAP, and THEM2) from the 6p22.2 region.

Results: The LCA dissected the phenotypes for ADHD and RD into nine genetically informative classes. Univariate and bivariate results indicated the presence of unique genetic components on each ADHD subtype and RD category, and also showed the existence of genetic factors for comorbid ADHD-RD. The association findings, using continuous data represented by scores of *DSM-IV*-defined ADHD and RD, showed two significant associations for ADHD and RD, whereas the association findings for the categorical data, represented by LCA, were richer as they showed 15 significant single-locus with ADHD and RD latent classes. Some of these association results were between ADHD candidate SNPs with RD latent classes and ADHD-RD comorbid classes. Some RD candidate SNPs were associated with ADHD latent classes and ADHD-RD comorbid classes. Haplotype block analysis detected a presence of one significant haplotype block containing two haplotype-tagging SNPs (ht-SNPs) of the

COMT gene (rs4680 and rs165599), including three risk alleles ('AA', 'GC', and 'AC') that were associated with some phenotypic RD components.

Conclusion: This study found that the use of ADHD-RD latent classes is more suitable for performing genetic association studies and haplotype block analysis than is *DSM-IV*-defined ADHD and RD definitions. Furthermore, there is an overlapping of genetic effect, as ADHD candidate genes contributed to RD phenotypes and vice versa. Thirdly, ADHD-RD comorbidity is caused by both ADHD and RD candidate genes.

CHAPTER 1: INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is a complex neuro-developmental disorder as classified in the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (*DSM-IV*). ADHD is distinguished by the presence of developmentally inappropriate levels of impulsivity, hyperactivity, and inattentiveness; by impaired ability to focus, sustain, and switch attention, and excessive and situationally inappropriate motor-activity (Stefanatos & Baron, 2007). ADHD is among the most frequent disorders in school-age children and the prevalence estimates for it in the school-age population range from 3 -10%, (Faraone, Sergeant, Gillberg, & Biederman, 2003; Graetz, Sawyer, Hazell, Arney, & Baghurst, 2001; Khan & Faraone, 2006). In addition, the *Diagnostic and Statistical Manual of Mental Disorders Text Revision (APA-TR, 2000)* reported a prevalence of 3-7%. ADHD is five times more prevalent in boys than girls (Kuntsi, McLoughlin, & Asherson, 2006), and is one of the most common causes of behaviour problems and poor school performance among school-aged children.

DSM-IV classifies ADHD into three subtypes: Predominantly Inattentive, Predominantly Hyperactive-Impulsive, and combined. *DSM-IV* uses categorical diagnosis for ADHD, requiring six or more symptoms: the Predominantly Inattentive subtype requires six or more inattentive symptoms and fewer than six Hyperactive-Impulsive symptoms; the Predominantly Hyperactive-Impulsive subtype require six or more Hyperactive-impulsive symptoms and fewer than six Inattentive symptoms; and the Combined subtype requires six and more Inattention symptoms and six or more Hyperactive-impulsive symptoms. Hay et al. (2001) illustrated that this categorical diagnosis may lead to a distorted identification of twins and their siblings, as one affected twin may have six symptoms of one subtype, while the other twin or sibling may have ten symptoms or be unaffected. Despite *DSM-IV* using a categorical diagnosis of ADHD based on cut-off scores, Levy, Hay, McStephen, and Wood (1997) found that ADHD is best presented as a continuum rather than as categories.

Several quantitative genetic studies have revealed that genetic factors are the major influence within family susceptibility; with heritability estimates for ADHD ranging from 60% to more than 90% (Biederman & Faraone, 2005). This indicates that specific genes play a role in the aetiology of ADHD. Pauls (2005) stated that the current advances in molecular genetics have accelerated the hunt for these candidate genes. Although the aetiology of ADHD is not fully understood, many molecular genetic studies have suggested the involvement of dopaminergic (Levy, 1991; Seeman & Madras, 1998), serotonergic (Manor et al., 2001), and noradrenergic genes (Comings, Gonzalez, Li S-C, & MacMurray, 2003). In addition, Comings et al. (2003) and Fisher et al. (2002) reported that ADHD can be strongly influenced by several genes of small effect; each contributing a small fraction of the total genetic variance. It has not been determined if all ADHD subtypes are influenced by the same genes or whether each subtype has its own particular candidate genes.

Reading Disability (RD) is also a complex neuro-behavioural disorder that affects approximately 5 -10% of school-aged children, irrespective of intelligence, education, and social environment (Shaywitz, Shaywitz, Fletcher, & Escobar, 1990). A person impaired with RD cannot interpret written words and would find it difficult or impossible to spell and decode words as a consequence of a deficiency in language phonology. This disability can lead to weak scholastic attainment (Lyon, Shaywitz, & Shaywitz, 2003). RD starts in childhood, continues into adulthood, and has a serious social impact (Bates, Luciano et al., 2007). According to *DSM-IV* (APA, 1994; 2000), the diagnostic criteria for Reading Disability are as follows: 1. a child with RD must show reading achievement that falls substantially below that which would be expected given their chronological age, measured intelligence, and age-appropriate education. 2. the disability in reading must considerably interfere with the academic achievement or with the daily living activities that require reading skills. 3. if a sensory deficit is present, the reading disabilities being experienced must be extreme considering individuals usually associated with the deficit.

Like the findings for ADHD, in twin studies the development of RD has a link to genetic factors, with a heritability of approximately 70%, and with interactions between genetic and environmental factors playing a substantial role in its manifestation (Bates, Castles et al., 2007). In addition, RD phenotypic components

show the presence of genetic effects, such as word recognition ($a^2=0.45$), orthographic coding ($a^2=0.58$), phonological decoding ($a^2=0.61$), and phonological awareness ($a^2=0.56$) (Gayan & Olson, 1999). Recent molecular genetic studies have also demonstrated genetic association between RD and candidate genes found on chromosome 6p (Cope et al., 2005; Francks et al., 2004; Paracchini et al., 2006). However, there is a lack of studies searching for candidate genes of RD phenotypic components.

There is a well-documented body of literature confirming that comorbidity between ADHD and RD is common, and co-occurs significantly more frequently than would be expected by chance because of a phenotypic overlap; however, this comorbidity is not well understood. (Friedman, Chhabildas, Budhiraja, Willcutt, & Pennington, 2003; Luca, Laurin, Misener, Wigg, Anderson et al., 2007).

Twin studies that confirm that ADHD and RD are influenced by genetic factors have also found a shared heritability between the two disorders (Gillis, Gilger, Pennington, & DeFries, 1992; Willcutt, Pennington, & DeFries, 2000). Studies have suggested that the substantial comorbidity between ADHD and RD is partially attributable to shared genetic influences (Willcutt et al., 2000a; Willcutt, Pennington, Smith, Cardon, Gayan et al., 2002). In addition, using the ADHD phenotype subcategories, the overlap between Inattentive ADHD and RD is stronger than Hyperactive-Impulsive ADHD and RD, based on twin studies that examined the genetic relationship of RD with these two subtypes of ADHD (Willcutt, Pennington, & DeFries, 2000). Twin studies have also demonstrated significant bivariate heritability between Inattentive ADHD and RD, estimated to be 0.39, whereas that between Hyperactive-Impulsive ADHD and RD was estimated to be 0.05 (Stevenson, 2001; Willcutt, Pennington, & DeFries, 2000).

In light of the findings reviewed above, there is convincing evidence that the two disorders are influenced by some of the same genes which act pleiotropically in the development of both disorders (Willcutt et al., 2002). Stevenson et al. (2005) stated that the latter finding offers primary evidence for 6p loci to be considered as an aetiological genetic factor for both disorders, and suggested that this may establish the basis for future studies on the aetiology of ADHD and its comorbidity with other

disorders such as RD. Furthermore, the same authors concluded that ADHD genetic studies have to examine ADHD alone, and ADHD comorbid with RD, as it is not known whether comorbid ADHD-RD is a distinct disorder from ADHD without RD.

The key to understanding the aetiology of ADHD and its comorbidity with RD may be in the recent advances that have been achieved in the field of quantitative and molecular genetics.

As recent findings suggest that ADHD and RD have substantial genetic components, researchers are working to identify the particular genes that are responsible for each disorder and for their comorbidity. Unfortunately, there is a lack of molecular genetic studies investigating these candidate comorbid genes: a search for this only found two studies (Luca et al., 2007; Stevenson, Langley et al., 2005). Therefore, it is recommended that additional work for the search of ADHD-RD comorbidity genes should be continued to help clarify the aetiology and classification of ADHD and its comorbidity with RD.

Using alternative ADHD and RD phenotypes, instead of those arising from the *DSM-IV*, may lead to more rapid success in the search for ADHD, RD, and ADHD-RD candidate genes. The reason that phenotypes based on *DSM-IV* criteria are not entirely successful as reference points in molecular genetic studies is that they are heterogeneous and therefore inadequate to detect the susceptible gene(s) contributing to these particular phenotypes (Szatmari et al., 2007). This may be one of the reasons for the delay in identification of the actual genes of ADHD, RD, and ADHD-RD; the most genetically informative phenotypes have not been fully identified.

One obstacle that Khan and Faraone (2006) raised is that *DSM-IV* diagnostic criteria for ADHD does not focus on its complexity, heterogeneity, and comorbidity, but only considers grouping symptoms. This leads to the conclusion that there is no distinct line between symptoms of ADHD and symptoms involving its comorbidity with RD. This makes it difficult to decide if symptoms of ADHD represent more than one disorder or whether these symptoms represent distinct subtypes of ADHD (Volk, Henderson, Neuman, & Todd, 2006). Another obstacle is that defining a child with *DSM-IV* comorbid ADHD-RD requires two sets of arbitrary cut-offs, which may lead to inappropriate classification, making the sample heterogeneous. One way

to avoid this is to utilise different definitions and criteria in order to appropriately identify and reduce this heterogeneity among the sample. Todd and his research team have demonstrated in several studies (e.g., Hudziak et al., 1998; Neuman et al., 1999; Rasmussen, Neuman et al., 2002; Todd et al., 2002; Todd et al., 2005; Volk et al., 2006) the efficiency of using Latent Class Analysis (LCA) in order to obtain homogeneously distinct groups that are appropriately classified, as LCA has the advantage of identifying naturally occurring clusters of symptoms without the need for symptom number cut-offs (Volk et al., 2006). Accordingly, LCA can also be used to refine the phenotypes of ADHD-RD comorbidity, to avoid heterogeneity and produce genetically and biologically informative phenotypes. Therefore, Neuman et al. (2005) encouraged the adoption of LCA in molecular genetic studies of ADHD as LCA seems to be a more suitable approach for such studies than *DSM-IV* diagnostic criteria.

This study aims to untangle the often disjointed web of our understanding of comorbid ADHD-RD by refining the phenotypes for both ADHD and RD, and exploring the genetic pattern of this comorbidity. Furthermore, the study aims to investigate if ADHD and RD together are influenced by the same or different genes, or if ADHD-RD comorbidity has distinct genes that differ from those acting on RD and on ADHD alone. Finally, the study explores the susceptibility of the 6p region to ADHD, as this region is confirmed to be susceptible to Reading Disability.

1.1 Dissertation Content

Chapter one introduces the thesis and explains its objectives. The following chapter is a literature review that includes the background of ADHD including definitions of its prevalence, classification and medication used for its treatment. It also contains a review of the molecular and twin genetic studies related to the disorder; an overview of Reading Disability, its definition and prevalence; and discussion about ADHD-RD comorbidity. Chapter two also covers Latent Class Analysis (LCA) and its importance in genetic studies with complex disorders such as ADHD and RD.

Chapter three looks at the methodology of this present study. It includes recruitment of participants, the measurements used to identify monozygotic and dizygotic twins, and the approach used to identify children with ADHD according to *DSM-IV* criteria.

In addition, Chapter three outlines the measurement of Reading Disability and its validity.

Chapter four provides a detailed description of the participants. It outlines the participants' allocation into particular groups and subtypes; and the MZ, DZ, and sibling numbers. This chapter also explains the gender differences and age groups among the *DSM-IV* ADHD subtypes and RD category. In addition, this chapter describes twin/sibling differences by gender differences and age groups.

Chapter five describes the univariate and bivariate analyses, using Mx to estimate the genetic correlation between ADHD and RD and to examine if the relationship between them is genetic, environmental, or both.

Chapter six discusses the significance of using Latent Class Analysis in genetic studies, and the methodology for defining ADHD and RD based on related cluster symptoms, in order to generate genetically independent classes. The chapter shows the endorsement of 18 *DSM-IV* ADHD symptoms in ADHD-RD latent classes identified through the analyses. The chapter also describes the overlap between cases found in *DSM-IV* ADHD subtypes and ADHD-RD latent classes, and the comparison between monozygotic and dizygotic twins in each latent class.

Chapter seven presents the genotyping analyses, including the family-based association study with both *DSM-IV* ADHD and RD categories and the ADHD-RD latent classes. This is a comparison of the genotyping analysis for *DSM-IV* ADHD and for RD as continuum data, and ADHD-RD latent classes as categorical data. This chapter also describes the findings of the haplotype-block analysis, which contributes to our understanding of ADHD-RD comorbidity.

Chapter eight is a general discussion of the three studies (Mx genetic modelling, Latent Class Analysis, and genotyping analysis). Together, this findings, frame the core outcomes of this research. The chapter draws general conclusions about the study and addresses its limitations regarding participant recruitment and methodological approaches. The chapter also includes recommendations and directions for future study.

CHAPTER 2: LITERATURE REVIEW

This chapter will give an overview of literature on Attention Deficit Hyperactivity Disorder (ADHD), Reading Disability (RD), and their comorbidity. It will also focus on the genetic research studies for both of these disorders, and the different approaches being used to define the phenotypes of complex disorders such as ADHD and RD.

2.1 *Attention Deficit Hyperactivity Disorder (ADHD)*

2.1.1 *What is ADHD?*

Attention Deficit Hyperactivity Disorder is a multifactorial, childhood-based behavioural disorder of unknown aetiology. There is strong quantitative evidence suggesting genetic causes for the disorder (Faraone et al., 2005; Levy & Hay, 2001; Shastry, 2004). The fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV*, American Psychiatric Association (APA), 1994) defines ADHD as a persistent disorder characterised by difficulty in sustaining attention, excessive motor activity, and impulsivity. The symptoms of ADHD include restlessness, difficulty with organising duties, distractibility, absentmindedness, and regularly interrupting (Fisher et al., 2002). It has been reported that ADHD is a clinical heterogeneous disorder that causes poor educational and vocational performance for the sufferers, and in turn causes social difficulties for the sufferer's family (Biederman, 2005; Kirley et al., 2002).

2.1.2 *Diagnostic Criteria*

Since 1980, the *Diagnostic and Statistical Manual of Mental Disorders*, criteria for ADHD diagnosis has changed in a number of ways.

In 1980, the *DSM-III* (APA, 1980) divided ADHD into three subtypes; namely, Inattentive, Impulsive, and Hyperactive. Each subtype consisted of particular symptoms: the Inattentive subtype had three symptoms, the Impulsive subtype also had three symptoms, and the Hyperactive had two symptoms. Thus, in total in 1980, there were three subtypes and eight symptoms.

However, seven years later, the revised edition of the *DSM-III* was released with a different number of subtypes and symptoms. The revised edition, known as the *DSM-III-R* (APA, 1987) contained only one subtype and the disorder was called Attention Deficit Disorder (ADD). Nevertheless, this subtype consisted of fourteen symptoms. This meant that the number of symptoms had almost doubled from the *DSM-III* edition. Furthermore, the *DSM-III-R* further decreed that if eight symptoms out of the total fourteen possible symptoms were present, ADHD was to be diagnosed.

The next shift in diagnostic criteria relating to ADHD, the *DSM-IV* (APA, 1994), was made seven years after the *DSM-III-R*. *DSM-IV* (APA, 1994) classified ADHD into three subtypes: the Predominantly Inattentive subtype, the Predominantly Hyperactive-Impulsive subtype, and the Combined subtype. Interestingly, after starting with three subtypes in 1980, and then condensing to just one subtype in 1987, the contributors felt it was necessary to move back to three subtypes in 1994: the Predominantly Hyperactive-Impulsive subtype, and a combination of the two: the Predominantly Combined subtype. The first two subtypes listed in the *DSM-IV* contained nine symptoms each, and the combined subtype consisted of the sum of the first two subtypes. The *DSM-IV* stipulates that if a child displays at least six out of the nine symptoms of the Inattentive subtype, or of the Hyperactive-Impulsive subtype, ADHD is to be diagnosed, and if the child displays at least six or more of the symptoms of both of the first two subtypes, Combined ADHD is diagnosed. (Levy, McStephen, & Hay, 2001).

Thus there have been significant changes in the diagnostic criteria for ADHD. This leads to a second aspect, the extent of the sufferer's impairment as a result of ADHD. Based on *DSM-IV* diagnostic criteria, in order to observe and diagnose ADHD impairment, the child must be seven years or older and show the symptoms in at least two out of the three following settings: the child's school, work, or home environments (APA, 1994).

2.1.3 Prevalence and Persistence

Faraone et al. (2003) reported that the prevalence of ADHD in the US ranged from 5-10% of school-age children, while Shastri (2004) reported that the prevalence of ADHD in

Western countries ranged from 3-5% of school-age children. Another publication by Faraone et al. (2005) stated out that the worldwide prevalence of ADHD ranges from 8-12%. These figures are an indication that ADHD is a serious worldwide epidemic. In the United States, it is estimated that ADHD affects about 4.4 million children between the ages of 4 to 17 years. Although the disorder frequently appears in the preschool years, the symptoms often persist into adolescence and adulthood for 50–80% of cases (Stefanatos & Baron, 2007). ADHD has been found in both males and females in ratios ranging from 1:4 to 1:9 respectively (Waldman & Rhee, 2002). Furthermore, it has been reported that the disorder is diagnosed more often in boys than in girls, with a ratio of 3-4:1 (Birleson, Sawyer, & Strom, 2000). In Australia, studies have shown that about 11% of children and adolescents meet the symptom criteria for ADHD (Birleson et al., 2000). However, this result has caveats. According to Hay (2006), sometimes psychologists or psychiatrists can incorrectly diagnose a child with ADHD. Taking this into consideration, 6% is a more reasonable estimate. Additionally, Hay et al. (2001) stated that an efficient tool to confirm ADHD impairment is the use of questionnaires, because it is a more conservative tool to estimate any behavioural problems. Hay and his colleagues (2001) reported that “our experience is that questionnaires can provide a conservative estimate of the extent of behavioural problems, in parents report more symptoms at interview than in questionnaire. On average, parents who reported five DSM-II-R ADHD symptoms in the questionnaire reported the eight needed to reach criterion at interview. It is reassuring that our rates of DSM-IV subtypes in 8- to 16- years old Australian female twins, obtained by questionnaire, were very close to the rates obtained by Hudziak and colleagues in telephone interview with US adolescents twins” (p. 13).

Biederman (2005) reported that an impaired ability to relate to others and to manage one’s life is not always a result of persistent of ADHD, based on his previous work assessing the education, and emotional and social functioning of adolescents with persistent ADHD. With a group of such adolescents, Biederman found that 20% performed well in all three areas, 60% displayed intermediate functioning, and only 20% of those studied functioned inadequately across all three areas. These results strongly indicate that the syndromic persistence of ADHD does not map to a single concomitant functional outcome, and in

fact is more closely allied with a broad spectrum of “emotional, educational and social adjustment outcomes that can be partially predicted” (Biederman, 2005, p.45).

2.1.4 The Importance of ADHD Studies

The importance of studying ADHD can be seen in the following reasons: firstly, the aetiology of ADHD is still not fully understood (Hay, McStephen, & Levy, 2001; Levy, McStephen et al., 2001). Secondly, ADHD is considered a worldwide public health problem and is among the most common childhood psychiatric disorders with a prevalence of 8-12% worldwide (Faraone et al., 2005). Thirdly, ADHD is a heterogeneous disorder that is comorbid with other disorders such as Conduct Disorder and Reading Disability. Both clinical and neurobiological perspectives have made ADHD one of the best-validated childhood disorders (Faraone, 1998; Faraone, Biederman, Spencer et al., 2000). Moreover, family members of children with ADHD often have marked academic failure, low self-esteem, poor peer relationships, parental conflict, and delinquency. Finally, as ADHD can show a pattern of psychological dysfunction, psychosocial disability, and psychiatric comorbidity, there is a high chance that ADHD adolescents are more exposed to injuries leading to frequent hospital visits, and to street violence, smoking, and alcohol/drug abuse (Biederman et al., 2004; DiScala, Lescohier, Barthel, & Li, 1998; Woodward, Fergusson, & Horwood, 2000).

2.1.5 Role of Environmental Factors

Lehen et al. (2007) highlighted several environmental factors that might contribute to causing ADHD: poor parenting strategies, family dysfunction, low parental socioeconomic status (SES), environmental deprivation, food additives, maternal smoking, maternal alcohol consumption, and traumatic brain injury. Swanson et al. (2007) reviewed the literature of the environmental factors involved in ADHD. This review reported that exposure to lifestyle factors such as smoking, alcohol, caffeine, stress, and to toxic substances such as nicotine and lead during pregnancy and the early childhood period might increase the risk for a child to develop ADHD (Linnett et al., 2005). Maternal smoking had been found to contribute genetically to the association between Alcohol Use Disorder (AUD) in mothers and the risk of ADHD development in the offspring (Knopik et al., 2006). The presence of significant genetic correlation was established,

suggesting that there is a major risk of environmental factors contributing to offspring developing ADHD due to correlated parent behaviour, which heightens the child's environmental exposure to toxic substances such as nicotine. Lead is also a risk factor in ADHD development as Braun, Kahn, Froehlich, Aulinger and Lanphear (2006) found that even minimal levels of lead were responsible for the development of ADHD. This demonstrates the importance of considering a potential gene-environment interaction model in the aetiology of ADHD.

Furthermore, Swanson et al. (2007) proposed that if a pregnant woman was exposed to a stress, this might affect the infant's development mechanisms. In addition, mothers with minor stress-related faults might develop behavioural deficits such as ADHD. This might be difficult to measure, as the minor damage found in the brain would not be obvious to detect. The authors also reported that studies had found that damage in the striatal-frontal cortical circuitry in ADHD children, as result of traumatic brain injury, is considered a cause of ADHD symptoms. Moreover, the authors also pointed out that Low Birth Weight (LBW) and Premature Birth (PB) are considered environmental risk factors for ADHD. These both can result from prenatal exposure to maternal smoking as well as passive exposure.

2.1.6 Medication

The clinical efficacy of ADHD medications results from the changes in dopaminergic and noradrenergic pathways, suggesting that these medications boost the inhibitory influences of frontal cortical activity on subcortical structures in the brain (Zametkin & Rapoport, 1987). One of these medications, a stimulant called methylphenidate (known as Ritalin), plays a role in treating individuals suffering from ADHD. Methylphenidate has the ability to distribute rapidly throughout the body as it contains highly soluble lipids and has a low protein-binding ability. This allows the accumulation of a high methylphenidate concentration in the central nervous system (CNS) in a short time (Kimko, Cross, & Abernethy, 1999; Masellis et al., 2002). This stimulant acts to inhibit the dopamine transporters. It also blocks dopamine and norepinephrine reuptake into the presynaptic neurons, increasing a amount of the monoamines' in the extraneuronal space, resulting in low ADHD symptoms in children (Elia et al., 1990).

In a press release by the Department of Health in Western Australia (2005), about their published report “Stimulant prescribing and usage patterns for the treatment of ADHD in Western Australia”, they reported that 2.2% of children from 2-17 years old, which included 0.4% of adolescents, used ADHD stimulant medication. The most-used ADHD stimulant medication in Australia is dexamphetamine (Dexedrine), which is used more frequently than methylphenidate (Ritalin), which is not frequently used in Australia, as it was only added to the Pharmaceutical Benefits Scheme (PBS) in late 2006. The report compared the consumption rate of ADHD medication between New South Wales and Western Australia and found that 81.8% of ADHD patients in NSW used ADHD medications, whereas only 61.6% ADHD patients from WA used the medications.

In July 2007, the PBS listed the drug Strattera (atomoxetine HCl) as an alternative to stimulant medications for the treatment of ADHD. Strattera is a selective norepinephrine (noradrenaline) reuptake inhibitor (Eli Lilly, 2007). It is indicated in the treatment of ADHD for children six years or older, adolescents and adults; it has been prescribed to 1400 children and 600 adults (Eli Lilly, 2007). The exact chemical mechanism responsible for the therapeutic effects of atomoxetine HCl in ADHD is unknown, however studies in *ex vivo* uptake and neurotransmitter usage conclude that it is by a selective inhibition of the pre-synaptic norepinephrine transporter (2007).

Because ADHD is a major public health problem of interest to many parents, teachers, and health care providers, the National Institute of Mental Health (NIMH, 2000) sponsored a multi-site, cooperative-agreement treatment study of children with ADHD, named The Multimodal Treatment Study of Children with Attention Deficit Hyperactivity Disorder (MTA) (The MTA Cooperative Group, 1999a, 1999b). The goal set was the evaluation of leading treatments for ADHD, inclusive of different forms of behaviour therapy and medications. The study ran across approximately 600 elementary school children aged 7 to 9 years. The children were assigned arbitrarily to one of four treatment modes: (1) medication alone; (2) psychosocial/behavioural treatment alone; (3) a combination of both; or (4) routine community care. The findings revealed that medication management alone (mode 1) and long-term combination treatments (mode 3)

are significantly superior to psychosocial/behavioural treatments (mode 2) and routine community treatments (mode 4) in reducing ADHD symptoms. The study demonstrated that the duration of the differential benefits achieved extended up to 14 months. It was further noted that the combined treatment approach was consistently superior to routine community care, whereas treatment with medication only or behavioural treatment only was not consistently superior. Mode 3 allowed children to be successfully treated over the course of the study with somewhat lower doses of medication compared to the mode 1 group. The MTA results can be generalised to include a wide range of children and families in need of treatment services for ADHD (NIMH, 2000). An overview of the behavioural genetics studies of families, adoption and twins is presented here.

2.2 Behavioural Genetic Studies

In order to investigate genetic and environmental influences, behavioural genetic research focuses on family, adoption, and twin designs. These designs can assist in determining the extent to which a trait or disorder is influenced by genetic and environmental factors. Heritability can measure the total phenotypic variance in a disorder that has a genetic influence. Environmental factors can be measured in two ways: “(1) The Shared Environmental (Common Family Environment) influence accounts for the similarity of individuals within a family in comparison to unrelated individuals in the population and non-shared environment. (2) The Non-Shared Environmental (Unique Environmental Factor) influence accounts for the differences among individuals in a family” (Plomin, De Fries, McClearn, & McGuffin, 2001, p. 115). The next three sections will discuss some of the behavioural genetic studies on ADHD.

2.2.1 Family Studies

Based on previous ADHD family studies, there is evidence that ADHD has a familial nature at childhood latescence (Faraone et al., 2001). Both earlier studies of ADHD – which at the time was defined as Hyperactivity by Cantwell (1972) and Morrison and Stewart (1971) – and more recent studies (Biederman, Faraone, Keenan, Knee, &

Tsuang, 1990; Faraone et al., 1992; Faraone, Biederman, Mick et al., 2000; Frick, Lahey, Christ, & Green, 1991; Schachar & Wachsmuth, 1990) which used the *DSM-III*, *DSM-III-R*, and *DSM-IV* symptoms identified the disorder as having a higher risk of occurrence among family members. Family studies have found that the relative risk for ADHD among first degree probands who have parents and siblings with ADHD is six to eight times higher than the base rate of ADHD in the population (Biederman et al., 1990; Cantwell, 1972; Faraone et al., 1992; Faraone, Biederman, Mick et al., 2000; Frick et al., 1991; Morrison & Stewart, 1971; Schachar & Wachsmuth, 1990). Studies also found no differences in risk of occurrence between boys and girls (Faraone, Biederman, Mick et al., 2000). Furthermore, a study by Samuel et al. (1999) indicated that families of both Caucasian and African-American probands exhibited evidence of the familial nature of ADHD, and suggested that the disorder can occur through other demographic groups.

In conclusion, even though family studies have shown that ADHD can pass through families, research has not been able to ascertain to what degree familial ADHD is caused by genetic or environmental factors.

2.2.2 Adoption Studies

Studies by Morrison and Stewart (1973) and Cantwell (1975) found evidence that biological relatives of hyperactive children are more likely to have hyperactivity than are the adoptive relatives of hyperactive children. In subsequent adoption research by Alberts-Corush, Firestone, and Goodman (1986), Van der Valk, Verhulst, Neals and Boomsma (1998) and Sprich et al. (2000), the findings illustrated that probands with adoptive parents who had ADHD were not considerably different from children whose adoptive parents did not have ADHD, while the biological siblings and parents of non-adopted children with ADHD displayed considerably higher rates of ADHD and associated attention difficulties.

Adoption studies of ADHD have observed that the disorder can involve a genetic aetiology, suggesting that ADHD is familial and that familial risk may be caused by genes rather than by shared environment.

2.2.3 *Twin Studies*

The third major method used to disentangle genetics from environment is the use of twin studies. Twin studies provide more precise estimation of the degree to which genes influence a particular trait and of shared and non-shared environmental factors, by contrasting the similarity of monozygotic (MZ) twins with dizygotic (DZ) twins (Plomin et al., 2001). This section highlights some of the important aspects of twin studies including the historical background and development of twin studies concepts based on a comprehensive article by Boomsma, Busjahn, and Peltonen (2002). Twins can be divided into two kinds: monozygotic or identical twins who are derived from a single fertilized egg, sharing 100% of their genetic composition, and dizygotic or fraternal twins who derive from two fertilized eggs, sharing on average approximately 50% of their genetic makeup. Boomsma et al.'s (2002) article reviewed the beginning of the classical twin study, which originated in 1875, conducted by Francis Galton, who is viewed as the pioneer of the classical twin method. Siemens in 1924, cited in Boomsma et al. (2002), introduced the systematic analysis of similarity between MZ and DZ twins concluding that any heritable disease will be more concordant in MZ twins than in DZ twins, while such concordance will be even lower in non-twin siblings. The higher genetic resemblance in MZ twins is associated with the greater similarities for their traits. It can be inferred that the comparison between MZ and DZ twin similarity provides an estimation of heritability.

Fundamentally, twin studies are used as a means to compare the occurrence of a disorder. Twin studies can also be used to compare MZ to DZ twins for disorder concordance rates, or correlations of continuous traits. Furthermore, to determine disorder incidence or prevalence among twins, it helps to compare MZ to DZ twins for observed concordance rates against expected concordance rates. This kind of study compares discordant twins for developmental, lifestyle, environment or medical care factors, using co-twin cohort studies or controlled trials. Twin studies can also compare levels of exposure to potential causes of disorders with co-twin case-control studies in discordant twins. Moreover, for co-twin studies, comparisons can be made between the

disorder's early markers or biological mechanisms in the unaffected member of the discordant MZ twin pairs. Finally, DZ twins can be used effectively in sib-pair methodologies for genetic linkage-and-association studies (Strachan, 2000).

Researchers of behavioural genetics use zygosity status in genotyping studies including family-based association studies, by selecting genetically informative twin families. The use of a genetically informative twin-family design can distinguish between two types of effect: genotypic association effects between and within family components (Strachan, 2000). Because MZ twins share the same genes, these twins tend to be concordant. In contrast, DZ twins share only about 50% of genes: this means DZ twins tend to be more discordant. This difference can be used to estimate the extent to which genes influence susceptibility to a particular trait, by comparing the degree of concordance in MZ and DZ twins.

Selecting MZ twins only for performing association studies is not considered to be genetically informative for the within-family association component; whereas, they are considered to be so for the between-family component. Using MZ twins for performing association studies can be genetically informative to the within-family association component when paired with non-twin siblings. On the other hand, DZ twins, either paired or not paired with non-twin siblings are considered to be informative for both the within-family and the between-family components.

2.2.3.1 Twin identification methods

There are five methods of identifying twins for research use: (1) Clinical case-series that identify twins having a certain disorder. The benefit of this method is that no twin register is needed: it can effectively pinpoint rare disorders and a wide range of conditions in the twin category including zygosity. The drawbacks are that the estimation of disorder prevalence can not be obtained, twin concordance is selective, and case definition is arbitrary and inflexible. (2) Record linkage to routine data is considered to be a highly efficient, representative, and comparative tool for twins versus single children. (3) A population-based, nationwide twin register is based on birth

records, can furnish more representative prevalence data, eliminate the inherent bias towards concordant twins, and provide flexibility in case definition. However, it is often difficult to set up and maintain, incomplete responses from participants may bias the prevalence, and zygosity may be partly definite (e.g. WATCH, Scandinavian countries).

(4) Volunteer twin series are based on appeals from the media for twins in order to establish a twin register. With this method, twin recruitment requires no twin registry. Responses to surveys or tests are high, with flexibility in case definition. An example of this kind of method is the Australian Nationwide Twin Registry (ATR). The disadvantages of this method are the difficulty in setting it up, developing and maintaining it; bias prevalence may arise because of incomplete responses, zygosity may be incompletely confirmed, and lack of population representativeness.

(5) Using questionnaires or tests with twins in order to ascertain systematically the presence of the disorder (e.g. The Australian Twin ADHD Project, 'ATAP'). This method can give flexible case definition and is less prone to concordance-related biases. However, not getting enough responses may bias the prevalence (Strachan, 2000). ATAP is one of the largest twin registers in the world. It established access with the Australian National Health and Medical Research Council (NHMRC) funded the Australian Twin Registry (ATR) to recruit the twins (Hay, McStephen, Levy, & Pearsall-Jones, 2002; Rasmussen, Neuman et al., 2002). ATAP was first described by Levy et al. (1996; 1997) and Hay et al. (2001).

2.2.3.2 Ascertainment of disorder

There are a variety of strategies used to ascertain disorders among twins. One type of strategy is the use of questionnaires, interviews or objective tests to assess twins for any particular trait or disorder. Another type of strategy is to use routine data linkages: records of births, deaths, disorder registrations or hospital admissions. Clinicians and mutual-support associations can also help to obtain patients with the disorder of interest. Finally, the media may be used to call for twins having the disorder of interest (Strachan, 2000).

2.2.3.3 Twin concordance measurement

Concordance means that both members are affected by a trait or disorder. Determination of absolute concordance rates relies on three methods: firstly, to identify a particular disorder in order to ascertain probands. Questionnaires or objective testing can be administered to twins recruited through a registry to give a better chance of selecting between a sensitive and inclusive case definition, or a more specific and exclusive definition. There is however, a limitation on ascertaining probands diagnosed or defined by studies that rely on routine data linkage or a clinical case-series. Secondly, co-twin studies of affected probands are more effective than the total population of twins, as the former can typically be assessed more comprehensively for the presence or absence of a disorder, allowing greater flexibility in disorder definition. Also, for estimates of concordance rates, more comprehensive criteria are ideal. Lastly, time-interval (longitudinal) studies of ascertained co-twins having the disorder can influence the absolute concordance level (Strachan, 2000). In conclusion, the concordance measurement is dependent on the twin-study designs used.

2.2.3.4 ADHD twin studies

Levy and Hay (2001) suggested that ADHD has a substantial, genetic component but how it contributes to the aetiology of ADHD is unknown. This conclusion was arrived at chiefly by examining the results of twin studies, as well as to a lesser extent family and adoption studies, which varied considerably in methodology and definitions of ADHD. Nevertheless, genetic component was determined to be responsible for a significant amount of the phenotypic variance present in ADHD cases, with heritability estimated at 0.70 or greater in most cases (Faraone & Doyle, 2000; Smalley, 1997; Tannock, 1998). In 20 ADHD twin studies a correlation was found between the phenotypic variance in ADHD symptoms and the non-shared environmental influences (average $e^2=0.27$) (Coolidge, Thede, & Young, 2000; Edelbrock, Rende, Plomin, & Thompson, 1992; Gillis, Gilger, Pennington, & deFries, 1992; Gjone, Stevenson & Sundet, 1996; Goodman & Stevenson, 1989; Hudziak, Rudiger, Neale, Heath, & Todd, 2000; Kuntsi & Stevenson, 2001; Levy et al., 1997; Martin, Scourfield, & McGuffin, 2002; Matheny & Brown, 1971; Nadder, Silberg, Eaves, Maes, & Meyer, 1998; Rietveld, Posthuma, Dolan, & Boomsma, 2003; Schmitz, Fulker, & Mrazek, 1995; Sherman, Iacono, &

McGue, 1997; Silberg et al., 1996; Stevenson, 1992; Thapar, Harrington, Ross, & McGuffin, 2000; Thapar, Hervas, & McGuffin, 1995; Willcutt, Pennington, & deFries, 2000; Willerman, 1973). These studies additionally demonstrated that shared environmental influences were not of consequence.

A twin model designed by Eaves, Silberg et al. (1997) showed that the heritability of ADHD measured by parent and teacher ratings was high, while the contrast effects (i.e. MZ-DZ similarity) did not show significant heritability. Moreover, Martin, Scourfield, and McGuffin (2002) discovered strong heritable evidence (approximately $h^2=0.75$) on ADHD twins, when tested with a univariate model (ACE) based on parent- and teacher-rated data. They concluded that ADHD have different genotypes which require more than one genetic design in order to detect those ADHD genes. Recently, Willcutt (in press), Biederman (2005) and Faraone et al. (2005), estimated the mean heritability (h^2) of ADHD from more than 20 studies (American, 1987) (Figure 2.1). They respectively reported the mean heritability for ADHD as 0.73, 0.76, and 0.77, which gives a total mean of 70.5. Therefore, ADHD is considered as a good candidate for molecular genetic studies. Levy and Hay (2001) stated that one of the key reasons for the ability to understand the aetiology of ADHD (phenotype, classification, and comorbidity) are the recent advances that have been achieved in the fields of molecular, quantitative, and behavioural genetics, which enable identification of the gene(s) that may contribute to this disorder. In addition, a recent study by Martin, Piek, and Hay (2006) has found evidence of similar rates in the genetic involvement in ADHD symptoms between genders, implying that the genetic factors involved in ADHD symptoms were found to be largely the same in boys and girls. This suggests that molecular genetic studies can validly examine the same candidate loci in both sexes. A study by Derks, Dolan, Hudziak, Neale, and Boomsma (2007) found high heritability in both boys and girls for ADHD. It also found that teacher assessment, based on Conners' Teacher Rating Scale-Revised: Short version (CTRS-R:S), could be related to the same latent variables in boys and girls. This originated from finding no gender differences in the factor structure of the CTRS-R:S, implying the absence of measurement bias.

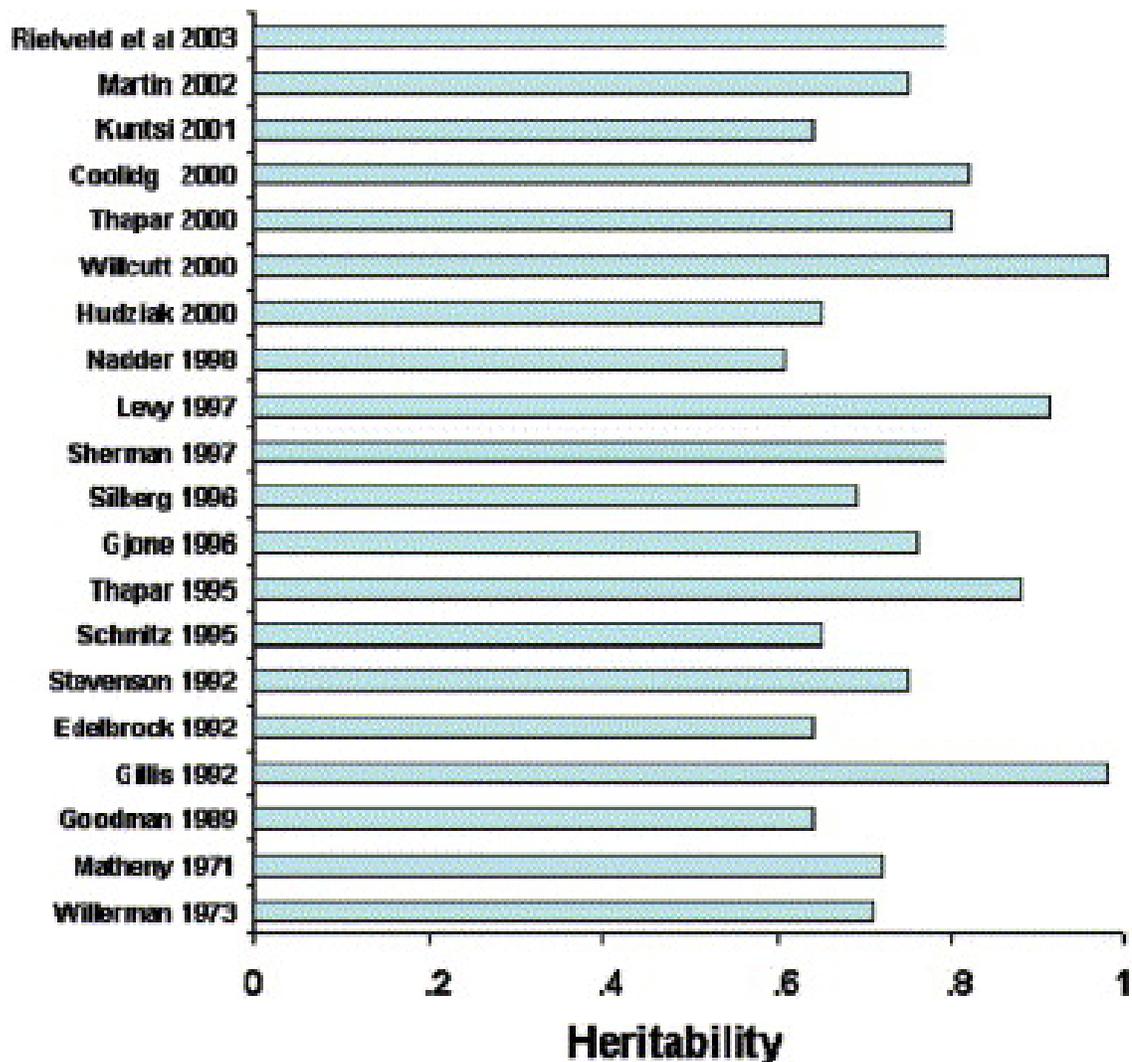


Figure 2.1 Estimated heritability of attention-deficit/ hyperactivity disorder, based on pooled results from 20 studies. (Source: (Faraone et al., 2005).

The Australian Twin ADHD Project (ATAP) is one of the world’s largest projects. Its goals are to understand the aetiological and developmental patterns of ADHD, to establish a robust database for performing quantitative genetic analyses and molecular genetic analyses (in order to determine the contributions of genetic and environmental factors to ADHD), and to understand the comorbidity of ADHD with other behavioural disorders such as Reading Disability (RD), Conduct Disorder (CD), Oppositional Defiant Disorder (ODD), and Generalized Anxiety Disorder (GAD) (Bennett et al., 2006). A study by Martin, Piek, and Hay (2006) on 1285 twin families from ATAP has shown that

comorbidity of ADHD subtypes with Developmental Coordination Disorder (DCD) exhibited a strong shared-additive genetic component, especially between the DCD-fine motor and ADHD-Inattentive subtype based on *DSM-IV* criteria, displaying an existence of a significant genetic element in all ADHD and DCD subsets. Another study by Martin, Levy, Piek, and Hay (2006) on 2040 twin families from ATAP has examined the comorbidity of ADHD with Conduct Disorder, Oppositional Defiant Disorder, and Reading Disability. The results showed a presence of a shared genetic heritability of 31% of the ADHD-Inattentive subtype with Reading Disability, indicating a strong comorbidity between them. Comparatively, 37% of a shared genetic heritability existed between the Hyperactivity-Impulsivity subtype with Conduct Disorder, and 42% of a shared genetic heritability was found between Oppositional Defiant Disorder (ODD) and the Hyperactivity-Impulsivity subtype, again indicating a strong comorbidity.

In the UK, Robert Plomin established the Twin Early Development Study (TEDS) as a large-scale twin study investigating issues related to language and cognitive development, including ADHD as well. TEDS is working on MZ and DZ twins by using twin methodology in order to investigate the genetic and environmental influences on three major (and common) childhood psychological problems: communication disorders, mild mental impairment, and behavioural problems (Plomin, 2003). A recent twin study by McLoughlin et al. (2007) on ADHD investigated the aetiological overlap between two ADHD subtypes (Inattentive and Hyperactive-Impulsive) based on the two subscales of the Conners' 18-item *DSM-IV* checklist, as well as it examined the genetic specificity among ADHD subtypes. The study found a high genetic overlap between the Inattentive and the Hyperactive-Impulsive symptoms, but also there was a significant unique genetic effect for each subtype, suggesting that there is a genetic heterogeneity for the Hyperactive-Impulsive subtype which is associated with specific genes for the Inattentive subtype. Therefore, refining the phenotypes of ADHD is the key issue to reducing the heterogeneity. These results also suggested that molecular genetic studies should investigate each subtype separately as it would be possible to group ADHD genes into three sets of genes, related to Hyperactivity-Impulsivity, to Impulsivity, and to both symptoms.

Because of the association between ADHD and Intelligence Quotient (IQ), twin studies were needed to examine whether the co-occurrence of ADHD and lower IQ is due to genetic or environmental causes. While the aetiology between ADHD and IQ is not fully understood, research has shown that ADHD and lower IQ differ in the population. Kuntsi et al. (2004) reviewed the relationship between ADHD and low IQ, and stated the following: (1) ADHD children can differ from children without ADHD by seven to twelve IQ points and the correlation between ADHD symptoms and IQ rating scale is -0.2 to 0.4; (2) the presence of common genetic factors may help discover ADHD and IQ candidate genes; (3) the co-occurrence of ADHD and IQ can imply that the low IQ scores may cause academic delay and failure to achieve occupational skills; (4) the heritability estimates of IQ can increase gradually with age (e.g., the heritability (h^2) at ages of 4-6 yrs old = 0.4, at ages 6-12 = 0.5, and in adulthood, 0.8); (5) twin studies implicated environmental factors that contributed to both ADHD and IQ because of the short unity of the heritability. Therefore, the genetic and environmental estimates for ADHD and IQ were studied separately.

Rucklidge and Tannock (2001) reported that in general population samples, the difference between children diagnosed with ADHD and control children is about 7–12 IQ points. In addition, Rapport et al. (1999) found the correlation between rating-scale measures of ADHD symptoms and IQ is -0.2 to -0.4. Kuntsi et al. (2004) revealed the presence of a shared genetic aetiology between ADHD symptom scores and lower IQ, inferred from a correlation of 0.86, between ADHD-symptom scores and IQ, as well as from a correlation of 100% between ADHD research diagnosis and IQ scores. The study also showed that the environmental factors that contributed to the ADHD alone and IQ alone did not significantly contribute to their co-variation in the population. The study concluded that the shared genetic aetiology of ADHD symptoms and lower IQ might be caused by pleiotropic effects of genes involving in the aetiology of ADHD, or genes related to IQ.

2.2.3.5 ADHD concordance rate and contrast effects studies

Comparing MZ and DZ twins for concordance rates is the usual method to measure heritability for a diagnostic category. In the ATAP, Levy et al. (1997) and Levy et al. (2001) examined the concordances between probands, based on *DSM-IV* criteria on 1167 same-sex twin pairs, and found that MZ pairs exhibited a significantly higher concordance rate (57-62%) than same-sex DZ pairs, who displayed a concordance rate of (8-30%). The disparity between the MZ and DZ concordance rates indicates that ADHD has a significant heritable component, and suggests that an MZ concordance of less than 100% may be due to environmental influences involved in the aetiology of ADHD.

The correlation of 0.62 in MZ twins exhibits a high intra-class correlation, while the correlation of 0.30 in DZ twins is considered a low intra-class correlation. Thapar et al. (1995) suggested that this lower correlation incorporates sibling competitive or contrast effects that tend to exaggerate true differences between DZ twins. Furthermore, Carey (1986) and Eaves et al. (1997) stated that “contrast effects” can result if DZ twins are treated more differently than are MZ twins; this differential treatment affects hyperactivity (Scarr, 1986). The correlations for DZ twins may also be a result of rating DZ twins as less similar than MZ twins based on their expected zygosity as perceived by informants (Goodman & Stevenson, 1989). Low DZ correlations may also arise from either genetic dominance effects or non-additive genetic interaction; however, this would not apply to correlations less than zero (Levy, Hay, Waldman, & McStephen, 2001).

Neale and Cardon (1992) reported that, in order to explain genetic and environmental factors in ADHD aetiology and the contrast effects influence on hyperactivity ratings, structural modelling has been used. Thapar et al. (1995) reported that model-fitting results from the Rutter A scale, on maternally-rated scores of hyperactivity, were found to be influenced by contrast effects and additive genetic factors with heritability estimated at 0.88 in a population-based sample of 376 twin pairs. Recent studies from ATAP and the Virginia Twin Study of Adolescent Behavioural Development (VTSABD) used the complete *DSM-IV* diagnostic criteria or similar data, allowing discrimination between the Hyperactivity-Impulsive and Inattentive subtypes of ADHD (Levy et al., 2001).

Results from Virginia Twin Study of Adolescent Behavioural Development (VTSABD) by Silberg et al. (1996) indicated that 70% of the observed variation in hyperactive behaviour in males and females was explained by additive genetic effects whereas sibling-bias interaction effect on the rater accounted for 1-5% of the variation. A similar study by Eaves et al. (1997), using VTSABD samples, reported that ADHD symptomatology was assessed using both maternal and parental ratings from a semi-structured face-to-face interview. After the removal of contrast effect, a heritability estimate of 0.6-0.8 was found (Eaves et al. (1997), as cited in Levy, McStephen et al., 2001). A study by Nadder et al. (1998) also used VTSABD subjects. They reported that rater-bias effects need to be corrected by the use of maternal, parental, and teacher ratings. Using only one rater does not differentiate between contrasts that are a result of rater bias versus those derived from competitive sibling interactions. They proposed that incorporating contrast effects provided the best fit for ADHD symptomatology. Simonoff et al. (1998, as cited in Levy, McStephen & Hay, 2001) applied hyperactivity ratings on VTSABD twin pairs from mother's and teachers' reports, in order to detect the origin of the contrast effects. In a proportion of twins studied, the independent teacher-reports were used. It has been implied that contrast effects from the maternal data models were operative, while teacher ratings were influenced by twin confusion or by rater bias.

2.3 *ADHD Candidate Genes*

Because ADHD is a heritable, complex disorder, it may be influenced by genes of small effect. This means that ADHD is not only caused by one gene but by several genes that, together, cause the disorder. In addition, it could be that the same phenotype of ADHD can be influenced by several genes. Both the genetic and environmental factors, and their interaction, contribute to cause ADHD susceptibility (Yeh, Morley, & Hall, 2004). As ADHD shows a complex pattern of inheritance, the challenge in understanding the aetiology of ADHD is to disentangle the genetic and environmental factors.

It is most likely that ADHD is a polygenic and multifactorial disorder that displays additive inheritance. This would mean that ADHD susceptibility is influenced by a number of gene variants that are present in the general population. Each gene variant is likely to have a small effect upon the risk of developing ADHD. None of these genes is

sufficient or necessary to cause the disorder (Yeh et al., 2004), and the same phenotype may be produced when different combinations of genes exceeds a threshold. The combined effects of variant genes, environmental factors, and their interaction all contribute to ADHD susceptibility. This complexity may make it difficult to identify genetic polymorphisms that influence ADHD liability (Yeh et al., 2004).

Todd (2000) asked the question, “Are we ready for ADHD molecular genetics?” In answer there have been many twin studies confirming strong evidence of the genetic factors, playing an important role in discovering the aetiology of ADHD. Technological and statistical advances in the field of molecular genetics have allowed researchers to move beyond the quantification of heritability to begin to hunt for candidate genes that contribute to the ADHD phenotype. By using the strategies of Linkage and Association with Quantitative Trait Loci (QTL), Todd (2000) concluded that advances in identifying allelic variations in genes causing ADHD are possible. However, ADHD molecular genetic study replications must await better characterization of the heritable phenotypic elements of ADHD and a better understanding of its genetic heterogeneity. The third ADHD Molecular Genetics Network meeting (Faraone, 2002) reported that there is more effort needed in order to understand the genetic mechanisms that contribute to ADHD, because of the failure to replicate findings, the complexity of the ADHD phenotype, the likelihood of genetic heterogeneity, and the probability that several genes of small effect are acting in concert.

2.3.1 DAT1 or SLC6A3 (Sodium-Dependent Dopamine Transporter) Gene

The Sodium-Dependent Dopamine Transporter gene (DAT1 or SLC6A3) is a candidate gene for ADHD and is located on chromosome 5p15.3 (DiMaio, Grizenko, & Joobar, 2003). Numerous genetic association studies have demonstrated a positive association between ADHD and polymorphism within this gene (Masellis et al., 2002). DAT1 is involved in the reuptake of dopamine from the synaptic cleft back into the presynaptic cell, and thus plays a major role in the regulation of functional dopamine levels in the brain (Waldman & Rhee, 2002). In the case of methylphenidate response and Adverse Drug Reactions (ADRs), the DAT1 gene is a candidate of paramount importance, given that methylphenidate binds to and directly inhibits its expressed protein.

The DAT1 gene as a transporter is the principal target for ADHD stimulant medications such as dexamphetamine and methylphenidate (Kirley et al., 2002; O'Rourke, 2003). These stimulant medications are the most popular and the most effective treatments for ADHD. They exert their therapeutic effects primarily by blocking presynaptic reuptake of dopamine (DiMaio et al., 2003), thus increasing functionally active levels of dopamine at the synapse (Waldman & Rhee, 2002). DAT1 is therefore of primary interest, since it is posited that methylphenidate (dexamphetamine) exerts an inhibitory effect on its function. A study of DAT1 'knockout' mice showed extreme levels of hyperactivity and greatly increased synaptic levels of dopamine in mice that were homozygous for DAT1 deactivation. The DAT1 knockout mice exhibited a fivefold to sixfold increase in motor activity and dopamine remained in their synaptic cleft 100 times longer than in wild-type mice. DiMaio et al. (2003), Giros et al. (1996) and Roman, Rohde, and Hutz (2004) reported that the sequence analysis of this gene revealed a VNTR (variant number of tandem repeats) polymorphism with a 40 bp unit repeat length, ranging from three to thirteen copies; therefore, focus was on the 3' VNTR marker, in particular the 10-repeat (480 bp) putative high-risk allele, as well as the 9-repeat 440 bp allele.

An early family-based study reported an association between ADHD and the 480 bp allele at VNTR in DAT1 (Cook et al., 1995). These results were later replicated in family-based studies using the Transmission/Disequilibrium Test (TDT) and Haplotype Relative Risk (HRR) methods (Daly, Hawi, Fitzgerald, & Gill, 1999; Gill, 1997). Terwilliger and Ott (1992) showed that HRR can account for parental marker alleles (or haplotypes) transmitted to an affected child, and they compared them with those parental alleles not transmitted. Moreover, Waldman et al. (1998) reported an association between the presence of linkage disequilibrium of DAT1 and ADHD. Their evidence was particularly strong for the *DSM-IV*-defined ADHD Hyperactive-Impulsive subtype. In addition, they found that the number of high-risk (480 base pairs) alleles was related to Hyperactive-Impulsive symptoms but not to Inattentive symptoms. Swanson et al. (2000) measured allele proportions of the 10-repeat VNTR polymorphism among ADHD populations. Using the HRR method, a significantly greater frequency of the 10-repeat allele was observed compared with control groups, indicating that the transporter gene is likely to be implicated in the aetiology of ADHD. In contrast, another study by Todd, Jong, et al.

(2001) examined the association using the Transmission/Disequilibrium Test in a population sample of twins. Using a number of ADHD diagnostic systems, they found no significant disequilibrium of VNTR alleles. Maher, Marazita, Ferrell and Vanyakov (2002) performed a meta-analysis of 11 studies examining DAT1, with a total of 824 informative meioses that yielded a non-significant pooled odds ratio of 1.27 and a p-value of 0.06.

Kirley et al. (2002) confirmed the association of DAT1 (480 bp allele) with ADHD, when a significant preferential transmission of DAT1 (480 bp, 10-repeat VNTR allele) (TDT: $X^2=4.57$; $p=0.042$; HRR: $X^2=7.5$; $p=0.0062$) was observed. DiMaio et al. (2003) also pointed out the strong possibility of a link between DAT1 and ADHD. They concluded that the replicated evidence of SLC6A3's implication in ADHD strongly indicated that the brain dopamine systems are involved in the pathogenesis of ADHD. A recent study by Lim et al. (2006) found evidence of association with the 10-repeat allele of a 40-bp 3' UTR VNTR polymorphism of DAT. This finding arose in 33 *DSM-IV*-defined ADHD Korean probands. Results showed evidence of increased transmission of the 10-repeat allele using TDT ($p=0.001$; OR=7.88; CI=2.20-28.29). A study by Langley et al. (2005) tested the DAT1 3' VNTR and three single-nucleotide polymorphisms (SNPs) in the putative promoter region of DAT1 for association with ADHD in 263 parent-proband trios. In contrast to Lim et al. (2006) their results showed no association between the disorder and DAT1 3' VNTR, with an additional three promoter variants. Based on the foregoing, it is likely there is an association between the 10-repeat allele of DAT1 3' UTR VNTR and ADHD, which has been reported in several recent studies. A more tenuous association between the DAT1 genotype and stimulant medication response has been observed; although not universally (Holmes, 2000; Roman et al., 2001; Smith et al., 2003; Swanson, Wasdell et al., 2000; Todd, Jong et al., 2001). The review by Roman et al. (2004) concluded that despite negative findings of any significant genetic association of a 40 bp VNTR polymorphism with ADHD, their results suggested that the allele with 10-repeat of the 40 bp sequence was the risk allele for ADHD.

A meta-analysis by Purper-Ouakil et al. (2005) on twelve published family-based association studies between ADHD and the 10-repeat allele of the DAT1 gene showed no

significant association ($p=0.21$); however, a significant heterogeneity between those studies was found. Moreover, they reported that the odds ratios increased with more recent studies and may decrease with larger sample size. DAT1 is the second-most replicated candidate gene in the field of ADHD molecular genetics; nonetheless, further studies are required to validate this genetic relationship.

It has been suggested that there is a direct pharmacological interaction between dopamine-system candidate genes with methylphenidate. This is of interest given that methylphenidate (Ritalin) is thought to inhibit the function of the dopamine transporter by preventing presynaptic reuptake (Giros, 1996).

2.3.2 *DRD4 (Dopamine Receptor D4) Gene*

The human Dopamine Receptor D4 (DRD4) plays an important role in attention (conscious, directed mental thought) and in disorders of attention (Swanson et al., 2000). It is considered a candidate gene for ADHD, given its expression in areas of the brain that are likely to underlie ADHD. Results from knockout studies in mice relating to the gene and novelty-seeking also deserve attention (Dulawa et al., 1999; Paterson, Sunohara, & Kennedy, 1999). DRD4 is found close to the telomere of chromosome 11p15.5 and is among the most variable of the human genes known (Ding et al., 2002; Swanson, Flodman et al., 2000). The majority of this diversity is due to the length and SNP variation in a 48 bp tandem repeat (VNTR) in exon 3, encoding the third intracellular loop of this dopamine receptor. The gene is thought to play a role in G-protein coupling (DiMaio et al., 2003). A genetic architecture study of the DRD4 gene was performed by Wang et al. (2004), which assumed that an extraordinary mutational event resulted in the 7-repeat allele, and positive selection then increased this allele to high frequency. Based on this assumption, a method for directly estimating haplotype diversity in specific geographic regions (Africa, Europe, Asia, North and South America and the Pacific Islands) was created by the team, the purpose of which was to entirely sequence DRD4 from 103 subjects homozygous for 2-allele, 4-allele, or 7-allele variants of the VNTR. Significantly, their findings in the 7-allele variants showed a strong worldwide Linkage Disequilibrium (LD) across disparate geographic locations ranging from sub-Saharan

Africa to areas of the South American rainforests, with 7R/7R individuals exhibiting the same alleles at most polymorphic sites.

Lowe et al. (2004) found the 48 bp sequence can be repeated up to ten times, with the 4- and 7-repeat alleles being the most common in the Caucasian population. One of the most replicated associations between ADHD and the dopaminergic system is the 7-repeat allele of the VNTR polymorphism of the DRD4 gene (Hawi et al., 2003). This was based on an association found between specific alleles of this extremely variable gene and certain behavioural phenotypes. The association has been confirmed by numerous analyses (Benjamin et al., 1996; Eisenbarth et al., 2001; La Hoste et al., 1996; Swanson, Flodman et al., 2000; Swanson, Wasdell et al., 2000). Initial studies seemed to indicate that the 7-repeat allele of the DRD4 gene was most likely linked to the behavioural trait of novelty-seeking (Benjamin et al., 1996; Ebstein et al., 1996); however, the association demonstrates a strong link between the 7R allele and ADHD (Benjamin et al., 1996; Ebstein et al., 1996; La Hoste et al., 1996; Swanson, Flodman et al., 2000; Swanson, Wasdell et al., 2000).

A meta-analysis performed by Faraone et al. (2001) on the association between the 7-repeat allele of DRD4 and ADHD demonstrated a small significant DRD4-ADHD association for both case-control and family-based studies (case-control: $p=0.00000008$; relative risk = 1.9; 95% Confidence Interval (CI) = 1.4 - 2.2; within-family: $p=0.02$; relative risk (RR) = 1.4; 95% CI = 1.1 - 1.6). This strongly implicates the role of DRD4 in ADHD, highlighting the importance of dopamine in the aetiology of the disorder. In addition, this study indicated that, even though there was a small risk of the 7-repeat allele conferring ADHD upon individuals, this allele might play an important role at the population level, because of its relatively high population frequency (DiMaio et al., 2003).

A recent study by Lowe et al. (2004) searched for other markers rather than the 7-repeat allele of the VNTR polymorphism of DRD4. This is because of lack of firm link between this VNTR polymorphism with ADHD as reported by previous studies (Bakker et al., 2005). This study sought to investigate additional markers (120bp, -616 SNP (C

allele), -521 SNP (A allele), -376 SNP (C allele), and 7-repeat allele VNTR at the 5'-end with potential influence on the expression of the DRD4. The findings showed a significant over-transmission of -616 SNP ($X^2=7.45$; $p=0.008$; $OR=1.63$), and an excess transmission of the A allele of the -521 SNP, although it was not statistically significant ($X^2=2.14$; $p=0.17$; $OR=1.25$). The results exhibited a weak but significant LD between the 120 bp duplication and the VNTR ($D'=0.151$), but no evidence of LD between either the -616 or -521 promoter SNPs and the VNTR. Accordingly, it was concluded that association between the -616 SNP and ADHD is independent of findings with the 7-repeat VNTR polymorphism; therefore, more investigation is needed to examine this, preferably in samples that had showed significant previous association with the VNTR.

Another recent study by the same group (Kirley et al., 2004) used the TDT on *DSM-IV* ADHD proband sample of 178. The results showed non-significant excess transmission of the *DSM-IV* ADHD Combined type with the DRD4 7-repeat allele. However, the results were significantly associated with ADHD children with comorbid Oppositional Defiant Disorder (ODD) ($X^2=6.7$; $p=0.01$; $OR=2.5$). Also, there was significant preferential transmission of this allele to ADHD children with ODD compared to those without ODD ($X^2=5.1$; $df=1$; $p=0.025$; $OR=2.8$). Association of the DRD4 -616 polymorphism to children with comorbid ODD was also observed ($X^2=5.3$; $p=0.03$; $OR=1.9$). These findings provided further support for the investigation of clinical subtypes within the ADHD phenotype.

Todd et al. (2005) examined whether population-based ADHD subtypes, defined by Latent Class Analysis, help to differentiate findings across ADHD-gene association studies. The data of this study was taken from three previous association studies that exhibited no association between polymorphisms of the DRD4 and DAT1 genes and *DSM-IV* ADHD symptoms. Todd's 2005 study showed significant association between the combined data sets for 440 base-pair 3' DAT VNTR polymorphism and population-defined severe Combined ADHD ($OR=1.25$; $p=0.01$). Another slightly significant association ($OR=1.20$; $p=0.16$) occurred between the 7-repeat DRD4 allele and population-defined severe Combined ADHD. These results offer preliminary validation that these population-defined ADHD subtypes may have different genetic associations.

The finding may also resolve some of the variable results presented in candidate gene association studies.

2.3.3 *DRD5 (Dopamine Receptor D5) Gene*

The dopamine receptor D5 is a candidate gene for ADHD and is located on chromosome 4p16.1 (Fisher et al., 2002). Four studies reported an association and linkage between DRD5 polymorphic loci and ADHD. The first report was by Daly et al. (1999). They reported an association between the 148 bp DRD5 allele and ADHD, as well as a preferential transmission of this allele (RR=1.67 (1.29-2.15); p=0.00005). They noticed that the transmission of it was stronger in non-familial cases (RR=1.59 (1.05-2.42)). A second study performed by Tahir et al. (2000) used a multiallelic version of the TDT to examine Linkage Disequilibrium between ADHD and DRD5, and found preferential transmission of a 151 bp allele ($X^2=2.38$; p=0.061; RR=1.28). In addition, modest support for this association was seen in a case-control sample of ADHD children diagnosed with Tourette's syndrome (Comings et al., 2000). Nonetheless, substantial evidence for biased transmission of the 148 bp allele was not observed by Barr et al. (2000). They did not observe any significance; however, they observed biased transmission of the 136 bp and 146 bp alleles. In their family-based study, Payton et al. (2001) were unable to detect a significant association between ADHD and DRD5; however, a trend was identified for preferential transmission of the 148 bp allele. A meta-analysis of family-based studies found a significant association between DRD5 and the disorder, suggesting that the non-significant findings were due to low statistical power (Maher et al., 2002). Fisher et al. (2002) did a genome-wide scan for loci involved in ADHD and reported that DRD5 coincided with sites of positive linkage for ADHD. A study performed by Mill et al. (2004) found that an allele of D4S615 (a dinucleotide repeat located 131 kb 3' of DRD5) demonstrated significant association with ADHD.

A family-based study by Lowe et al. (2004) found a significant association of the 148 bp allele with ADHD (OR=1.2; 95% CI=1.1 - 1.4) in the Inattentive and Combined subtypes. The same group found two additional 5' microsatellite markers and an SNP in the 3' untranslated region which showed a significant association with ADHD (Hawi et al., 2003). All these studies suggest a strong role for DRD5 in increasing the risk for ADHD.

2.3.4 Dopamine Beta-Hydroxylase (DBH) Gene

Dopamine-beta-hydroxylase (DBH) is an enzyme responsible for the conversion of dopamine to norepinephrine. DBH is released along with catecholamines from the adrenal medulla and from sympathetic nerve endings (DiMaio et al., 2003). This enzyme is expressed within the secretory vesicles of norepinephrine and epinephrine-producing neurons and neuro-secretory cells. DBH is also present in human plasma and cerebrospinal fluid (CSF), where its activity and level are highly stable and correlated.

The DBH gene is located on chromosome 9q34.2 (Fisher et al., 2002), and is closely linked to the ABO blood group locus (Roman et al., 2001). The DBH gene is a candidate for ADHD and has several polymorphisms that have been described in this locus, all of which are correlated with the level of DBH activity (Tang et al., 2006). Comings et al. (1996) examined a *TaqI* restriction site polymorphism in intron 5 (T-allele SNP) of DBH and found evidence of significant association in a sample having Tourette's Syndrome with ADHD. A follow-up study by Daly et al. (1999) also reported a significant association at the A2 allele of *TaqI* polymorphism of the DBH gene. They found that the allele was preferentially transmitted to ADHD children 124 times and not transmitted 95 times in 86 trios and 19 parent-proband pairs ($p < 0.05$). Payton et al. (2001) reported no association between DBH and ADHD in a family-based study. Roman et al. (2002) detected an association between ADHD and the DBH *TaqI* A2 allele in a sample encompassing 88 Brazilian nuclear families. In performing Haplotype Relative Risk (HRR) analysis of the DBH *TaqI* restriction site polymorphism, Roman's group found that a preferential transmission of the *TaqI* A2 allele in the entire ADHD sample ($X^2=3.61$; $p=0.03$) was evident.

A recent study by Tang et al. (2006) found an association of DBH SNPs with ADHD. They also found Linkage Disequilibrium with two putative functional SNPs. These three single SNPs are -1021C→T (rs161115; SNP1), DBH *TaqI*A (rs2519152; SNP3)(which has been found associated with ADHD (Comings et al., 1996), and +1603C→T (rs6721; SNP3). Tang et al. (2006) examined whether SNP2 associates with plasma dopamine beta-hydroxylase activity ($pDBH$), and whether Linkage Disequilibrium between SNP2 and the other SNPs explained this association. The study concluded that SNPS2 was significantly

associated with *p*DBH activity, with the T-allele associating with lower *p*DBH activity (also it has a significant LD with SNP1 and SNP3). DiMaio et al., (2003) concluded that although replication with larger samples is needed to support the association in any working model of ADHD, these findings do shed light on the possible association between DBH and ADHD.

2.3.5 MAO (Monoamine Oxidase) Gene

Monoamine Oxidases (MAO) are gene-encoded enzymes that metabolize dopamine and other neurotransmitters (Kirley et al., 2002). MAO genes are located on chromosome Xp11.3 (Fisher et al., 2002), and are considered as candidates for ADHD. Jiang et al. (2001) reported an association with the DXS7 locus on the X chromosome, a microsatellite marker closely linked to MAO genes. TDT was used by the same group to test for linkage between a VNTR polymorphism at the MAO-A or MAO-B locus and ADHD diagnosed according to the *DSM-III-R* criteria in 82 Chinese nuclear families. A linkage was found between ADHD and the MAO-A locus ($X^2=15.25$; $p<0.05$), however, this linkage was not evident at the MAO-B locus ($X^2=11.18$; $p>0.05$). Recently, Domschke et al. (2005) tested four polymorphisms of the MAO-A gene and two markers in the MAO-B gene in a sample of 179 Irish ADHD nuclear families. The four polymorphisms of MAO-A gene are 30 bp promoter VNTR, CA microsatellite in intron 2, 941G/T SNP in exon 8, and A/G SNP in intron 12. The two polymorphisms of the MAO-B gene are CA microsatellite in intron 2 and T/C SNP in intron 13. Results showed a significant association between the MAO-A 941G allele with ADHD ($X^2 =5.1$; $p= 0.03$; OR=1.7) by TDT, and also a significant increased transmission of a haplotype, consisting of the shorter allele of the promoter VNTR (allele), the 6-repeat allele of the CA microsatellite and the G-allele of the 941G/T SNP ($p=0.01$). These results suggest that MAO-A (941G) might be a susceptibility factor for ADHD.

2.3.6 SNAP-25 (Synaptosomal-Associated Protein of 25 kDa) Gene

The gene of the presynaptic plasma-membrane protein SNAP-25 (synaptosomal-associated protein of 25 kiloDaltons) is expressed highly and specifically in the nerve cells, and encodes a protein which is necessary for synaptic vesicle fusion and neurotransmitter release. SNAP-25, along with syntaxin 1a and VAMP-2

(Synaptobrevin-2), make up the core essential for docking and holding synaptic vesicles at the presynaptic membrane in preparation for neurotransmitter exocytosis triggered by Ca⁺ (Söllner et al., 1993).

The SNAP-25 gene is reported in different locations: Maglott, Feldblyum, Durkin and Nierman (1996) reported that the gene is located on chromosome 20p11.2, while Fisher et al. (2002) reported that it is located on chromosome 20p12.3. SNAP-25 is potentially related to dopamine transmission and may be implicated in the aetiology of ADHD (Wilson, 2000). Mill et al. (2002) identified a novel microsatellite repeat in SNAP-25 located between the 5'UTR and the first coding exon, and have tested it for association with ADHD. The analyses of case-control suggest there may be a role for this polymorphism in ADHD, with one allele over-represented in probands. Within-family tests of linkage-and-association confirmed these results. Another study by Mill et al. (2004) tested eight polymorphisms of the SNAP-25 gene; two microsatellite and six SNPs. The results exhibited a significant association between three individual SNPs: SNP-2015 A/T located in the putative promoter region; a microsatellite in intron 1; and 80609 G/A located in intron 7 with ADHD. The haplotype analysis detected evidence of strong association of these three markers rather than just individually, and the pooled analyses for T1065G showed significant evidence for association with the disorder.

2.3.4 Serotonin Receptors (HTR1B and HTR2A) and Transporter (5-HTT or SLC6A4) Genes

Two different 5-HT receptors may be involved in the aetiology of ADHD (Kirley et al., 2002). First, 5-HTR1B is an auto receptor found on presynaptic serotonergic neurons and functions to modulate the release of 5-HT. This receptor is also found in areas known to be involved in motor control, such as the striatum, frontal cortex, medulla, hippocampus and pituitary. The polymorphism of the HTR1B gene encodes for the 5-HTR1B serotonin receptor. Second, the 5-HTR2A serotonin receptor, encoded by the HTR1B polymorphism, has recently been implicated in ADHD by linkage-and-association studies using a 452Tyr polymorphism in the HTR2A (Quist et al., 2003). This receptor is known to encode a protein involved in signal transduction mediated via phosphoinositol

hydrolysis and intracellular Ca^{2+} mobilization. Both polymorphisms at either HTR1B or HTR2A have been postulated to contribute to the development of ADHD. Evidence suggests that the disorder is a consequence of the pathophysiological interplay of the dopamine and serotonin systems (Hawi et al., 2002). Hawi et al.'s study involved an examination of polymorphisms in HTR1B and HTR2A, which involved the respective encoding of the serotonin receptors 5-HT1B and 5-HT2A in a European ADHD sample. It was discovered that the total sample revealed that the allele 861G of the HTR1B had a considerable transmission for HRR ($X^2=7.4$, $p=0.0065$) and TDT ($X^2=6.4$; $p=0.0014$). It was also found that the total sample showed little association between the His452Tyr polymorphism and ADHD but a slight increase in transmission of the allele 452His was recorded in an Irish sample ($X^2=4.9$; $p=0.0026$) (Quist et al., 2003). Hence, it was concluded that the development of ADHD was a result of the pertinent role of the serotonin system.

A study by Kent et al. (2002) investigated two more 5-HTT polymorphisms and the VNTR in the interon 2 and the 3' TUR SNP ($X^2=4.06$, $p=0.04$). A subsequent haplotype analysis in ETDT revealed important preferential transmission of haplotypes that had the T allele of the 3' UTR SNP with the long allele of the promoter polymorphism ($X^2=13.18$; $p=0.004$) and the 10-repeat of the VNTR ($X^2=8.77$; $p=0.03$). Quist et al. (2003) conducted a separate study based on the hypothesis that the 5HT1B receptor could be a positive candidate for ADHD genetic studies. Based on a sample of 115 families using TDT, they tested for associated disequilibrium between the 5HT1B G861G polymorphism and ADHD. Their study revealed that there was a trend of excessive transmission of the 861G allele ($X^2=2.91$; $p=0.09$), which, when a more thorough analysis for parental allele transmission was conducted, showed that in an affected child, there was higher paternal allele transmission of the G allele ($X^2=4.80$; $p=0.03$).

Previous findings as well as other studies suggest an association between the HTR1B gene, but not HTR2A, and ADHD; however, this needs further confirmation (Faraone et al., 2005). A recent study by Smoller (2006), using an independent sample of 299 families,

examined whether the G861G SNP is associated with ADHD, and also tested for linkage disequilibrium of the HTR1B locus with ADHD and its diagnostic subtypes. Combining TDT analyses of G861C SNP in two previous studies (Hawi et al., 2002; Quist et al., 2003) with the current one, show significant evidence of association with the G allele ($p=0.0009$), though the overall effect size is modest ($OR=1.35$; $95\% CI=1.13 - 1.62$) (Smoller et al., 2006). Also, the study exhibited significant paternal over-transmission of the G allele ($p<0.0001$) but not maternal transmission ($p=0.22$). The haplotype block analysis showed three SNPs were found to be associated with the Inattentive subtype. It was recommended to investigate whether identification of ADHD phenotypes can be defined by the contribution of HTR1B variants.

A more recent study by Li et al. (2006) found evidence of association of ADHD with serotonin 4 receptor (HTR4) gene polymorphisms in a Han Chinese sample. These polymorphisms (C/G haplotype of T allele of the 830997 C>T and 83198 A>G polymorphism ($X^2=8.783$; $p=0.003$), and the C/G/C haplotype of these and the -36 C>T polymorphism ($X^2=5.762$; $p=0.0016$)) of the HTR4 gene exhibited an association ($X^2=5.762$; $p=0.0016$) when haplotype TDT block analysis was applied. These results suggest that HTR4 gene may contribute to ADHD aetiology. Serotonin is used to regulate dopaminergic neurotransmission in certain parts of the brain through a few 5-HT receptors. The actions of the neurotransmitter serotonin (5-HT) are terminated by reuptake via a sodium-dependent serotonin transporter (5-HTT). The gene encoding the human serotonin transporter is located at chromosome 17q11.2 (Fisher et al., 2002). Another study by Kent et al. (2002) stated that there are three common polymorphisms associated with the serotonin transporter gene (5-HTT or SLC6A4): an insertion/deletion in the promoter region; a variable number tandem repeat (VNTR) in intron 2; and a 3' untranslated region (UTR) G/T single nucleotide polymorphism. Seeger, Schloss, and Schmidt (2001) and Beitchman et al. (2003) reported an association between a 44 bp insertion/deletion polymorphism (5-HTTLPR) in the promoter region of SLC6A4 and ADHD based on four case-control studies. Manor et al. (2001) found significant association in 98 trios of Combined ADHD with 5-HTTLPR, when applying a family-based approach. Kim et al. (2005) examined the two polymorphisms of the serotonin transporter gene (5-HTTLPR and the intron 2 VNTR) in 126 families with ADHD in

Korea. There was a definite linkage between 12-repeats of the intron 2 VNTR and the Inattentive subtype ($p=0.031$) based on the use of QTDT and haplotype analysis, which supported the premise of associated disequilibrium between SLC6A4 and ADHD.

Furthermore, Curran et al. (2005) tested 5-HTTLPR, 5-HTTVNTR, and 3'-UTR SNP polymorphisms as Quantitative Trait Loci (QTL) for ADHD. Their findings found a significant association between ADHD subjects and the Long (L) allele of the 5-HTTLPR ins/del marker ($X^2 = 5.1$; 1 df; $p=0.019$), and significant association with five further SNPs out of ten being tested. However, no significant association was observed with either 5-HTTVNTR, or 3'-UTR SNP polymorphisms. There was a positive association for two of the first haplotype blocks in the haplotype analysis, along with the association of a twin primary and secondary test in the global as well as local tests ($p=0.0054$; $p= 0.00081$). They concluded that the serotonin transporter gene (SLC6A4) can be a QTL for ADHD.

2.3.5 *COMT (Catechol-O-Methyltransferase) Gene*

As stated by Waldman and Rhee (2002) a number of studies have been published examining association and linkage between ADHD and COMT. This enzyme (COMT) plays a role in the metabolism of dopamine, adrenaline, and norepinephrine. The COMT gene is located on chromosome 22q11.2 (Qian et al., 2003). Researchers have examined an amino acid substitution (valine to methionine) in exon IV that is functional and has been shown to substantially affect COMT enzyme activity. Since higher activity of valine can lead to less synaptic availability of dopamine than methionine can, it is reasonable to consider valine the high-risk allele. The polymorphisms in exon IV of COMT, where a G/A transition at codon 158 of the membrane-bound COMT, results in a valine-to-methionine substitution. This substitution leads to differences in enzyme activity and thermal stability in red blood cells. The Val/Val homozygote confers high enzyme activity and enhances thermal stability, while the Met/Met homozygote is reported to cause a threefold to fourfold reduction in activity compared with Val/Val homozygotes.

Qian et al. (2003) hypothesised that the activity of COMT may contribute to the aetiology of ADHD based on the role of COMT in catecholaminergic transmission, and the hypothesised role of catecholaminergic dysfunction in ADHD (Faraone, 1998). A study by Eisenbarth et al. (2001) found an association between the Val/Met polymorphism of COMT and ADHD ($X^2=4.72$; $p=0.03$) using a Haplotype Relative Risk design with 48 ADHD triads from Israeli samples. Subsequent research groups (Barr et al., 1999; Hawi et al., 2000; Tahir et al., 2000) tested for association between the COMT gene and ADHD using family-based association study methods such as TDT and HRR, but found no significant association. Qian et al. (2003) used TDT and HRR to conduct a case-control association study within a sample of 202 unclear ADHD families in a Han Chinese population. It was found in the HRR analysis that there was a preferential transmission of the low-activity enzyme COMT Met allele in ADHD boys ($X^2=3.858$; $p=0.05$).

A study by Turicet et al. (2005) examined the functional Val158Met for association with ADHD in a family-based association sample of 279 probands and their parents. They also tested two other markers (rs737865, rs165599) of the COMT gene. Their findings showed no evidence for association with any single marker or haplotype in the sample. A recent meta-analysis was performed on a total of 12 family-based and case-control studies (Cheuk & Wong, 2006). This study investigated the association between the Val158/108Met polymorphism of the COMT gene and ADHD. Their results showed no significant association. Cheuk and Wong (2006) expected that further studies investigating this genetic association would be unlikely to detect it unless a large sample of homogenous subjects was employed. They recommended that in addition to further investigation to find an association with this gene, future studies must test moderator variables, and gene-gene and gene-environment interactions.

2.3.9 *The Adrenergic α_{2A} (ADRA2A) Gene*

Comings, Gonzalez, Li, and MacMurray (2003) introduced a new approach called the “Line Item” to examine the association between genotypes of G-1291 Msp I promoter SNP region of the Adrenergic α_{2A} (ADRA2A) gene with ADHD and other complex

disorders, based on *DSM-IV* criteria. This gene was chosen because of its importance in the mode of action of clonidine, a common method of treatment of Tourette's syndrome, and its likely involvement in ADHD. However, the involvement of this polymorphism with ADHD is inconsistent. A study by Park et al. (2005) examined two SNPs of the ADRA2A gene, Hhal and Dral, which have been found in linkage disequilibrium with the MspI polymorphism. Their TDT results showed confirmation of the relationship between the Dral polymorphism with the ADHD combined type ($p=0.03$). Similarly, the QTDT demonstrated a relationship between this polymorphism and the Inattentive ($p=0.003$) and Hyperactive-Impulsive ($p=0.015$) symptom dimensions, thus underlining the association of the Dral polymorphism of ADRA2A gene with a causative polymorphism.

To demonstrate the findings of the above candidate genes, Table 2.1 shows the genetic association studies for ADHD.

Table 2.1
Genetic linkage and association studies for ADHD

DAT1 gene (5p15.3)			
Publication	Polymorphism	Allele	Finding
Cook et al. (1995)			Positive association
Gill et al. (1997)			Positive association
Waldman et al (1998)			Positive association
Daly et al. (1999)	40 bp 3 VNTR exon15	10-Repeat (480 bp)	Trends ($0.05 < P < 0.15$)
Swanson et al. (2000)			Positive association
Holmes (2000)			Negative association
Todd et al (2001)			Negative association
Roman et al. (2001)			Negative association
Smith et al. (2003)			Negative association
Roman et al. (2004)			Positive association
Langley et al.			Negative

(2005)		association
Lim et al. (2006)		Positive association

DRD4 gene (11p15.5)

Publication	Polymorphism	Allele	Finding
Benjamin et al. (1996)			Positive association
Ebstein et al. (1996)			Positive association
Eisenbarth et al. (2001)			Positive association
La Host et al. (1996)	48-bp VNTR repeat exon	4-repeat allele 7-repeat allele	Positive association
Swanson, Flodman et al. (2000)			Positive association
Swanson, Wasdell et al. (2000)			Positive association
Faraone et al. (2001)			Positive association
Lowe et al. (2004)		-616 SNP -512 SNP 120 bp	Positive association Negative Association Positive association
Kirley et al. (2004)		7-repeat allele	Negative association
Todd et al. (2005)			Positive association

DRD5 gene (4p16.1)

Publication	Polymorphism	Allele	Finding
Daly et al. (1999)	(CA)n repeat, 5 UTR	148-repeat allele	Positive association
Tahir et al. (2000)			Trends (0.05<P<0.15)
Barr et al. (2000)		136-repeat allele 148-repeat allele	Trends (0.05<P<0.15) Positive association
Payton et al. (2001)	(CA)n repeat, 5 UTR	148-repeat allele	Negative association
Maher et al. (2002)			Positive association
Fisher et al. (2002)			Positive linkage

Mill et al. (2004)	D4S615 allele	Positive association
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DBH gene (9q34.2)

Publication	Polymorphism	Allele	Finding
Comings et al. (1996)	<i>TaqI</i> polymorphism, Intron 5	B1 repeat (A1)	Positive association
Daly et al. (1999)		A2 –repeat allele	Positive association
Payton et al. (2001)	G/T SNP, exon 6		Negative association
Roman et al. (2002)	<i>TaqI</i> polymorphism, Interon 5	A2 –repeat allele	Positive association

MAO gene (Xp11.3)

Publication	Polymorphism	Allele	Finding
Jiang et al. (2001)	MAO-A:(CA)n repeat	144-bp repeat allele	Positive association
		122-bp repeat allele	Positive association
Jiang et al. (2001)	MAOB:(GT)n repeat, intron 2		Negative association
Domschke et al. (2005)	MAO-A:(CA)n repeat	-941 G allele	Positive association
		-6-repeat allele CA microsatellite	Positive association
		-G-allele 941G/T SNP	Positive association

SNAP-25 gene (20p11.2)

Publication	Polymorphism	Allele	Finding
Mill et al. (2002)	(ATTT)n repeat, intron 1	5-repeat 2-repeat	Positive association
Mill et al. (2004)		SNP-2015 A/T Microsatellite intron1 80609 G/A intron7	Positive association

5-HT1B gene

Publication	Polymorphism	Allele	Finding
Hawi et al. (2002)	861 G/C SNP	R: G P: C	Positive association
Kent et al. (2002)	G/T SNP, 3 UTR	R: T P: G	Positive

Smoller et al. (2006)	G861G SNP	R: G P:C	association Positive association
5-HTR2A gene			
Publication	Polymorphism	Allele	Finding
Quist et al. (2003)	His452Tyr SNP	R: Tyr P: His	Positive association
HTR4 gene			
Li et al. (2006)	830997C>T 83198 A>G -36 C>T Polymorphism	C/G haplotype of T C/G haplotype of T C/G/C haplotype	Positive association
5-HTT gene			
Publication	Polymorphism	Allele	Finding
Seeger et al. (2001)	44-bp insertion/deletion, promoter region	R: Long p: Short	Positive association
Manor et al. (2001)			Positive association
Kent et al. (2002)	44-bp insertion/deletion, promoter region	R: Long p: Short	Trends (0.05<P<0.15)
Kim et al. 2005	5-HTTLPR intron 2 VNTR	12 repeat allele	Positive association
Curran et al. (2005)	5-HTTLPR	L-allele-ins/del marker	Positive association
Curran et al. (2005)	5-HTTVNTR 3-UTR SNP	L-allele-ins/del marker	Negative association Negative association
COMT gene (22q11.2)			
Publication	Polymorphism	Allele	Finding
Eisenbarth et al. (2001)	Val 108 __ met	R: val P:met	Positive association
Qian et al. (2003)	exonIV(158 G/A SNP)		Positive association
Barr et al. (1999)			Negative

Hawi et al. (2000)			association
Tahir et al. (2000)			Negative association
Turicet et al. (2005)	Val158Met	rs737865 rs165599	Negative association
Cheuk & Wong(2006)	Val 108 __ met exonIV(158 G/A SNP) Val158Met		Negative association

ADRA2A gene (10q24-28)

Publication	Polymorphism	Allele	Finding
Comings et al. (2003)	492 C/T SNP (Cys to Arg)		Positive association
Park et al. (2005)	-1291 C/G SNP	R:m(<i>MspI</i>) P:M	Negative association
		HhaI DraI	Positive association

2.4 Neuropsychology

Research that identifies ADHD neuropsychological deficits is well recognised and documented (Swanson et al., 2007). Stefanatos and Baron (2007) explained that the development of ADHD and its expression could be traced back to the deficiency in particular neuropsychological processes; which was basically neuropsychological impairment, as previously proposed by Barkley (1997a). An important neuropsychological theory of ADHD proposes that the symptoms are a consequence of the lack of Executive Functions (EF), defined as neurocognitive processes that ensure sufficient problem-solving ability to achieve a subsequent goal (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). Willcutt, Pennington, Olson, Chhabildas, & Hulslander (2005) reported findings that showed a variation on a range of neurocognitive measures between groups with ADHD and those without. It was also found that children with ADHD performed poorly on neuropsychological tests of executive functions, which are used to determine the integrity of frontal systems, particularly the prefrontal cortex (Doyle et al., 2005).

Willcutt et al. (2005) explained that, based on the similarity between ADHD symptoms and the behaviour of people with frontal lobe injuries, it could be concluded that ADHD is the result of a primary deficiency in particular areas that control executive functions. Willcutt et al. (2005) described executive functions as “top-down” cognitive inputs that help decision-making through analysis of various options to decide on the best course of action. The components included in EFs are: Inhibitory Control (response inhibition), working memory, planning or set shifting, abstraction, organisation, fluency, aspects of effortful attention, and Reaction Time variability (RT), also known as Delay Aversion, which is considered a regular deficiency in ADHD (Doyle et al., 2005; Nigg, 2005). Willcutt et al. (2005) reported that previous research had concluded that the symptoms of ADHD were caused by the lack of a specific executive function, such as response inhibition or working memory, or a more general weakness in executive control. Barkley (1997b) suggested that the primary deficit that consequently affects other executive functions contributes to the underlying theory of ADHD with deficient inhibitory control. Inhibitory control, which is vital in response control to environmental factors in daily life (for example, the ability to make a complete and sudden halt during an activity), is among the most important executive functions considered as deficient in ADHD. Thus, a deficiency in inhibitory control can act as a strong indicator for ADHD (Barkley, 1997b; Schachar, Mota, Logan, Tannock, & Klim, 2000). Working memory is another executive function that contributes to ADHD assessment. Baddeley (1996) (cited in Stefanos & Baron, 2007) defined working memory as the ability to sustain information or to focus on goal achievement in the presence of interference. Working memory is the interaction between perception, attention, memory and action. Barkley (1997b) reported that a deficiency in a type of working memory, such as verbal or spatial working memories, underlies inattention problems.

Willcutt et al. (2005) conducted a meta-analysis that examined the hypothesis that ADHD symptoms were the result of a lack of executive control. It was concluded in the study that executive dysfunctions, such as response inhibition, planning, vigilance, and working memory, contribute to the complex neuropsychology of ADHD. However, a weakness in executive functions may not necessarily be the cause of all cases of ADHD. Nigg (2005) offered a conclusion through a meta-analysis that specified the

neuropsychological tasks that illustrate the greatest discrepancy in performance by children with and without ADHD. It was concluded that 35%-50% of Combined ADHD individuals demonstrated deficiencies on commonly-studied measures of inhibition, interference control, and processing speed or set shifting. However, the study could not conclude that a particular neuropsychological deficit is enough to explain ADHD; however, a lack of vigilance/attention, cognitive control and motivation was evident throughout the cases.

Stefantos and Baron (2007) expounded that the problem in identifying neuropsychological processes that explain ADHD is the inherent heterogeneity of the Disorder. The Combined subtype was the focus of most neuropsychological studies, with insufficient study done on the Inattentive subtype. Efforts to characterise ADHD subtypes based on the neuropsychological patterns of performance or to confirm current behaviourally-defined subtypes based on neuropsychological data produced mixed results. Chhabildas, Pennington and Willcutt (2001) cited in Stefantos & Baron, (2007) tested the hypothesis that Hyperactivity-Impulsivity symptomatology could be due to behavioural inhibition deficits, whereas Inattentive symptomatology could be due to the lack of processing speed and vigilance. Children with the combined subtype may show deficits in both areas. In contrast to commonly held beliefs, similar patterns of neuropsychological impairment were present throughout the three subtypes and symptoms of the Inattentive subtype particularly were reliable indicators across all measures and ADHD subtypes.

The aim of this paragraph is to highlight Pennington's (2005) commentary on three studies, by Nigg et al. (2005), Sonuga-Barke (2005) and Sergeant (Sergeant, 2005) respectively, which examined the issue of ADHD neuropsychology (all three articles are cited in Pennington, 2005). Pennington (2005) commented on the first study by Nigg et al. (2005) that despite nearly 80% of children with ADHD demonstrating a deficit on at least one measure of executive function, the same can be said of nearly half the number of children without ADHD. As such, Nigg et al. (2005) proposed for a need for an EF-deficit subtype of ADHD because there is proof that this subtype is familial, more

damaging than ADHD without EF dysfunction, and can be isolated from other potential subtypes of ADHD (eg. a Delay Aversion (DA) subtype). This proposal was made with the assumption that the heterogeneity of ADHD could be distinguished using several different single-deficit subtypes, with the EF-deficit subtype being an important component.

The second article, by Sonuga-Barke (2005) similarly suggested single-neuropsychological-deficit subtypes of ADHD, in particular an EF-deficit and a motivational-deficit subtype. However, alternatives to single-deficit models, namely dual or even multiple-deficit models, have been proposed by Sonuga-Barke (2005). Thus, ADHD researchers need to consider the number of valid cases of ADHD that can be attributed to a single neuropsychological deficit, whether cognitive or motivational, and the number of cases involving combinations of deficits. The third article by Sergeant (2005) reviewed another motivational model of ADHD, the Cognitive-Energetic (CE) model. This model isolated ongoing information processing and action selection from energetic pools that impact on cognitive processes. Pennington (2005) concluded from these articles that the next step in the development of a neuropsychological model of ADHD is to directly assess the validity of single-deficit subtypes of ADHD, in particular the suggested Executive Function (EF), Delay Aversion (DA), and Cognitive–Energetic (CE) subtypes. It will be necessary to explain the interactions among these three models of ADHD and to trial multiple-deficit models in which the kinds of deficit, postulated by the EF, DA, and CE subtypes, interact.

Nigg et al. (2007) examined two possible resiliency factors in ADHD children: genotype, and neurocognitive response inhibition. These suggested factors are based on hypotheses that these protect children against the development of ADHD and related externalizing problems in difficult times. The test was carried out on three candidate genes, DRD4, DAT1, and ADRA2A, with the results showing that both genotype and strong response-inhibition abilities provided significant defence against the expression of ADHD and CD in situations that present psychosocial adversity. In family, twin and adoption studies of ADHD neuropsychology, the following observations were made by Doyle et al. (2005): (1) the genetic

defects of an ADHD family might explain the impairment on neuropsychological measures related to executive function, processing speed, visual attention, and response variability; (2) that familial-genetic similarities are most evident on multiple neurocognitive measures; (3) there is a difference, between the low scale of bivariate heritability and the moderate effects of deficit in ADHD-susceptible relatives, in their genetic contributions to ADHD. Otherwise, other factors may explain the problems of identifying the degree of common genetic causes. Hence, additional research is necessary to assess the impact of diagnostic and neuropsychological heterogeneity and to ascertain the relations between various executive functions, as well as the associations between EFs and other neurocognitive and emotional–motivational factors (Willcutt et al, 2005a).

2.5 *Endophenotypes of ADHD*

Great interest has been generated in molecular genetic studies with regard to endophenotypes, so as to comprehend the aetiology and pathophysiology of common mental disorders such as ADHD and RD (Cannon & Keller, 2006; de Jong, Oosterlaan, & Sergeant, 2006; Doyle et al., 2005). However, Waldman (Waldman, 2005) warned that there remains a large discrepancy between what we know about candidate genes and the manifest symptoms of disorders such as ADHD as commonly determined by interviews or rating scales. It is conceptually and empirically necessary to ascertain valid and meaningful constructs that can explain the discrepancy. Cannon and Keller (2006) defined endophenotypes as "intermediate phenotypes that form the causal links between genes and overt expression of disorders" (p.7.2)" and "an alternative method for measuring phenotypic variation that may facilitate the identification of susceptibility genes for complex psychiatric disorders" (p.7.1).

Candidate endophenotypes of psychiatric disorders – biochemical, neurophysiological, endocrinological, neuroanatomical, cognitive, neuropsychological, neuroimaging, or electrophysiological – that can be used in molecular genetic studies have been proposed in several studies (Almasy & Blangero, 2001; de Jong et al., 2006; Doyle et al., 2005; Gottesman & Gould, 2003; McGrath, Smith, & Pennington, 2006),. Gottesman and Gould (2003) explained that the term "endophenotypes" was initially used 35 years before to describe psychiatric disorders by Gottesman and Shields in 1967, in their validation for the

genetics of schizophrenia as “internal phenotypes discoverable by a biochemical test or microscopic examination.”

Cannon and Keller (2006) suggested innovative means for intervention and prevention of ADHD based on the knowledge of the molecular mechanisms that explain disease risk and expression. This suggestion was made on the premise that endophenotypes make it possible for the detection of the genetic and environmental structure of common mental disorders. The genes influencing liability to mental disorders are likely to affect multiple neural systems thought to be involved in these illnesses, including cortical and subcortical dopaminergic, serotonergic, and glutamatergic systems that intervene with a number of neurocognitive and affective processes, such as attention, learning, memory, language, stress sensitivity, emotional regulation, and social cognition. Thus, it was concluded that the potential endophenotypes for these syndromes may be located through measuring performance on neuropsychological tests affecting these brain systems or in more obvious physiological or anatomical tests. Figure 2.2 illustrates the varying levels of phenotypic impact between a genetic sequence variation and a diagnostic syndrome. Any candidate endophenotypes involved in ADHD could be detected at any of the intermediate stages. (Cannon & Keller, 2006).

Six properties of endophenotypes that can be validly used in genetic analyses have been suggested in this thesis, based on previous literature (eg. Gottesman & Gould, 2003): (1) endophenotypes should be at least moderately heritable to help in the genetic classification of complicated traits; (2) Instead of the symptoms of disorders or their treatment, endophenotypes should be investigated as possibly being involved in causing disorders, from disease-promoting alleles (as shown in Figure 2.3); (3) endophenotypes should be less genetically complicated than the disorders they cause; (4) endophenotypes should fluctuate in the general population; (5) candidate endophenotypes should be ascertained preferably through several stages of analysis (Figure 2.2); (6) endophenotypes that cause multiple disorders should be investigated for genetically-associated problems.

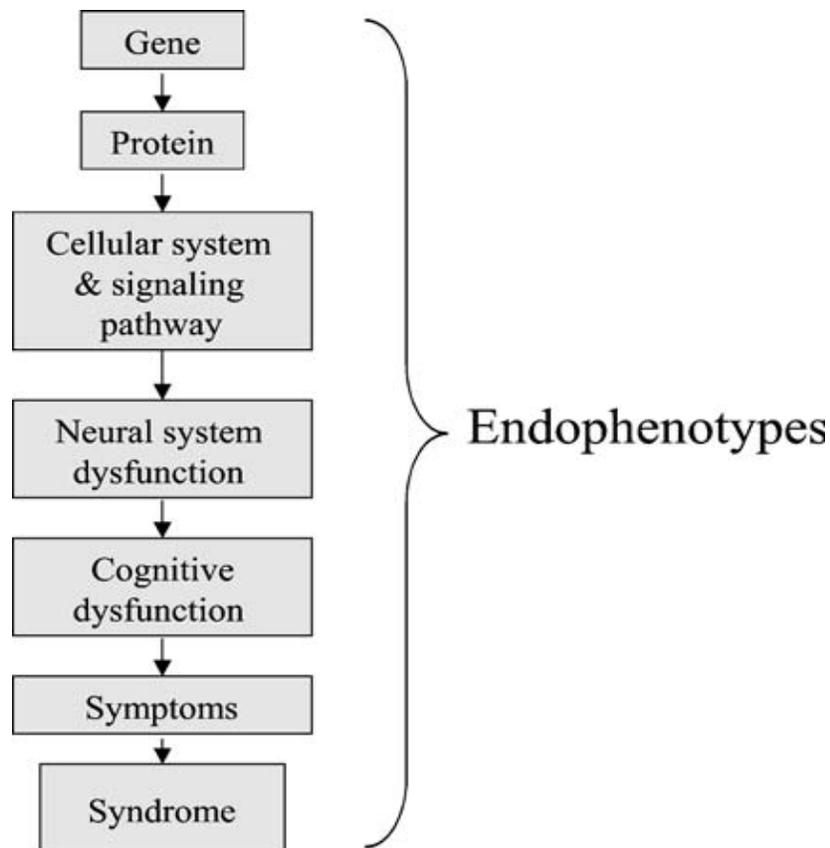


Figure 2.2 Candidate endophenotypic measures, which include all aspects of the disorder (Source: Cannon and Keller, 2006, p.7.3.)

Waldman (2005) detailed ten criteria for assessing the validity and utility of putative and candidate endophenotypes related to ADHD. These criteria include (1) the ability to measure endophenotypes psychometrically; (2) the relation of endophenotypes to the Disorder and its symptoms in the general population; (3) the stability of endophenotypes through time (i.e., they are expressed in the same way regardless of the current manifestation of the Disorder); (4) the expression of endophenotypes in a higher degree in kin of probands without the Disorder than in randomly selected people from the general population; (5) the association of endophenotypes and disorders within families (i.e., they “co-segregate”); (6) the heritability of endophenotypes; (7) similar genetic influences foundational to the endophenotypes and the Disorder; (8) the relationship and/or linkage of the endophenotypes with one or more of the candidate genes that trigger the Disorder, which should show the contribution of the gene over and above the gene’s association with the diagnosis or symptoms; (9) the reconciliation of the

endophenotype with the candidate gene and/or its relation to the candidate gene and the Disorder, implying the expression of the effects of a specific gene or locus on a disorder, in full or in part, through the endophenotype; and (10) the moderate association and/or linkage of the endophenotype between the candidate gene and the Disorder, implying the stronger effects of a particular gene or locus on a disorder in individuals with the Disorder where the endophenotype is present. It must be highlighted that criteria 1–7 are useful for assessing the validity of candidate endophenotypes and for assessing their potential for inclusion in molecular genetic studies, whereas criteria 8–10 denote the activity of candidate endophenotypes in candidate gene studies.

Doyle et al. (2005) provided a comprehensive review of the endophenotypes of ADHD. A summary of the main points of that paper involve the following: (1) ADHD symptomatology phenotypes are dependent on biological mechanisms controlled by genetic elements that produce the Disorder's pattern. Both behavioural and molecular genetic studies have proved that genes act substantially in causing the Disorder. However, the findings are inconsistent and, as a result of low sample size, fail to uniformly identify genes of small effects and heterogeneity. This increases the need for the involvement of endophenotypes in ADHD molecular genetic research. (2) The genetics of endophenotypes are less complex than is the Disorder. This is due to the endophenotype's relative closeness to genetic products in human mechanisms leading from gene to behaviour, and to its ability to detect the underlying pathophysiological problems. As endophenotypes are influenced by fewer genetic and environmental risk factors than the Disorder as a whole, they can help to detect the effects of the individual genes by increasing the statistical power of the measurements, and also give more details about the suspected pathophysiological basis of the Disorder. (3) Resulting from the success of stimulant medications, neuroimaging, electrophysiological, and neuropsychological studies have found dysfunctions in frontostriatal pathways in individuals with ADHD, and have revealed dopamine pathways involved with hyperactivity. (4) Neuroimaging, electrophysiological, and neuropsychological studies for ADHD endophenotypes provide an important path to progress using molecular genetic research, especially considering the problems caused by genetic heterogeneity

and measurement. The investigation of endophenotypes can untangle the problem of ADHD complexity. (5) The study of ADHD endophenotypes can increase understanding of the neuropsychological factors in ADHD. Assessment tools are cost effective and relatively easy to implement because of familial or genetic overlap between neuropsychological impairments, neuroimaging measures, and electrophysiological paradigms. (6) As the aetiology of ADHD is not fully understood and is still ambiguous, it may be that the use of the new and promising area of endophenotypes can help to understand the aetiology and pathophysiology of ADHD provided problematic issues such as heterogeneity, measurements and lack of statistical power can be overcome.

2.6 Reading Disability (RD)

Reading Disability, also known as Specific Reading Disability or Developmental Dyslexia, is a clinical disorder aetiologically and functionally heterogeneous with ADHD (Lyon et al., 2003). This common, albeit complex, cognitive childhood neuro-behavioural disorder interferes with the reading ability of children and adults, even if these children possess average intelligence and reasonable education. Critchely (1970) described Reading Disability as a disability in reading even in the provision of adequate learning facilities and proper educational coaching. Lyon et al. (2003) believed that RD was a result of the person affected being unable to recognise, spell and decode words as a consequence of a deficiency in language phonology, which then led to weak scholastic attainment. Frost and Emery (1995) explained that RD is characterized by deficits in phonemic awareness, sound-symbol relations, and retrieval of phonological information in memory.

2.6.1 Prevalence and Heritability of RD

A dimensional model is suited to the understanding of RD as reading ability is not a discrete disorder: but may be measured on a continuum. It must be noted, however, that the distinction between Reading Ability and Reading Disability is not always clear, as a diagnosis of RD may only indicate the lower end of a normal distribution (Cardon et al., 1994; Grigorenko et al., 1997; Pennington et al., 1991). Reading Disability is a chronic disorder, with a prevalence of 5-17.5%. In addition, males are diagnosed with RD at a much higher rate than are females. This can be attributed to the fact that males often

display disruptive behaviours more frequently than do females. When individuals are carefully assessed using stringent criteria, the disorder is found to occur at more equal rates across genders (APA, 2000).

Furthermore, RD is considered a family disorder, with several family studies showing that there exists a 23-65% chance that other members may be dyslexic if there is at least one family member with RD (McGrath, Smith, & Pennington, 2006). Raskind (2001) reported that although the causes of RD are considered largely unknown, it is generally accepted that its causes have a genetic origin. Other studies such as Grigorenko et al. (1997) confirmed a genetic effect in RD with a heritability of 0.45 to 0.61. Furthermore, researchers believe that because of the complex nature of reading, multiple genes of relatively small effects contribute to its phenotype variability. The chance of family members developing the Disorder is heightened for monozygotic twins over dizygotic twins. Likewise, it is greater for DZ twins than for siblings and the risk is increased for siblings more than for cousins. The American Psychiatric Association (2000) mentioned that it is generally understood that RD is more prevalent among first-degree biological relatives.

Frost and Emery (1995) reviewed RD and considered it as language disorder because of its relation with phonological deficit according to the difficulty to utilise the phonological information when dealing with written and oral language. Phonological deficits include phonemic awareness, sound-symbol relations, and retrieval of phonological information in memory. Problems of phonemic awareness, or ones understanding of and access to the sound structure of language, are the most prevalent characteristic of RD. A child diagnosed with RD may also have a difficulty in fractioning words into their individual syllables or phonemes. Moreover, a deficit in the retrieval of phonological information during reading, results in slow and inaccurate recall of phonological codes, such as pronunciations of letters, word segments, or entire words, from long-term memory. In addition, a deficit in the storage of phonological information may be caused by a malfunctioning working memory resulting in inaccurate application of sound rules (Frost & Emery, 1995).

Previously, RD was diagnosed based on the ability achievement discrepancy model (Elbro & Petersen, 2004). For example, a two-step procedure to diagnosing RD considered the most efficient strategy as using the standard ability achievement discrepancy model yields an over diagnosis of RD (Leong, 2001). In turn to separate the garden-variety poor readers from those suffering from RD, cut scores was suggested based on performing two procedures: 1. a procedure requires a child to have a word list reading score below 90, which ensures that the child is a poor-for-age reader, 2. a procedure requires that this reading score is at least 10 points lower then their Full Scale IQ. Thus, if a child has a Full Scale IQ of 130 and has a word list reading score of 110, they would not be considered for a RD diagnosis, which has not been the situation in the past (Dykman & Ackerman, 1992).

Elbro and Petersen (2004) reported a presence of various interventions for children with RD including the phoneme awareness training and letter sound training, showing long-lasting possibilities and significant positive effects. The former training intervention includes lessons related to phoneme deletion, identification, and discrimination. The letter training intervention includes lessons such as letter naming, word decoding, and pronunciation accuracy. Contrary, Harm, McCandliss, and Seidenberg (2001) explained that the phonemic interventions has a limitation in their improvements due to the fact that they are creating poor representations that neglect the phonemic and orthographic connection.

Frost and Emery (1995) provided other RD-related interventions such as the one focused on teaching comprehension by presenting main vocabularies to an RD child, which stimulate the conceptual skills before primary reading, recalling the story, answering questions that related to analyse the story, as well as helps to teach the fundamental elements of the story such as characters, places and tenses. Also, this intervention helps the RD child utilising these elements to recall the story's events. One more intervention teaches automaticity, attempting to expose individuals to decidable words in order to

help them read these words in simultaneous way until these individuals get use to these words, then they can be exposed to more irregular words aiming to increase their reading's accuracy.

Frost and Emery (1995) listed various RD assessment measures helping to determine the phonological core deficits of RD. First, for measuring general reading ability, four assessment techniques can be used: Metropolitan Achievement Test-Reading, Gray Oral Reading Tests, 3rd Ed, WRAT-R-Reading, and WRMT- Word Identification. Second, for measuring Storage and Retrieval, four assessment techniques can be utilised: SB-4-Memory for Sentences, Verbal Selective Reminding Test, Rapid Automatised Naming Test, and Boston Naming Test . Last, for measuring phonological awareness, five assessment techniques can be applied: Test of Awareness of Language Segments (TALS), Test of Auditory Analysis Skills (TAAS), Lindamood Auditory Conceptualisation Test, and Decoding Skills Test. Furthermore, Bell, McCallum, and Cox (2003) focused on other RD assessment techniques that's related to the cognitive processes such as the Woodcock-Johnson Tests of Cognitive Abilities, Tests of Achievement third edition (W-J III) which assesses the auditory processing, visual processing/speed, memory, orthography, rapid naming, and reading skills, the Wechsler Individual Achievement Test Revised (WIAT-R), which assesses the phonetic decoding and phonological awareness, the Qualitative Reading Inventory-3 which assesses the reading comprehension and fluency, and the orthographic measures of Illinois Test of Psycholinguistic Abilities-3, which assesses spelling ability.

2.6.2 Phenotype Definition of Reading Disability

Phenotypically, RD has been defined by partitioning reading skill development into its major contributing cognitive components; namely, phonological awareness, phonological coding, orthographic coding and rapid serial naming (Zumberge, Baker, & Manis, 2007). Behavioural and molecular genetic studies on each of these cognitive components individually have indicated that they can be successfully used in isolation as RD phenotypes. These results supported the idea that the specific cognitive components involved in reading skill development may each map neatly to specific genomic regions

(McGrath et al., 2006). McGrath et al. (2006) reported that the correlation between these cognitive components, however, has shown this idea to be overly simplistic. In addition, the complexity of the reading process, and its dependence on language abilities, working memory and attention, as well as the previously mentioned cognitive processes, means that the precise contribution of each component and its overlap with other components is not currently known (Willcutt, Pennington, & DeFries, 2000). McGrath et al. (2006) explained that notwithstanding the abovementioned findings, behavioural genetics results do support the theory that there exist partially independent genetic influences on the separate cognitive components of reading ability. Current RD linkage-and-association studies are used as the basis for research. They search for convergence among the multiple cognitive measures of reading. Performing molecular genetic studies using multivariate analysis, which would capture covariance data for the cognitive processes involved in reading, may potentially enable researchers to exploit RDs precisely-defined phenotype (Willcutt, Pennington, & DeFries, 2000).

Castles, Bates, and Coltheart (2006) categorised RD phenotypes into two main groups: surface RD, and phonological RD. The surface RD can be assessed by testing performance on reading irregular words (e.g. yacht) out loud as vocalising the word shows how the word is processed. According to Bates (2006), such words are easy to correctly pronounce according to regular grapheme–phonological correspondences; however, the study suggested that children affected with surface RD have the inability to store or access the sounds linked with familiar words. Phonological RD demonstrates poor non-lexical reading which is caused by the inability to process grapheme–phoneme correspondences, which distinguish many words in different languages. Phonological RD is due to decline in the ability to decode phonology and is demonstrated by translating written (but meaningless) text into spoken words. This can be assessed by reading written non-words out loud.

Samuelsson et al (2005) introduced six reading ability categories based on a factor analysis of a collection of preschool measures: 1. Print knowledge: this category gives a strong indication that a preschool child knows the name of the letters and thus predict

that he or she is not affected with RD. This also includes word notions as units of print and the direction of words across the page. 2. Phonological awareness: this is considered as a powerful predictor of reading ability and is the most important cause of Reading Disability, as it has the capacity to separate and influence sublexical speech segments, including words within compound words, syllables, rhymes, and phonemes. 3. Phoneme identity learning: this is the top predictor of word-reading ability in preschool and the early grades; however, preschool children did not succeed in the phonological tasks at the level of the phoneme. 4. Rapid naming: this is considered an important predictor for reading ability in later stages of life. Rapid naming needs visual processing of objects and retrieval of the proper words in rapid sequence. 5. General language skills: this also is a preschool predictor of reading ability in later stages of life. This category contains many skills such as vocabulary, including naming of pictures and the definition of words.

2.7 RD Twin Studies

Twin studies of RD exhibited convincing evidence for a strongly genetic influence as well as major environmental factors (Grigorenko, 2001; Olson & Gayan, 2001). Raskind (Raskind, 2001) explained that the levels of genetic and environmental contributions to the variance of the phenotype can be estimated throughout the comparison of concordance rates between MZ and same-sex DZ twins, as the former is more similar than the latter for the a particular trait which has a genetic origin. Consequently, the author also reported that when the trait is completely heritable, this means that MZ twins are completely concordant as the twins have the same gene structure. On the other hand, DZ twins are less similar as their gene structures only share the half. Thus the selected DZ co-twins of probands for a particular trait in one tail of the distribution should regress to half of the complete population's mean. The phenotypes that influenced entirely by the environmental factors in MZ and DZ twins should be equally concordant as the co-twins of the both types MZ and DZ twins share the same environmental factors for the selected phenotype, plus the non-shared environmental factors should be also considered. Moreover, the relative contributions of the genetic, shared and non-shared environmental effects for a particular trait can be estimated from the observed concordance patterns (Raskind, 2001).

Several RD twin studies (e.g., Pennington et al., 1991) suggested that the concordant rates of RD MZ twins is not entirely 100%, despite the former is usually higher than the concordant rates of DZ twins. Olson, Wise, Connors, Rack, and Fulker (1989) reported a presence of significant phonological coding heritability, and presence of insignificant orthographic coding heritability, however, Olson et al. (1994) reported a presence of similar heritability between orthographic coding and phonological coding. The heritability of RD components including the word recognition, phonological coding, phonological awareness and orthographic coding ranged from 47 to 60%, however, the phenotypic variance of the shared environmental factors ranged from 29-48% (Olson et al., 1994). Another study by Olson, Datta, Gayan and DeFries (1999) showed individual differences in phonological decoding and orthographic coding are due to shared as well as independent genetic influences.

Olson and Gayan (2001) reviewed several behavioural genetic studies and concluded that MZ and DZ twins may share in their similarity in the environmental factors, this leads to suggest that the gene differences between MZ and DZ twins may help to select different environments. Their review also involved a variety of reading and sub-reading skills such as phonological and orthographic awareness. For instance, word recognition exhibited a genetic effect of 0.45 and a shared environmental effect of 0.15. However, the phonological and orthographic awareness showed higher genetic effect of 0.56 and 0.58 respectively, whereas the shared environmental effects were low (0.24 for the phonological awareness and 0.20 for the orthographic awareness), and the non-shared environmental effects were high (the phonological awareness was 0.20 and orthographic awareness was 0.22). The authors observed that reason of presence of reading individual differences is the presence of partly shared genetic effects and partly shared and non-shared environmental effects, concluding that that these effects are linked with early literacy development.

Research teams who have done significant work in the twin studies of RD include The International Longitudinal Twin Study (ILTS), and The Colorado Learning Disabilities Research Centre (CLDRC).

2.7.1 The International Longitudinal Twin Study (ILTS)

The International Longitudinal Twin Study is a developmental behavioural-genetic study of early reading development in preschool through the second grade in Australia, the United States (Colorado), Norway, and Sweden (Samuelsson et al., 2005). ILTS recruited about 900 sets of twins, and assessed them in seven successive cohorts (Byrne, 2006). The study aims to investigate the genetic and environmental influences that affect Reading Disability across children during their early progress in literacy. ILTS focuses on the several categories of pre-reading skills assessed in preschool children, such as print knowledge, phonological awareness, phoneme identity learning, rapid naming, general language skills, and visual-perceptual skills.

Byrne et al. (2002) performed a longitudinal twin study of early reading development in Australia, the United States, and Norway. This study introduced preliminary evidence for genetic influence on individual differences in some pre-reading skills including phonological awareness, phoneme learning, and verbal memory, and also the influence of a shared-family environment on other skills including print knowledge and vocabulary. Another study by Samuelsson et al. (2005) examined the individual differences for the pre-reading skills in 4- to 5-year-old twins' print environments in Australia, Scandinavia, and the United States. This study found lower levels of print knowledge in the Scandinavian sample, related to less frequent shared parent-child literacy activities. The univariate analyses showed phonological awareness, verbal memory, and rapid naming were more affected by genes than by shared environment; whereas vocabulary, grammar/morphology, and print awareness were more affected by shared environment. The multivariate analyses demonstrated an overlap of both genetics and shared environment on abilities such as phonological awareness, rapid naming, and print awareness. Genetic influence on these abilities was similar to genetic influence on general verbal ability, but each was influenced independently by genes too.

Two recent papers by Byrne et al. (2006; 2007) also investigated the genetic and environmental influences on early literacy of children in kindergarten and preschool to

Grade 1. The first paper (Byrne et al., 2006) used genetic modelling for pre-reading and early reading skills in preschool twins in Australia, Scandinavia, and the United States, by addressing the question, “which of the reading phenotypic components (such as spelling, verbal learning and memory, phonological awareness, rapid memory, or overall reading ability) can be affected by the same or different genes, by a shared environment or by both factors?” The findings showed a strong genetic influence on preschool phonological awareness, rapid naming and verbal memory, whereas print awareness, vocabulary and grammar/morphology were affected by a shared environment. In addition, spelling was equally affected by genes and shared environment.

The second paper (Byrne et al., 2007) also applied genetic modelling to study preschool through Grade 1 literacy skills of Australian and US (Colorado) twins. The study also found that there was a strong genetic influence on word and non-word identification, reading comprehension, and spelling. Furthermore, rapid naming showed more a modest, though still reliable, genetic influence. Individual measures of memory and learning were also more affected by genes than non-shared environment. However, phonological awareness was subject to a high non-shared environment influence, with no reliable genetic effects.

2.7.1.1 The Colorado Learning Disabilities Research Center (CLDRC)

The Colorado Learning Disabilities Research Center (CLDRC) is a multidisciplinary, multi-site collaborative effort at locations in Colorado, Nebraska and Australia. The goals of CLDRC are to investigate the definition, aetiology, and treatment of learning disabilities (LD) and ADHD; to assess the genetic and environmental causes of reading deficits and ADHD and their comorbidity; and to assess possible predecessors of reading deficits, as well as their genetic and environmental origins (Wadsworth et al., 2001). As of the time this study was undertaken, CLDRC was running five projects: twin studies, reading and language processes, validity of ADHD subtypes, genomic analyses, and early reading, language, and attention development. CLDRC performed several important studies in the field that showed evidence for genetic etiologies of RD and ADHD.

The DeFries-Fulker regression model (DF model) (DeFries & Fulker 1985, 1988) is frequently applied when probands twins are selected for extreme scores on a phenotypes which allows a proband's score to be predicted from their co-twin's score, based on the differential regression to the mean of MZ and DZ co-twins. Gayan and Olson (1999) applied a DeFries-Fulker regression model to 1031 MZ and DZ twins (618 pairs had at least one twin with RD, and 413 were normal twins), which demonstrated a presence of significant genetic and environmental factors on individuals with different RD phenotypes including Word Recognition ($h^2_g=0.45$; $c^2_g=0.49$), Orthographic Coding ($h^2_g=0.58$; $c^2_g=0.20$), Phonological Decoding ($h^2_g=0.61$; $c^2_g=0.24$), and Phonological Awareness ($h^2_g=0.56$; $c^2_g=0.24$). Despite their results, the authors recommended further investigations to determine if the genes responsible for RD are the same or different genes. An ongoing twin study by CLDRC in 2001 selected 245 MZ twins and 195 DZ twins identified with at least one twin met the criteria for RD. The MZ twins were significantly higher (65%) than DZ twins (35%), indicated a presence of genetic factor that might be contributed to RD (De Fries & Fulker, 1985, 1988).

A multiple regression analysis called deFries-Fulker (DF) model is being used by CLDRC in order to analyse twin data; it is a flexible and powerful test of genetic influence on the extreme scores of a disorder's dimensional phenotype (Spector, Snieder, & MacGregor, 2000). Willcutt, deFries, Pennington, Olson, Smith, and Cardon (2003) explained that the transformation of RD dimensional data into discrete data causes loss of significant dimensional data. The DF regression equation utilises the regression of MZ and DZ co-twin (the other member of twin pair) scores in order to estimate the scores of affected individuals (probands). According to the assumptions of the classical twin model, DF equation presents a straight heritability estimate, as it is versatile and can be extended to include covariates (Willcutt, DeFries et al., 2003. p.231). Willcutt et al. (2003) reported that although the scores of MZ and DZ co-twins were expected to regress toward the mean of the unselected group, it was found that regression of the scores of DZ co-twins were higher than the regression of MZ co-twins to the extent that extreme scores are influenced by genes. The importance of the contrasting regression by zygosity utilisation in the DF model is a powerful tool to estimate heritability.

The basic DF model equation is $C = B_1 P + B_2 R + K$ as explained by Willcutt et al. (2003) “where C is the expected cotwin score, P is the proband score, R is the coefficient of relationship (1 for MZ pairs, 0.5 for DZ pairs), and K is the regression constant. The B_1 coefficient represents the partial regression of the cotwin score on the proband score, and provides a measure of twin resemblance irrespective of zygosity. The B_2 parameter represents the partial regression of the cotwin score on the coefficient of relationship, and after appropriate transformation of the data provides a direct estimate of the heritability of extreme scores on the trait under consideration (h^2_g). After adjustment of the standard errors of the regression coefficients to correct for the double entry of concordant pairs, the significance of the B_2 parameter provides a statistical test of the extent to which extreme scores are attributable to genetic influences” (Willcutt, DeFries et al., 2003, p. 231).

2.7.1.2 Other RD twin studies

A study by Zumberge et al. (2007) performed genetic modelling analyses on normal variation in reading ability with its components: attention and intelligence (IQ in order to investigate the phenotypic and genetic relationships between them). The results showed that variation in reading, inattention and IQ and the covariation between them have substantial genetic effects. The results also showed a presence of partial shared genetic contribution between reading ability and IQ, inattention and phonological ability; however it did not show this connection with impulsivity. Their results also showed a presence of substantial shared environmental contribution among the reading ability variations. Another recent study by Friend et al. (2007) investigated the genetic and environmental influence on word recognition and spelling deficits as a function of age, based on a hypothesis from earlier findings: that genetic influence might decrease for word recognition and increase for spelling recognition across age, based on a possible dissociation between genetic influences on word recognition and spelling disorders. Their results showed that genetic influences decreased across age for reading and increased for spelling disorders, which matched the dissociation suggestion.

2.8 *RD Candidate Genes*

Two recent comprehensive reviews by Fisher and Francks (2006), and McGrath, et al. (2006) will give a background about the candidate genes of Reading Disability. There were more candidate genes for ADHD than for Reading Disability; therefore, the search for RD candidate genes started by performing genome-wide linkage studies (McGrath et al., 2006). Genome-wide linkage studies by Fisher and De Fries (2002) and a bivariate linkage scan by Gayan et al. (2005) reported a presence of seven linkage regions: 1p36-p34 (Dyslexia-susceptibility-8 or DYX8), 2p16-p15 (Dyslexia-susceptibility-3 or DYX3), 3p12-q13 (Dyslexia-susceptibility-5 or DYX5 or ROBO1), 6p22.2 (Dyslexia-susceptibility-2 or DYX2), 15q21 (Dyslexia-susceptibility-1 or DYX1), 18p11.2 (Dyslexia-susceptibility-6 or DYX6), and Xq27.3 (Dyslexia-susceptibility-9 or DYX9). As a result, several studies performed to hunt for RD candidate genes 15q21 (DYX1), 3p12-q13 (DYX5 or ROBO1), and 6p22.2 (DYX2).

2.8.1 *DYX1C1 on Chromosome 15q21*

A study by Taipale et al. (2003) on a Finnish sample found both the -3G→ SNP and the 1249G→T SNP of 15q21 in RD cases as well as in the control group; however, the two SNPs expressed more in RD cases as a result of haplotype transmission. Fisher and Francks (2006) suggested that both SNPs might significantly influence DYX1C1 function in different ways; the -3G→A has the ability to alter the amount of the DYX1C1 protein because it is located in a regulatory region, while the 1249G→T might cause the production of a shorter DYX1C1 protein because its located in the stop codon region. Fisher and Francks (2006), and McGrath et al. (2006) reported on subsequent studies investigating the association between these two SNPs with RD. Although most of the studies could not replicate Taipale's et al. (2003) finding, two studies showed association of these two SNPs with RD; however, the association was the opposite to the previous results. One finding showed an association of RD with -3G and 1249→G alleles rather than with -3A and 1249→T alleles (Wigg et al., 2004). The other finding, by Scerri et al. (2004) in the UK, found that although there was a minor association of 1249→G with one out of six RD, it appeared as a non-significant association with further replication. This led to the conclusion that the association of DYX1C1

polymorphisms found in the Taipale's et al's 2003 Finnish sample were unlikely to be valid. Both Fisher and Francks (2006), and McGrath et al. (2006) explained that the presence of the unique Finnish pattern of association, and occurrences of false positive association, were possible reasons for the inconsistency between Taipale's et al. (2003) study and the replicated studies. Therefore, further investigations are needed to confirm Taipale's et al. (2003) finding.

2.8.2 *DYX5 (ROBO1 genes) on Chromosome 3p12*

Fisher and Franckes (2006) stated that mutations occurring in the Robo gene (ROBO1, fruit-fly version) can cause abnormalities in the fruit-fly's Central Nervous System. Hannula-Jouppi et al. (2005) investigated ROBO1 on chromosome 3p12 in the Finnish family that Taipale's et al. (2003) study examined. Hannula-Jouppi et al. (2005) stated that the presence of some SNPs in the ROBO1 gene could have contributed to RD in the Finnish sample; therefore it concluded that this might be a susceptible gene for RD. Hannula-Jouppi's et al. (2005) study found the expression of the ROBO1 gene was lower in affected individuals than in unaffected individuals, suggesting that low expression of ROBO1 protein in the CNS can cause Reading Disability.

2.8.3 *DYX2 (KIAA0319 and DCDC2 genes) on Chromosome 6p22*

Linkage studies on chromosome 6p.21-23 found it to be the most well replicated Quantitative Trait Loci (QTD) of RD (Fisher & Francks, 2006; McGrath et al., 2006). Several studies found linkage evidence of 6p markers with RD: the D6S291 marker (Fisher et al., 1999), the D6S105-TNFB marker (Cardon et al., 1994; Cardon et al., 1995); the D6S109-D6S306 marker (Grigorenko et al., 1997); D6S276-D6S105 marker (Gayan et al., 1999); D6S464-D6S273 marker (Grigorenko, Wood, Meyer, & Pauls, 2000); and the D6S109-JA01, D6S299-D6S621, and D6S105-D6S265 markers (Grigorenko et al., 2003). Recent association studies suggested KIAA0319 and DCDC2 as candidate genes for RD (Cope et al., 2005; Deffenbacher et al., 2004; Francks et al., 2004). These two genes are located on the 6p chromosome, and might play a function in neural migration, as findings by Meng et al. (2005) and Paracchini et al. (2006) showed a significant reduction in neural-migration distance of the transfected vectors targeted

against DCDC2 and KIAA0319 genes into cells at the cerebral ventricular area of living rat embryos. The expression of the KIAA0319 gene produces a protein that helps to control the interactions and adhesion between flanking neurons on the surfaces of cells (Francks et al., 2004; Paracchini et al., 2006). Deffenbacher et al. (2004) was first to refine the 6p22 region between D6S273-D6S105 markers (Figure 2.3) at a distance of ~680kb, and found that out of ten genes screened in this region by a high-density SNP map of 31 SNPs, 13 SNPs from five genes showed association with RD: 2 SNPs in VMP; 8 SNPs in DCD2; 1 SNP in KIAA0319; 1 SNP in TTRAP; and 1 SNP in THEME.

Meng et al. (2005) and Schumacher et al. (2006) attempted to replicate Deffenbacher's et al (2004) results. Both studies showed an association of the DCDC2 gene with RD but not with the KIAA0319 gene. Furthermore, Francks et al. (2004) performed an association study to identify the QTL that influence RD on chromosome 6p22.2, by investigating 57 SNPs from eight genes (DCDC2 gene was excluded) in two UK samples and in one US sample (89 families, and 175 families, 159 families respectively). Their results showed evidence of association in the 77-kb region spanning the TTRAP and KIAA0319 genes.

Consequently, Cope et al. (2005) carried out an association study by using a high-density SNP map on the VMP, DCD2, KAAG1, MRS2L, KIAA0319, TTRAP, THEME and, C6orf genes. Their results showed significant associations with three SNPs in the KIAA0319 gene (rs4504469, rs2179515, rs6935076), one SNP in MRS2L (rs2793422), and one SNP in the THEME2 gene (rs3777664).

A recent family-based association study and haplotype spanning by Luciano et al. (2007) examined ten SNPs from the KIAA0319, TTRAP, MRS2L, THEM2, and C6orf62 genes in 440 families tested on Reading and Spelling Ability. Among those ten SNPs the study found only two SNPs associated with the phenotype: rs6935076 from the KIAA0319 gene and rs2143340 from TTRAP. In addition, the results showed association with three SNP haplotypes spanning the KIAA0319 and TTRAP genes. This study concluded that

Reading Disability can represent the low tail of a normal distribution of reading ability in the population, suggesting that the effect of the KIAA0319 gene or loci near it may influence variations in Reading Ability.

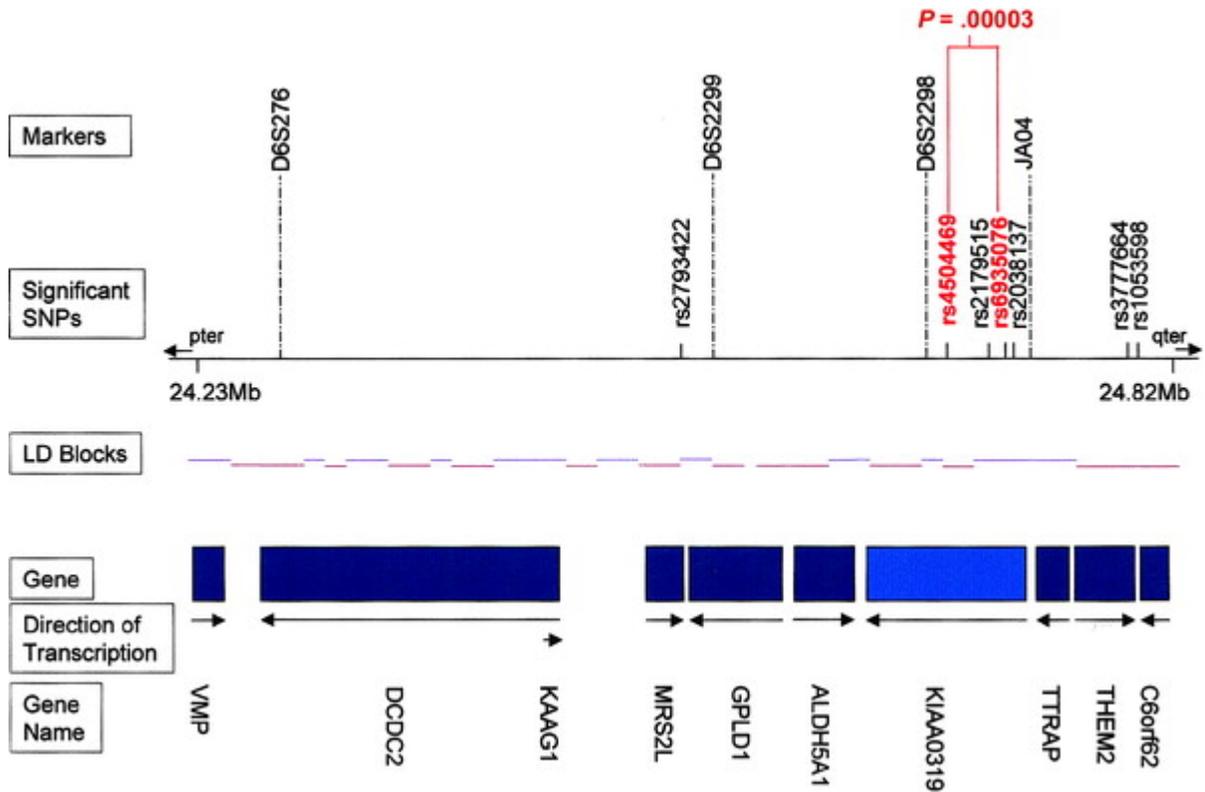


Figure 2.3 Location of RD candidate genes on chromosome 6p. (Source: Cope et al., 2005, p.589).

To sum-up with the previous findings, Table 2.2 reviewed the genes that found contributed with Reading Disability. McGrath et al. (2006) concluded that the KIAA0319 gene is the most likely RD candidate gene, with a possible biological role in the brain development. However, the functional variants of this gene have not yet been identified; therefore, it is unknown how this gene contributes to the aetiology of RD. Also it is not yet understood how the mechanisms of this gene can affect the brain development, but researchers believe that these functional variants can work as a regulator. Further studies are needed to reveal the contribution of this gene to RD.

Table 2.2
Genetic association studies for Reading Disability

<u>DYX1C1 on chromosome 15q21</u>			
Publication	Polymorphism	Allele	Finding
Taipale et al. (2003)		-3G SNP 1249G-T SNP	Positive association
Scerri et al. (2004)		1249G-T SNP	Trends (0.05<P<0.15)
<u>DYX5 (ROBO1 genes) on chromosome 3p12</u>			
Publication	Polymorphism	Allele	Finding
Hannula-Jouppi et al. (2005)	3p12	ROBO1	Positive effect with RD
<u>DYX2 (KIAA0319 & DCDC2 genes) on chromosome 6p22</u>			
Publication	Polymorphism	Allele	Finding
Deffenbacher et al. (2004)	D6S273 –D6S105 of -680 kb	VMP, DCD2, KIAA0319, TTRAP, THEME	Positive association
Meng et al. (2005)		DCD2	Positive association
Schumacher et al. (2006)		DCD2	Positive association
Francks et al. (2004)		TTRAP & KIAA0319 of 77-kb region	Positive association
Cope et al. (2005)	KIAA0319,	rs4504469, rs2179515, rs6935076	Positive association
	MRS2L THEME2	MRS2L(rs2793422), THEME2(rs3777664).	

2.9 *The Comorbidity of ADHD with RD*

Several studies indicate that ADHD-RD comorbidity occurs significantly more frequently than would be expected by chance; however, the causes of this comorbidity are still not understood well (Friedman, Chhabildas, Budhiraja, Willcutt, & Pennington, 2003; Stevenson, 1993; Willcutt, Pennington, & DeFries, 2000; Willcutt & Pennington, 2000a). Literature by Angold, Costello and Erkanli (1999), Caron and Rutter (1991), Stevenson et al. (2005) and Willcutt et al. (2007) discuss five hypotheses that might

result in ADHD-RD comorbidity, with only the last one demonstrating validity. (1) Sampling artefacts: ADHD-RD comorbidity could be due to sampling artefacts that resulted in a biased sampling procedure or measurement problem. However, the possibility of a sampling defect was remote, with ADHD-RD comorbidity present in both clinical referred and community samples (Willcutt & Pennington, (2000a). (2) Shared-method variance and rater-bias effects are not possible as ADHD symptoms are determined by teacher and parent reports, and RD by direct assessment of the child. (3) Comorbidity is not due to symptom overlap as RD and ADHD are separate occurrences. (4) Phenocopies and cross-assortment: it was hypothesized that a chance existed of either ADHD or RD being a phenocopy of the other. It was believed that ADHD could be demonstrated by children with RD in the absence of the ADHD deficit in executive function. Cross-assortment was also hypothesized to trigger comorbidity among individuals with ADHD and learning disabilities. Both of these hypotheses could not be repeated and confirmed in subsequent studies (Willcutt, Pennington et al., 2007). (5) Shared common genetic aetiology: as several bivariate twin analysis studies demonstrated that genetic factors were underlying causes in both RD and ADHD, it was proposed that this was an apparent reason for a shared genetic aetiology. Willcutt et al. (2005; 2007) concluded that this remains the most likely explanation for RD and ADHD comorbidity.

Willcutt and Pennington (2000a) reported the following findings about ADHD-RD comorbidity. (1) measures of RD and ADHD exhibit moderate correlations and the two disorders co-occur in about 15-40% of cases. (2) A common genetic aetiology could be the most convincing reason to cause comorbidity of RD and ADHD, because several family and twin studies have revealed that both RD and ADHD are heritable ($h^2=0.57-0.93$). (3) RD appears to be more robustly associated with Inattentive symptoms, showing a bivariate heritability estimate for Inattentiveness and RD ($h^2_g \text{ RD/Inatt.} = 0.45$), which was higher than that for hyperactivity/impulsivity and RD ($h^2_g \text{ RD/Hyp.-Imp.} = 0.5$).

Willcutt, Pennington, and deFries (2000) found that the rate of RD, in any typical samples chosen for ADHD, tended to be from 25-40%, while 15-35% of children with RD fell into the category of ADHD. Several twin studies have indicated that genetic factors contributed to ADHD-RD comorbidity; however, the question to be asked is whether it is same or different genetic influences that contribute to this comorbidity. Gillis et al. (1992) discovered that both ADHD and RD were due to distinct genetic factors. A statistical trend, on the other hand, proposed that children with comorbid ADHD-RD might characterise an aetiological subtype, and also concluded that some cases of comorbid ADHD-RD might exist as a separate disorder with a genetic aetiology different to either ADHD or RD diagnosis in isolation. This was despite findings that most cases of RD and ADHD were not directly due to the same genetic effect (Willcutt et al, 2000). Light et al. (1995) found significant bivariate heritability for RD and ADHD ($h^2_{g\text{RD/ADHD}}= 0.45$). Willcutt et al. (2000) also found that RD heritability ranged from 0.4 to 0.6 whereas heritability for ADHD ranged from 0.6 to 0.9.

The authors hypothesised that common genetic influences may cause ADHD-RD comorbidity, based on the evidence that RD is more likely to cause the Inattentive, rather than the Hyperactive-Impulsive, subtype (Willcutt & Pennington, 2000a; Willcutt, Pennington et al., 2005). For example, the prevalence of extreme Inattentiveness in RD is greater than the prevalence of extreme Hyperactivity-Impulsivity in children, despite those with RD being more likely to show both Inattentive and Hyperactive-Impulsive symptoms relative to children without RD, (Willcutt, Pennington, & DeFries, 2000; Willcutt & Pennington, 2000a). In contrast, RD is more apparent among children diagnosed with the Inattentive subtype than among those with Hyperactive-Impulsive subtype (Levy, Hay, Bennett, & McStephen, 2005). Evidence for substantial, but not absolute, genetic overlap between Inattentiveness and RD was established in twin studies ($h^2_{g\text{RD/Inatt.}} = 0.39-0.70$) (Gayan et al., 2005), suggesting that both shared and independent genetic influences exist in these two domains (Luca et al., 2007). Zumberge et al. (2007) reported that shared genetic effects have been asserted by behavioural genetics studies as a probable reason of this comorbidity, since both ADHD and RD demonstrated 50% heritability. Nevertheless, the determination of genetic overlap

between ADHD and RD appears to change based on the diagnosis of the ADHD subtype (Inattentive, Hyperactive/Impulsive or Combined).

Furthermore, Stevenson (2001) stated that the Australian Twin ADHD Project (ATAP) data, which examined the relationship between a history of reading intervention and the *DSM-IV* diagnostic criteria for RD, showed that all of the genetic variance in Inattentiveness is shared with the genes affecting reading. Hyperactivity/Impulsivity is partly genetically independent. For RD, evidence indicates that being raised in the same home with the same approaches to reading (shared environment) has some effect. In addition, there are some specific genetic effects, reflecting that part of RD is not necessarily related to ADHD. Stevenson (2001) also stated that individuals with RD were significantly more likely than individuals without RD to exhibit elevations on both symptom dimensions, but the difference is larger for Inattentiveness than Hyperactivity/Impulsivity. He found significant bivariate heritability of RD and Inattentiveness (h^2_g RD/Inatt. = 0.39), whereas the bivariate heritability of RD and Hyperactivity/Impulsivity was minimal and non-significant. Additionally, only 21% of the phenotypic overlap of RD and Hyperactive/Impulsive symptoms was not attributable to common genetic influences, compared to approximately 95% for Inattentive symptoms and RD.

A recent study by Willcutt et al. (2007) conducted a twin analysis and observed moderate to high heritability for all RD measures and ADHD for the univariate analyses. The bivariate analyses demonstrated that the connection between RD and the Inattentive subtype is due to similar genetic influences, with bivariate heritability estimates being immaterial for Hyperactivity-Impulsivity and any of the RD measures. Their study also established that both RD and ADHD symptoms were more heritable if the proband satisfied factors for both disorders as opposed to RD or ADHD separately, recommending that future molecular genetic analyses of comorbid ADHD-RD may aid in the detection of defective genes for RD, ADHD, and their comorbidity.

2.9.1 Genetic Studies of ADHD-RD Comorbidity

ADHD and RD are significantly comorbid in both clinical and community samples (Willcutt, Pennington, & DeFries, 2000). The established genetic correlation between RD and ADHD suggests that their comorbidity is due at least in part to genes that have an impact on several phenotypes, a phenomenon known as pleiotropy (Willcutt et al., 2002). According to this, some twin studies revealed significant bivariate heritability of ADHD and RD which ranges for both disorders from 0.37 to 0.70, suggesting that the pleiotropic effects of a common gene or genes increase susceptibility to both disorders (Light, Pennington, Gilger, & DeFries, 1995; Stevenson, 1993), but no evidence has been found to localize the genes for both disorders yet.

There are several twin studies that indicated that genetic factors do contribute to ADHD-RD comorbidity; however, the question being asked is whether it is the same or different genetic influences contribute to this comorbidity. Willcutt et al. (Willcutt, Pennington, & DeFries, 2000) reported that RD heritability ranged from 0.4-0.6 whereas heritability for ADHD ranged from 0.6-0.9. In addition, ADHD-RD comorbidity is contributed to by shared genetic effects, as the bivariate heritability between RD and the Inattentive subtype ($h^2_g \text{ RD/Inatt.}=0.39$) was significantly higher than the bivariate heritability between RD and Hyperactive-Impulsive subtype ($h^2_g \text{ RD/Hyp-Imp.}=0.05$), which was minimal and insignificant, and 21% of the overlap between RD and the Hyperactive-Impulsive subtype was attributable to common genes (Willcutt, Pennington, & DeFries, 2000).

Willcutt et al. (2002) reported that the Quantitative Trait Loci for RD on chromosome 6 is also associated with increased susceptibility to ADHD. This study indicated that comorbidity of ADHD and RD might be due at least in part to pleiotropic effects of a QTL on chromosome 6p, which spans the Human Leukocyte Antigen (HLA). This was based on several linkage studies that identified potential RD loci on chromosome 6, with several consistent findings in the 6p21.3-22 region (Cardon et al., 1994; Cardon et al., 1995; Fisher et al., 1999; Gayan & Olson, 1999; Grigorenko et al., 2000; Kaplan, 2002).

A study by Gayan et al. (2005) attempted to explain the pleiotropy for these two disorders and has discussed four previous genome-wide linkage studies, which revealed an overlap between eight linkage loci: 3q13, 4q12-13; 6p22-q16; 10cen-q11; 13q12-33; 15q15-21; 16p13; and 17p11-q22 (Bakker et al., 2003; Loo et al., 2004; Ogdie, 2003). The authors argued why the previous studies have identified various overlapping loci regions (3q13, 4q12-13, 6p22-q16, 10cen-q11, 13q12-33, 15q15-21, 16p13, and 17p11-q22), and introduced the following reasons. Firstly, results of these studies were considered likely to be falsely positive because of their weak linkage; therefore, the authors recommended replication should be applied to independent subjects. Secondly, low-power sample size, marker informativeness, and sampling error are the main reasons for having relatively broad chromosomal regions, suggesting that this overlap could be due to the influence of two separate genes, moderately at a distance on the same chromosome. Finally, linked genes that influence each disorder separately, the occurrence of pleiotropy, or genes that act together could be due to the genetic effect on those overlapped loci regions. Consequently, the authors have suggested that a bivariate linkage approach can be applied to overcome the above obstacles, by examining simultaneously if both ADHD and RD in the same families exhibit linkage with those loci.

Gayan et al. (2005) used a bivariate linkage analysis on 182 sibling pairs diagnosed as having comorbid ADHD-RD which might help identify the pleiotropic loci. The results have showed three loci that might be implicated in ADHD-RD pleiotropy. Those three loci are 14q32, 13q32, and 20q11. This result highlights the value of this bivariate linkage to investigate pleiotropy. Moreover, the study reported that RD and ADHD must show simultaneous linkage to some loci; possibly a stronger linkage exists between RD and Inattentiveness symptoms, as the RD and Hyperactivity-Impulsivity symptoms genetic correlation is lower ($r_g=0.37-0.40$) than RD and Inattentiveness symptoms ($r_g=0.39-0.70$) and lower than RD and total ADHD symptoms ($r_g=0.43-0.63$).

Up to now, the only study which tested for a bivariate linkage of RD and ADHD was performed by Willcutt et al. (2002), which identified a potential linked locus on chromosome 6p21. This study has revealed that the 6p21 region may contain a locus

with pleiotropic effects on both RD and ADHD. The key issue to ADHD-RD comorbidity is molecular genetic methodology, as many genetic linkage-and-association studies have implicated a number of chromosomal regions that may harbour susceptible genes for ADHD-RD comorbidity.

Certainly there have been few molecular genetic studies focusing on candidate genes for ADHD-RD comorbidity. A search found two studies only that attempted to investigate particular candidate genes for ADHD-RD comorbidity. The first study by Stevenson et al. (2005) examined the hypothesis that the ADRA2A receptor gene is a susceptible gene for ADHD-RD comorbidity. The study showed evidence of association between ADHD-RD comorbidity and the ADRA2A polymorphism. This finding supports the hypothesis that children with comorbid ADHD-RD may represent a distinct group, showing an association with a particular genetic variant in ADRA2A (Stevenson, Langley et al., 2005).

The second study by Luca et al. (Luca et al., 2007) examined the dopamine receptor D1 (DRD1) as a candidate gene for ADHD-RD comorbidity. The results showed no evidence for a relationship between reading and working memory skills and any DRD1 markers. However, Transmission-Disequilibrium testing showed significant evidence for association of the DRD1 gene with the Inattentive ADHD subtype. Although no associations were found with RD, with reading component skills or with verbal memory, the study found evidence for association with the Inattentive subtype, suggesting that DRD1 contributes uniquely to inattentiveness, without overlap on RD (Luca et al., 2007).

2.9.2 Endophenotypes of ADHD-RD Comorbidity

de Jong et al. (2006) reported the ambiguity of the role of some executive functions deficits, such as timing and naming, in the comorbidity of ADHD and RD deserved further study. In addition, it was reported that the phonological deficits do not seem to be evident in ADHD individuals and hence present an interesting platform for neuro-endophenotypic research. The following assessments about the endophenotypes of

ADHD-RD comorbidity were detailed by de Jong, Oosterlaan, and Sergeant (2006). (1) Several studies showed that Executive Functioning appears to be a candidate endophenotype of comorbid ADHD-RD, thus demonstrating more about the genetic basis of both ADHD and RD and their comorbidity. (2) An endophenotype could be a neuropsychological marker of a disorder and is a demonstration of the genotype rather than the phenotype of the Disorder. (3) More studies are needed for the subtypes of ADHD, with RD, and their relation with dyscalculia (difficulty in learning or comprehending mathematics) as ADHD subtypes and RD possibly has varied endophenotypes with potential distinct genotypes. (4) Twin and family research has demonstrated that Executive Functioning and working memory are heritable, which makes people with deficits in Executive Functioning candidates for possessing the endophenotypes of ADHD. (5) Genetic factors affect phonological skills, and a lack of phonological skills may be due to candidate endophenotype of RD. (6) “The double dissociation when two disorders are linked with opposite patterns of defect in two different cognitive domains” (Willcutt, Pennington et al., 2005), provides appreciation of the neuropsychological mechanisms of ADHD and RD and their comorbidity, as well as for the search for endophenotypes. De Jong et al. (2006) also identified several ADHD and RD studies that used the double-dissociation design to examine executive functions such as working memory, as well as functions such as timing, naming, and phonological skills. The research concluded that executive functioning deficits appear in children with ADHD and children with both ADHD and RD.

An instance of this study is that by Willcutt, Pennington, Olson et al. (2005), where the neuropsychology of ADHD-RD comorbidity was investigated. The following findings were reported: (1) both disorders are related to deficiency in multiple neuropsychological domains; (2) phonological-processing weaknesses were only evident in the RD groups but no deficiencies were specifically linked with ADHD; (3) the neuropsychological profile of the comorbid group was in line with the additive combination of the deficiency recognized in the groups with RD and ADHD alone. From this, the authors proposed that the phenocopy and cognitive subtype hypotheses could not be used to substantiate comorbidity of RD and ADHD in this sample.

2.10 Phenotype Definitions of Complex Disorders by Latent Class Analysis (LCA)

This study aims to identify the susceptible genes of ADHD and to find out whether candidate genes of comorbid ADHD-RD are same ones or whether each disorder has its own genes. By achieving these aims, this study may help to define the subtypes of ADHD. Despite the phenotypic characterisations of ADHD, defined through *DSM-III* (APA, 1980), *DSM-III-R* (APA, 1987), *DSM-IV* (APA, 1994) and *DSM-IV-TR* (APA, 2000), understanding ADHDs aetiology is still ambiguous. The validity of the disorder, because of its co-occurrence with other disorders, and the suitability of dimensional versus categorical models is debatable. As mentioned earlier, ADHD has been classified through *DSM-IV* into three subtypes: predominantly Inattentive, predominantly Hyperactive-Impulsive, and Combined. ADHD has been associated with significant rates of comorbidity with Oppositional Defiant Disorder (42%), Conduct Disorder (37%), Reading Disability (31%) (APA, 1987), Learning Disabilities (20%), and Depressive Disorders (20%) (APA, 1994). These rates of comorbidity have led to questions about the validity of the ADHD diagnosis.

Although *DSM* editions III through IV-TR have defined ADHD using categorical phenotypes which have marked heritability, the use of these categories has potential problems for genetic analyses. For example, an individual can have ten symptoms of *DSM-IV* ADHD, but if five are Inattentive and five are Hyperactive-Impulsive the individual is classified as unaffected. Another person may have ten symptoms of ADHD, six Inattentive and four Hyperactive-Impulsive, and would be categorized as having the Inattentive subtype, while yet another individual with ten symptoms of ADHD, four Inattentive and six Hyperactive-Impulsive, would be categorized as Hyperactive-Impulsive.

Even the argument about the suitability of continuous versus categorical models of ADHD is still debated. Levy et al. (1997) and Sherman et al. (1997) reported that ADHD symptoms are better described as continuously distributed in the general population.

As explained by Levy and Hay (2001), the classification and phenotypes of ADHD are still unconfirmed. Research goes in an attempt to understand the disorder. It may help to distinguish genetically between the ADHD subtypes, which can, in turn help delineate the phenotypes of ADHD subtypes more distinctively. This study attempts to isolate genes that contribute to the cause of the disorder. It may help to recognize the symptoms of the combined subtype. For example, some particular gene(s) may contribute to one subtype but not to another, and would be responsible for the subtype's phenotype. Moreover, this study may help to determine whether the Inattentive subtype and its comorbidity with RD have a unique genetic base with many different genes such as DRD4 and/or KIAA0319 gene on the 6p22 chromosome. In other words, which of these genes, either candidate ADHD genes or RD genes, are responsible for the genetic correlation?

Hallmayer et al. (2005) attempted to identify a familial subtype of schizophrenia, to identify the susceptible genes of the disorder. They adopted a novel phenotyping strategy; that is, aiming to identify composite profiles of cognitive performance. They used the GoM, which is a form of Latent Class Analysis (LCA), to define a particular number of latent groups from complex data sets. They employed a battery of tests, targeting different neurocognitive domains, neurobehavioural features, and selected personality traits. This strategy allowed them to identify a homogenous familial subtype of schizophrenia, characterised by pervasive neurocognitive deficit. Their proposed abbreviated battery of tests should facilitate phenotype characterisation for future genetic analyses and allow focus on a precisely-defined schizophrenia subtype, thus promoting a more informed search for susceptible genes.

Given the success of this approach, Latent Class Analysis will be used in order to define the significant classes of ADHD and RD so as to provide an informed basis for searching for susceptible genes. Previous studies (Eaton et al., 1989; Eaves, Silberg, & Hewitt, 1993; Faraone & Biederman, 1994) suggested that the use of Latent Class Analysis can help clarify appropriate symptom clusters and determine whether categorical or continuum models are more informative.

Hudziak et al. (1998) successfully applied the pioneer work of McCutcheon (1987) on Latent Class Analysis; for the first time using *DSM-IV* ADHD symptoms to determine whether different types of impairment (academic, peer, or family) are differentially associated with those subtypes and also to determine whether the distribution of ADHD symptomatology is more consistent with dimensional or categorical models. The results showed at least eight classes were needed to account for the 926 unique symptom profiles reported by parents about their adolescent female twins for 1629 families (the Missouri Adolescent Female Twin Study).

These classes were found to corresponded to class I, which showed no or few symptoms with a prevalence of 53.5%; class II exhibited the Talkative-Intrusive with a prevalence of 9.2%; class III represented the Intermediate Inattentive subtype; class IV illustrated the Intermediate Hyperactive-Impulsive subtype with a prevalence of 5.0%; class V represented by the Intermediate Combined subtype with a prevalence of 9.4%; class VI showed severe attentive problems with a prevalence of 4.0%; class VII demonstrate the Severe Hyperactive-Impulsive subtype with a prevalence of 2.0%; and class VIII demonstrated the Severe Hyperactive-Impulsive with Inattention subtype with a prevalence of 3.7%. Severe latent classes, corresponding to the predominantly Inattentive, predominantly Hyperactive-Impulsive, and combined subtypes were identified, with lifetime prevalence estimate of 4.0%, 2.2%, and 3.7%, respectively. Subjects of the Severe Inattentive class exhibited academic problems, family problems, and referral to health care providers, while subjects of the Hyperactive-Impulsive and Combined subtypes exhibited impaired social relationships. The researchers concluded

that these results provided validation of the *DSM-IV* criteria for ADHD and suggested the continuous model of the three symptom domains of ADHD subtypes.

A follow-up study by Neuman et al. (1999), on a sample from the Missouri Adolescent Female Twin Study, and male/female children and adolescent twin pair samples from the Collaborative Study on the Genetics of Alcoholism (COGA), reported that a pattern Latent Class Analysis suggested that ADHD consists of an Inattentive and a Combined subtype, each of which contains further continuous subtypes. The analyses performed indicated that genetic factors have a significant role to play in determining latent class membership. The study results also demonstrated two ADHD subtypes, a combined Inattentive and Hyperactive-Impulsive type and a primarily Inattentive subtype, each of which could be characterised dimensionally. These results support the application of Latent Class Analysis to the ADHD symptom endorsement profiles found in each distinct data set.

Research by Todd et al. (2001) investigated the familial effect and heritability of *DSM-IV* ADHD subtypes versus ADHD latent classes in a population sample of the Missouri Adolescent Female twins. They calculated sibling recurrence ratios (λ_s) for the three *DSM-IV* subtypes and the eight latent class subtypes, and found the eight latent classes have greater sibling recurrence ratio (λ_s) values for monozygotic than for dizygotic twins. This is consistent with a genetic contribution to liability, and appears to be independently transmitted in families. It has been suggested that these classes may be more fitting subjects of molecular genetic studies for ADHD.

A replicated approach to Latent Class Analysis was performed on population-based Australian twin samples diagnosed with *DSM-IV* ADHD (Hudziak et al., 1998; Neuman et al., 1999; Todd, Rasmussen et al., 2001). The aim of this replication was to examine the validity and heritability of eight ADHD latent class subtypes (Rasmussen et al., 2004) in an independent and culturally-different population. The collaborative work between the Missouri Adolescent Female Twin Study (MOAFTS) and the Australian

ADHD Twin Project (ATAP) hypothesised that the two ATAP samples (male/female twins) would exhibit the same latent class ADHD subtypes.

The results showed that most latent ADHD patterns of symptom endorsement probabilities were compatible with the *DSM-IV* ADHD subtypes across samples, including the "few symptoms" class, the Mild Inattentive class, the Inattentive-Impulsive class, the Talkative-Impulsive class, the Severe Inattentive class, and the Severe Combined class. However, there was also a rare Hyperactive-Impulsive class and another class that displayed a distinctive structure across all samples. The three samples illustrated how the *DSM-IV* subtypes were distributed across the eight ADHD latent classes. It was also found that the Severe Combined latent class contained the entire *DSM-IV* Combined subtype cases for Australian and Missouri females (100%), based on the compatibility of these results with results from MOAFTS Latent CLASS Analysis (Hudziak et al., 1998; Neuman et al., 1999; Todd, Rasmussen et al., 2001). Similarities also existed, across all samples in each of the stable classes, between the mean symptom endorsement and individual symptom endorsement probabilities.

As observed in previous studies (Hudziak et al., 1998; Neuman et al., 1999; Todd, Rasmussen et al., 2001), significant commonality exists between the *DSM-IV* Inattentive and Combined subtypes and the Severe Inattentive and Severe Combined latent classes. Rasmussen et al. (2002) concluded that of the eight latent class subtypes identified by MOAFTS, six have been replicated with ATAP samples, and that the separate ADHD subtypes identified by the LCA and *DSM-IV* schemes represent different phenotypic groups. An extended study by the same group (Rasmussen et al., 2004) examined the *DSM-IV* ADHD and Latent Class criteria for familial clustering of ADHD subtype combinations in a general population sample of children and adolescents from ATAP and MOAFTSA. Findings from both samples show significant same-subtype clustering with MZ probands, DZ probands and their siblings across all *DSM-IV* and ADHD subtypes, with the exception of the *DSM-IV* Hyperactive-Impulsive subtype and the Severe Hyperactive-Impulsive latent class. Additionally, a combination was found to

occur with both the *DSM-IV* Inattentive and Combined subtypes resulting in significant clustering among an MZ sibling pair in the Australian sample.

In addition, a regression-based approach has been used to determine odds ratios independently, for MZ versus DZ probands and to calculate odds ratios for zygosity to learn if genetic influences explain some variation in subtype clustering among siblings. The results suggest that the latent class approach may be useful in studying the genetics of ADHD, particularly in enabling a molecular genetic approach to determining the loci relevant to the aetiology and expression of symptoms of the Inattentive and Combined ADHD subtypes. Rasmussen et al. (2004) stated that the general pattern in both ATAP and MOAFTSA samples, for *DSM-IV* and latent class subtypes, indicated that although there were important sample differences, significant familial clustering of same-subtypes and combinations have been reported, along with significant contributed genetic influences corresponding to these patterns of subtype concordance.

The previous results from Latent Class Analysis in defining ADHD subtypes have implicated the molecular genetic approach in identifying the ADHD candidate genes. A recent study by Todd et al. (2005) attempted to examine if population-based ADHD subtypes (ADHD Latent Class Subtypes) defined by latent class analysis helped to resolve variable findings across individual gene-association studies. They hypothesised that population-based ADHD might represent distinct genetic entities that can be tested by comparing monozygotic / dizygotic twin concordance rates for twins of the same or opposite ADHD subtype.

The data from the above study was taken from three previous association studies which exhibited no association between polymorphisms of the DRD4 and DAT1 genes and *DSM-IV* ADHD symptoms. The study has analysed these data using population-based and *DSM-IV* subtypes, showing significant association between the combined data set for the 440 base-pair 3' DAT VNTR polymorphism and population-defined severe Combined ADHD (OR=1.25; p=0.01). Also, another slightly significant association (OR=1.20; p=0.16) has been exhibited between the 7-repeat DRD4 allele and

population-defined Severe Combined ADHD. These results have offered preliminary validation that these population-defined ADHD subtypes may have different genetic associations, and also may aid to resolve some of the variable results presented for candidate-gene association studies (Todd et al., 2005).

2.11 Molecular Genetic Approaches

The genetic architecture of polygenic disorders such as ADHD can be conveniently dissected under a quantitative model, in which the genetic factors are analysed (Haley & Anderson, 1997). The word 'quantitative' is typically substituted for 'continuous', and phenotypic variation displays standard distribution. Nonetheless, the scale of measurement for particular quantitative traits may also be discrete (Zhang & Cookson, 2002a). Traits controlled by several loci and environmental effects are referred to as 'quantitative', 'polygenic', 'multifactorial', or 'complex'. A polygene is the name given to each gene influencing a quantitative trait, and the locus or loci of a polygene is called quantitative trait locus/ loci (QTL) (Shalom & Darvasi, 2002).

QTL detection and mapping aims to uncover the genetic blueprint underlying a given complex trait by identifying specific chromosomal segments, and, ultimately, specific genes or regulatory elements that influence the phenotypic expression of the trait (Shalom & Darvasi, 2002). The detection of linkage between a QTL and genetic markers provides a more powerful and robust method of identifying QTLs. Linkage maps and DNA informative markers provide the basic tools with which to study the variation underlying quantitative traits. Haley and Andersson (1997) stated that the detection and mapping of QTLs is valuable because it gives insight into the actions and interactions of individual genes, which in turn allows more realistic modelling of phenotypic variations, responses to selection, and evolutionary processes. Zhang and Cookson (2002) asserted that the major advance in the genetics of complex traits was the development of statistical methods that take account of the fact that multiple genes make different quantitative contributions to the phenotype. QTL mapping has now become commonplace and has accelerated the analysis of polygenic susceptibility to various diseases. The development of a comprehensive chromosomal map of microsatellites and Single Nucleotide

Polymorphisms has made it possible to carry out mapping studies for quantitative traits such as ADHD.

2.11.1 QTL Association Procedure

Association is considered a more powerful strategy than linkage for finding genes of small effect in complex disorders (Plomin, Owen, & McGuffin, 1994). Performing a genetic association study allows a researcher to determine if a particular form of a DNA polymorphism occurs more frequently in individuals with a trait of interest. A particular allele in a gene is associated with a particular trait when it has significantly high frequency of alleles in the affected individuals. This significant difference can be measured by a Pearson Chi-square test of homogeneity of proportions. The genetic association can occur when the allele likely to cause the disorder is in linkage disequilibrium with the disorder-causing gene, or through a population admixture (Eley & Rijdsdijk, 2005). Some times a particular trait is more frequent in one ethnic group in heterogeneous populations and this might give a spurious association, which is known as population stratification. To overcome such a problem, the Transmission Disequilibrium Test (TDT) (Spielman, McGinnis, & Ewens, 1993) was introduced, using a within-family design, which includes both biological parents of the affected child. The genetic association can be detected based on the preferential transmission of particular alleles.

Allison (1997) and Rabinowitz (1997) introduced family-based linkage tests like TDT for quantitative traits, and stated that two alleles that form the child's genotype are inherited from the parents, and are called the transmitted alleles, while the remaining non-transmitted alleles act as internal controls. As all alleles come from one family there is no possibility of the results being false positives due to ethnic stratification. Such designs can offer direct tests of linkage disequilibrium and are efficacious fine-mapping tools, as they are robust and thus appropriate to identify candidate genes (Abecasis, Cardon, & Cookson, 2000).

Iles (2002) clarified that association between non-interacting alleles in a random mating population will only persist if they are linked. This association between linked loci is

known as Linkage Disequilibrium (LD). LD can be used to identify genes or regions involved in disorder susceptibility. Barr, Swanson, and Kennedy (2001) explained that the basis of association depends on the marker not being separated by recombination during meiosis over many generations such that the marker allele and the allele responsible for the phenotype remain together in a population. Therefore, the marker must necessarily be located much closer to the aetiological gene than can be found using a linkage study.

Association has two major advantages over linkage studies, the first of which is that it displays greater power in identifying susceptible alleles of small effect, such as those predicted to be involved in the influence of genes on behaviour. Second, samples for association studies are gathered more easily as it is only necessary to find a single affected individual from each family (Nuffield, 2002). Schulze and McMahon (2002) also highlighted that genetic association can identify a QTL region with only a minimal effect on the trait under study, along with the fact that population stratification (also known as “structure” or “substructure”) is not introduced via case selection when using association. Association provides an effective complement to linkage studies, although to employ association techniques across the entire genome would require immense numbers of markers, compared to the few hundred required for linkage, and is thus currently unfeasible. Therefore association is used primarily with candidate genes.

As reported by Vink and Boomsma (2002), genetic association studies can be carried out on candidate genes or candidate regions. On the other hand, linkage is usually genome-wide and requires the use of pedigrees (families and sibling pairs). It is possible to detect an association between a disorder and a specific allele in groups of cases and controls that are not related. Consequently, association studies become necessary in order to identify the susceptible genes that contribute to complex disorders such as ADHD.

2.11.2 Significance of Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms (SNPs) are single DNA base-pair variations. SNPs confer a base-compositional difference at a polymorphic site that can be detected in an amplified Polymerase Chain Reaction (PCR) fragment and are the most common type of

polymorphism in the human genome, with an approximate frequency of one kilobase (Le Hellard et al., 2002). Due to their frequency and distribution, SNPs may serve as superior genetic markers for assembly of a high-resolution map, aiding in the identification of disease-related loci (Weiner & Hudson, 2002). Werner et al. (2002) stated that over 4.1 million SNPs are available in public databases (dbSNP). Due to their vast number, stability, and simplicity compared to microsatellite markers they are considered to be useful markers for mapping genes that contribute to complex disorders. Le Hellard et al. (2002) found that SNPs are relatively easy to genotype compared with Variant Number of Tandem Repeats (VNTRs) and microsatellites.

Although linkage studies have been successful in the identification of genes underlying diseases with a simple Mendelian pattern of inheritance, it has been shown that they are not as powerful as association studies in detecting modest effects expected in complex disorders. As an alternative it has been suggested that case-control association designs could be used to identify susceptible genes, with dense sets of SNPs covering the region of interest. Lechner, Lathrop, and Gut (2001) have described SNPs as a source of markers for the dissection of complex traits in human genetics. These researchers pointed to three main reasons for this. First, these markers are abundant and easy to identify, thus, they provide a plentiful source of markers for association studies and Linkage Disequilibrium mapping. Second, they are relatively easy to characterise by a variety of techniques. Third, they are binary in nature and their analysis can be automated. As a result of this, a large proportion of the effort of genome centres is now focused on the identification and the mapping of large collections of SNPs. Ross, Hall, and Haff (2000) stated that current large-scale genome sequencing efforts continue to uncover SNPs at a frequency of approximately one SNP/ kilobase DNA. As SNPs are used in applications such as gene localization and disease diagnostics, a concomitant increase in the rate of routine SNP characterisation will be necessary.

2.11.2.1 How SNPs are used to find genes contributing to disorder

In some cases, particular SNP alleles are the actual functional variants responsible for increasing an individual's risk of contracting a disorder. Thus, those with this type of SNP allele are at greater risk of developing a specific disorder than those without the SNP allele. Most SNPs, however, are not the actual functional variants of a disorder, but serve an important purpose as markers for finding the genes that play a role in certain disorders. Comparison of the frequencies of SNP alleles, both in individuals that have and do not have them, is undertaken to determine what regions have genes that play a part in a disorder. Those regions which have SNP alleles present in greater frequency in individuals that have the disorder are thus determined to be associated with the disorder. Such associations in turn highlight the possibility that the genes in that region may contribute to the disorder (National, 2001).

2.12 Linkage Disequilibrium and Haplotype Block Analysis

2.12.1 What is Linkage Disequilibrium?

Linkage disequilibrium (LD) is the term used in genetics to indicate that association between non-interacting alleles in a random mating population will only exist if these alleles are found in linked form (Iles, 2002). Weiss & Clark (2002) explained that LD occurred when a mutation occurred to Single Nucleotide Polymorphisms along chromosomes, leading to association among a population of unrelated individuals and that the presence of one variant on SNPs provides information about the presence of other variants on those SNPs. Moreover, Sklar (2005) explained that LD takes place in the population more often than expected by chance when particular alleles at adjacent polymorphisms occur together on chromosomes. The genetic association in LD underlies all forms of genetic mapping. Since 2002 the use of linkage disequilibrium in genetic association studies by researchers has increased because of its ability to estimate the correlation of linked alleles among unrelated individuals. In addition, LD provides a measure of variation within genes, which is important information for gene-finding projects in population genetics.

Although both linkage analysis and LD both measure a correlation (or co-segregation or association) between a genetic marker and how the disorder affects a person, there are still major differences between them. Linkage analysis is based on measuring co-segregation in well-characterised pedigrees, and focuses on loci, whereas Linkage-Disequilibrium measures co-segregation in a population of unrelated individuals, and focuses on alleles. In addition, linkage is produced from recombination processes in the previous two to three generations, whereas Linkage-Disequilibrium is produced from much earlier, ancestral recombination incidents.

2.12.2 Patterns of LD

Research undertaken into patterns of LD has reached different conclusions regarding the distances at which LD can be detected, and the number of SNPs required for whole-genome association studies. After undertaking a simulation study, Kruglyak (1999) reported that LD could not extend more than an average of 3 kb in most populations, and in some extreme populations, more than 500,000 SNPs were necessary for whole-genome association studies. The same study found that while LD is typically detected for markers 1 megabase or further from the disorder gene, significant LD is often also present in markers between 50 and 100 kb from the said gene. Understanding these LD patterns in candidate genes and genomic regions is said to be critical in designing statistically powerful genetic association studies. Reich et al. (2001), however, reported quite different conclusions following research into LD patterns. They found that LD could extend up to 60 kb in some populations; and such this region has need of 50,000 SNPs in order to perform LD analysis.

Several studies into LD pattern variations have further elucidated correlations between LD and distance. Weiss and Clark (2002) have reported that weak LD can be detected between 10 and 20 kb from the disorder gene, and that strong LD is unlikely to exist between SNPs less than a few kilobases apart. Other Studies concluded that long-range LD spread over several hundred kilobases also contain areas of short-range LD present within only a few kilobases along the chromosome. Research undertaken by Daly et al.

(2001), Johnson et al. (2001), Patil et al. (2001) and Gabriel et al. (2002) into LD and haplotype block diversity found that the human genome could be segregated into blocks with limited haplotype diversity, with most haplotypes caught in small proportions of SNPs.

2.12.3 LD and defining haplotypes in the human genome

Weiss and Clark (2002) stated that high levels of LD in human genome are characterised by a low number of haplotypes, which are defined as a set of closely linked alleles present in one chromosome which tend to be inherited together. Ardlie et al. (2002) and Nordborg and Tavaré (2002) stated that in perfect LD, a novel mutation of all alleles happens, which re-assembles the chromosomes and disintegrates LD over consecutive generations. Ardlie et al. (2002) showed that it is less probable that sites in closer proximity to the new mutations are separated by the re-assembly. Hence, the current observation made on the pattern of LD between two loci is dependent on the age of the new mutation and the actual distance from alleles close by, which then allows positional cloning to use LD information (Weiss & Clark, 2002). In addition, they theorised that both gene conversion and recombination would cause an erosion in LD and the age of mutations involved, and the size of the past human population and structure would then give rise to the amount of LD. When a single SNP causes an increased risk of a disorder, a relationship between that risk and other SNPs in LD with the causal SNP may exist. As it is possible that co-occurring sites may contain unwanted information, the reliance on haplotypes, which depends on parts of the sites, may decrease the number of SNP markers that need genotyping to ascertain disorder- associated variants (Weiss & Clark, 2002).

2.12.4 Measures of Linkage Disequilibrium

The LD phenomenon can be used as a statistical measure of polymorphisms within genes. Hartl and Clark (1997) stated that since there are two combined alleles for each chromosome in a haplotype, it is difficult to directly observe haplotypes; therefore, the estimation of haplotype frequencies and LD measurement is a statistical enterprise. Weiss and Clark (2002) and Sklar (2005) stated that two measures of LD are commonly used: the absolute value of D' (Ardlie et al., 2002) and the squared correlation

coefficient r^2 (sometimes denoted Δ^2) (Wall & Pritchard, 2003). They measure the strength of LD between pairs of markers or across genes. The division of the genetic population parameter D , after adjustment for the allele frequencies at the SNP pair, provides the measurement D' . The absolute value of D' is 1 when complete LD exists for an SNP pair with no possibility of a past recombination incident happening between the two SNPs. In such cases, the fact that the second SNP in the pair was based on only a chromosome, as compared to the two alleles of the first SNP, only three of the four likely haplotypes that could arise with two SNPs alongside each other will be monitored. As the absolute value of D' ranges between 0 and 1, LDs become stronger when values near 1, while they gradually diminish when values approach 0 (Sklar, 2005; Weiss & Clark, 2002).

If D' is equal to 1 or -1, this means that there is no evidence for recombination between markers. If allele frequencies are similar, a high D' means the markers are good surrogates for each other. Ardlie et al. (2002) noted that a D' value of 1 equals complete LD; D' values greater than 0.8 equals strong LD; D' values ranging from 0.2 to 0.8 equal incomplete LD; and D' values of less than 0.2 equal negligible LD. A D' value of greater than 0.33 is considered as the minimum useful amount of LD (Abecasis et al., 2001). D' estimates can be inflated in small samples and when one allele is rare but it must be remembered that D' values in small samples are extrapolated and care is necessary in the interpretation of such values (Sklar, 2005; Weiss & Clark, 2002).

The squared correlation coefficient, r^2 , is another helpful tool to measure LD. r^2 is arrived at by the division of D^2 by the product of the four allele frequencies at the two SNPs. Perfect LD, when r^2 is 1, happens when only a pair of haplotypes are considered to have recombined, and the allele frequency remains the same, which could mean that SNPs have separated. However, this has not occurred.. Useful predictive information about behaviour can be achieved with the intermediate values of r^2 of the second SNP. r^2 is the preferred calculation of population geneticists, with its values varying between zero and one: the former indicates the two markers are in complete

equilibrium while the latter indicates that the same information exists in the two markers (Pritchard & Przeworski, 2001; Wall & Pritchard, 2003).

2.12.5 The Concept of Haplotype Blocks

At present, there is interest in statistically powerful genetic association studies to detecting variations responsible for common human diseases (Zhang et al., 2002). In addition, Wall and Pritchard (2003) opined that LD mapping can make use of the haplotype-block model to heighten the probability of accurate prediction of the recombination of alleles at unseen positions. Furthermore, Hoehe (2003) has highlighted that haplotype-based approaches to the analysis of candidate genes and genome-wide Linkage Disequilibrium mapping has recently received intense interest. Accordingly, several recent studies on human genomic variation recommend clustering SNP markers together into haplotype blocks (Daly et al., 2001; Gabriel et al., 2002; Goldstein, 2001; Patil et al., 2001; Wall & Pritchard, 2003). Cardon and Abecasis (2003) defined the haplotype block as “a discrete chromosome region of high linkage disequilibrium and low haplotype diversity. It is expected that all pairs of polymorphisms within a block will be in strong linkage disequilibrium, whereas other pairs will show much weaker association. Blocks are hypothesized to be regions of low recombination flanked by recombination hotspots.” (p.135).

Daly et al. (2001) carried out such a study, with an examination into a 500 kb region on human chromosome 5q31. Through genotyping a genetic variant of 103 SNPs is known to exist. The area was segregated into 11 blocks, and only four common haplotypes comprise almost all haplotypes studied. Haplotype structure in lesser areas, studied by Johnson et al. (2001) through the genotyping of 122 SNPs in a 135 kb area for nine genes, discovered that only 34 SNPs were needed to ascertain the haplotypes in 384 people. A broad investigation on the complete haplotype structure on chromosome 21 for 24,047 SNPs was performed by Patil et al. (2001). A rodent-human somatic cell hybrid technique was used to ascertain 20 haplotypes, which were then segregated into 4135 haplotype blocks, with repeated haplotypes responsible for more than 80% of the haplotypes studied in every block. In all, 4563 SNPs in repeated haplotypes were

haplotype-tagging SNPs (i.e. a cluster of SNPs selected to stand for all haplotypes in a particular DNA region is known as htSNPs). Subsequently, Zhang, Deng, Chen, Waterman and Sun (2002) decreased the block numbers and tagged SNPs to 2575 and 3582 respectively with the same information using a powerful programming algorithm. As such, it is possible to achieve 80% of all the haplotypes in each block with 15% (3582) of all the SNPs (24,047).

2.12.6 Haplotype-Tagging SNPs (htSNPs)

Johnson et al. (2001) stated that each haplotype block, in which the genome is largely made up of regions of low diversity, can be characterised by a small number of SNPs called htSNPs. They suggested that linkage disequilibrium and haplotype diversity within the region can be captured by those htSNPs. Ding et al. (2005) concluded that haplotype blocks and the choice of htSNPs offer a probable method to decrease the difficulties with association mapping of complex diseases. In addition, Arnheim, Calabrese and Nordberg (2003) and Wang et al. (2002) reported that haplotype blocks are assumed to rely on recombination hotspots, lesser regions in which the likelihood of recombination exceeds that of the nearby areas. The alleles at the SNPs are carried through generations due to the lesser likelihood of recombination within each block. Hence, every haplotype block can be taken as an individual marker alongside the set of alleles at the SNPs in the block made up of its allele (Cardon & Abecasis, 2003). It would be desirable that haplotype blocks allow less genotyping of SNP markers in LD mapping investigations as fewer htSNPs can be used to ascertain the common alleles in every block (Zhang et al., 2001; Zhang, Calabrese et al., 2002). Sklar (2005) regarded the statistic r^2 as an accurate tool to work out LD and to ascertain the SNPs that are tested and those that are not. Pritchard and Przeworski (2001) suggested that the choice of a set of SNPs should be made at an appropriate density so that the causal SNP would exist in a powerful LD with one of the genotyped SNPs. The r^2 statistic between a pair of SNPs is an accurate measurement of the ability to locate an untyped SNP but it is affected by SNPs with low r^2 values, which cannot be properly tested regardless of their closeness.

Zhang et al. (2002) noted that haplotype blocks, including the haplotype-tagged SNPs and common haplotypes determined by haplotype block-partitioning algorithms, can be effectively used in genome-wide association studies and in the fine-scale mapping of the genes of complex diseases, in order to detect genetic variations responsible for complex human disorders. This approach can significantly reduce the genotyping cost (Johnson et al., 2001).

In order to perform haplotype block studies, Zhang et al. (2002) pointed out that ten to twenty subjects can be genotyped at a very dense SNP map in a region. These subjects' haplotypes are identified during the genotyping process. Then, a haplotype block-partitioning algorithm is used to facilitate identification of haplotype block structure and a set of well-spaced tag SNPs. Software packages such as Haploview (Barrett et al., 2005) and Hapblock (Zhang et al., 2005) are based on these algorithms, and are designed to select the most efficient set of tagging SNPs. Then genotyping is performed on a larger number of samples only at the tag-SNP marker loci identified. Finally, the small and large genotype samples are combined, and with knowledge of the haplotype block structure, association studies are carried out (Zhang et al., 2002).

2.12.7 The International HapMap Project

The International HapMap Project (International HapMap Consortium, 2003, 2005) was established to develop a haplotype map of the human genome (the HapMap), so as to provide a description of the common patterns of human genetic variation. The Project has enabled scientists to produce a high-density haplotype map of the human genome for several target populations. This map enables the efficient selection of htSNPs, will help to detect the haplotype blocks of common complex disorders, and increase researchers' capacity to target high-risk alleles (International HapMap Consortium, 2003, 2005).

2.13 Rationale, Aims, and Design

2.13.1 The Study Rationale

The current study investigated the genetic components of ADHD, RD, and the comorbidity between them. The ultimate goal was to identify some of the genes and risk alleles that contributed to each one individually and to both of them as a comorbid condition. However, as these aspects are complex disorders, there are obstacles that can make it difficult to achieve this goal. Firstly for ADHD, despite the large volume of research that has been achieved on in last the decades, our understanding of this disorder is still incomplete. This can be confirmed by the 1998 National Institute of Health (NIH) Consensus Statement on Diagnostic and Treatment of ADHD stating that “Finally, after years of clinical research and experience with ADHD, our knowledge about the cause or causes of ADHD remain largely speculative. Consequently, we have no documented strategies for the prevention of ADHD” (NIH, 1989, p.3).

Todd (2005) reported that although there are many ADHD genetic studies, still there is no complete agreement, and the debate continues whether ADHD is best studied as a continuous or categorical disorder, as this plays an important role in genetic study designs. Thapar et al. (2006) stated that both dimensional and categorical approaches to studying the genetics of ADHD are appropriate. The best model for studying ADHD as dimensional data is the quantitative Trait Loci (QTL), whereas the best model for studying ADHD as categorical data are the linkage and the association approaches which have both produced valid results in identifying susceptible genes for ADHD. Levy et al (1997) showed that the heritability of *DSM-III-R*-defined ADHD was not statistically different whether ADHD was defined as a continuum or a category. Rasmussen et al. (2002) and Thapar et al. (2006) concluded that neither approach could be declared superior due to two major factors: 1) Existing phenotype classification tools display shortcomings which may inhibit the identification of common genotypes (Todd, 2000); and 2) Both categorical and continuous approaches have proven successful in demonstrating the association of ADHD with several genes (Thapar et al., 2006). Stevenson et al. (2005) recommended the dimensional approach for investigating the genetics of ADHD, whilst

Thapar et al. (2006) do favoured the use of a categorical approach over a dimensional approach for identifying susceptible genes for ADHD.

RD is a neuro-developmentally complex disorder. There are several consistent studies that showing that genetics plays an important role in the aetiology of RD. Unlike ADHD, there are no subtypes for RD based on *DSM-IV* diagnostic criteria. Bates (2006) stated that there is lack of information about whether the diagnostic categories of RD are valid or not and RD is one common disorder or whether has several phenotypic subtypes. From a genetic point of view, Bates (2006) argued that “RD is not a wholly distinct diagnostic entity, but is embedded within an overarching network of genetic effects” (p. 42). However, more research is needed to investigate the genetics of RD.

Secondly, there is no definite conceptualisation of whether genes are associated with Inattention will also be associated with the Hyperactive-Impulsive and Combined subtypes, or whether there is genetic heterogeneity between the three subtypes. McLoughlin et al. (2007) stated that it is still not well understood to what extent there is genetic overlap between Inattention and Hyperactivity-Impulsivity, despite the numerous linkage and association studies performed on ADHD. It has been suggested that there was no genetic specificity among ADHD subtypes (Smally et al, 2000). A further suggestion is the presence of genetic specificity between Hyperactivity-Impulsivity and Inattention subtypes, but no specificity between the Combined subtype and Inattentive subtype (Farone et al 2000, Todd 2001). A recent investigation by McLoughlin et al. (2007) suggested no genetic difference between Inattention and Hyperactivity-Impulsivity symptoms; however, to some extent there is a significant genetic heterogeneity among them. For RD, Bates (2006) reported that genes contributing to RD are not specific to RD alone, as these genes also can overlap with other disorders such as ADHD, autism, general intelligence and specific processing deficits. However, there is a lack of studies investigating this issue.

Thirdly, as explained earlier, use of the *DSM-IV* diagnostic criteria to identify children with ADHD has a potential problem, especially if these children are needed for genetic studies. This is because the use of six out of nine cut-off symptoms is an arbitrary point

along the dimensions of Inattention and Hyperactivity-Impulsivity (Levy et al., 2006). This criteria leads to uncertainty in identifying the children with the appropriate ADHD subtype (Neuman et al., 2005) because of the Inattentive and Hyperactive-Impulsive ADHD symptoms overlap, resulting in a heterogeneous phenotype. In addition, Barr, Swanson, and Kennedy (2001) stated that the phenotype of ADHD is very complex making one problem is that the *DSM-IV* diagnostic criteria focuses on the phenomena of ADHD and deliberately ignores the aetiology; therefore *DSM-IV* is not specifically suited for selecting ADHD children for genetic studies. Another problem is that, since 1980, is that the *DSM* revision process has generated three changes of the definition of ADHD phenotype.

RD contains several phenotypic components, with the presence of strong genetic effects, for word recognition, orthographic coding, phonological decoding and phonological awareness (Gayan & Olson, 1999), and also word and non-word identification, reading comprehension, and spelling (Byrne et al., 2007). Bates (2006) also reported that several studies that propose that there are certain genes contributing to all RD phenotypic components, and also there are specific genes that only contribute to particular RD components. However, investigating the general and specific genes for RD without refining its phenotype will not help to detect and distinguish between these two groups of genes, as the current definition of RD contains heterogeneous phenotypes.

RD exhibited a strong influence on many behavioural problems such as ADHD, leading to the conclusion of a high level of comorbidity (Bates, 2006). The comorbidity between RD and ADHD is highly heritable and frequent. However, this comorbidity relationship is still not well understood and needs more investigation. One problem is defining the exact locations of the possible genes underlying ADHD-RD comorbidity. Willcutt et al. (2002) suggested that the comorbidity of ADHD-RD is due to many possible candidate genes, which may have a pleiotropic effect; however, the specific genetic causes have not yet been identified. Stevenson et al. (2005) stated that the latter finding offers primary evidence that the 6p22.2 loci should be considered as an aetiological factor for both disorders, and suggested that this may establish the basis for future studies of the aetiology of ADHD and its comorbidity with RD. Furthermore, the same authors

concluded that ADHD genetic studies have to examine ADHD alone and ADHD comorbid with RD. But because of the phenotypic complexity and heterogeneity of both ADHD and RD, the genetic results would be ambiguous.

The proposed solution for a problem is to refine the ADHD and RD phenotypes by producing a ‘genetically informative phenotype’ (Szatmari et al., 2007). Latent Class Analysis (LCA) is a tool that can refine complex phenotypes as it produces more aetiologically-homogenous ADHD phenotype subtypes based on statistically defined clusters of symptoms (Volk et al., 2006). This occurred when Todd and his colleagues (Hudziak et al., 1998; Todd et al., 2002) applied an alternative definition of ADHD using LCA, hoping to overcome the inappropriate classification of ADHD individuals based on the *DSM-IV* six cut-off symptoms, and also to diminish the ADHD phenotype heterogeneity. However, to the best of my knowledge, this technique has not been applied to RD until now. Moreover, Szatmari et al. (2007) argued that identifying psychiatric disorders (e.g., ADHD) based on *DSM-IV* contained a wide range of phenotypes, and thus heterogeneous disorder. Although molecular genetic studies on ADHD have highlighted some of the susceptible genes that contribute to the aetiology of ADHD, there is no definite identification of specific genes that contributing to each ADHD subtype.

However, the subtypes produced by LCA are robust enough to use in molecular genetic studies. Evidence for this was seen in a study by Todd et al. (2005) where three previous ADHD studies had reported no association with DRD4 and DAT1 genes identified based on *DSM-IV*; these studies were then reanalysed using a population-based sample and *DSM-IV*-defined ADHD subtypes. The results showed significant associations of the polymorphisms of these two genes but did not show significant associations with the ADHD individuals identified based on *DSM-IV*. The recent methodological advance represented by the haplotype block analysis makes it possible to identify risk-allele genetic variants related to ADHD subtypes and RD phenotypic components, especially if their phenotypes are genetically informative.

2.13.2 *The study aims and design*

As this is a genetic study, a twin design was selected. Monozygotic (MZ) and Dizygotic (DZ) twins and their siblings aged from 4 to 18 years old recruited from the Australian Twin ADHD Project (ATAP). ATAP designed the ‘Twin and Sibling Questionnaire’, which has been extensively assessed against other measures of behavioural problems, in order to assess twins and siblings who have ADHD and RD.

The study was designed to use the *DSM-IV* diagnostic criteria to identify ADHD subtypes and used the continuum cut-off score to identify individuals with Reading Disability, aiming to:

- 1 examine the efficacy of *DSM-IV* ADHD subtypes and Reading Disability as one phenotypic group (RD) in genotyping analysis.
- 2 test the uncertainty of *DSM-IV* ADHD diagnostic criteria and to demonstrate that *DSM-IV* classification creates heterogeneous phenotypes.

The study utilised the Latent Class Analysis approach that has the ability to split individuals into phenotypically similar groups to produce distinctive and heritable classes (Volk et al., 2006) aiming to:

- 1 refine the ADHD alone, RD alone, ADHD-RD phenotypes in order to have homogenous genetically-informative phenotypes, based on related cluster symptoms.
- 2 investigate if RD phenotypes can be represented in more than one distinctive group or subtype.
- 3 compare the efficiency of *DSM-IV* diagnostic criteria and Latent Class Analysis (LCA) in the genotyping analysis and find which one can give clearer results.

The study implemented the genetic fitting model (Mx) on Monozygotic (MZ) and Dizygotic (DZ) twins to:

- 1 study the genetic and environmental effects of each ADHD subtype and RD separately, and to investigate the genetic effects of the comorbidity of each ADHD subtype with RD.
- 2 investigate whether children who are diagnosed with ADHD-RD are a genetically distinctive group from those with ADHD without RD.

Both LCA and genetic modelling in this study needed a large sample size to facilitate a powerful genetic analysis.

The study performed a family-based association analysis aiming to:

- 1 replicate some of the previous findings of ADHD and RD candidate genes on an Australian twin sample.
- 2 test ADHD candidate genes on RD phenotypes, and to test RD candidate genes on ADHD phenotypes, in order to examine the genetic overlap between the two domains.

The study designed to carry out a haplotype block analysis aiming to:

- 1 detect the risk alleles of ADHD alone, RD alone, and ADHD-RD comorbidity.

2.13.2.1 Selection of ADHD and RD candidate genes and their SNPs for this study

The study used twenty-one Single Nucleotide polymorphisms (SNPs) from nine genes in the genotyping analysis, which included family-based association study and haplotype block analysis on Australian twin families. The DNA extraction and the genotyping analyses were carried out at the Queensland Institute Medical Research (QIMR) laboratories. Out of the nine genes, there were five ADHD candidate genes: DRD4, DAT1, SNAP25, COMT, and HTR1B, and also there were four RD candidate genes: MRS2L, KIAA0319, TTRAP, and THEME2 from chromosome 6p22. The selection of

these genes was based on selecting the most common candidate genes for ADHD and RD. The study could not include all candidate genes were reviewed earlier, because of funding limitations. Therefore, I selected the common candidate genes for ADHD and RD in order to examine if ADHD candidate genes overlap with and contribute to RD, and also to examine if RD candidate genes overlap with and contribute to ADHD. In addition, I examined which candidate gene group (ADHD or RD) was more related to ADHD-RD comorbidity, and whether both groups of genes have the same genetic contribution? The following review gives a brief overview of some of the SNPs that were used in the genotyping analysis.

Feng, Crosbie et al. (2005) studied the association between SNAP-25 and ADHD by using two polymorphisms identified in the 3' untranslated region of 20p12.2 by screening all the coding exons. Based on previous studies, this study has focused on four SNPs: rs1051312; rs362549; rs362987; and rs362998. In addition, it has investigated the serotonin receptor HTR1B gene on the 6q14.1 region by focussing on four SNPs: rs130058; rs6296; rs6297; and rs6298. These SNPs were also investigated by Ickowicz et al. (2007) to detect their possible association with ADHD. The COMT gene on chromosome 22q11.21 was also examined because Cheuk and Wong (2006) reported that there were inconsistencies relevant to the association between the Val158/108Met polymorphism of the COMT gene and ADHD. The rs165599, rs4680, rs737865 DNA variant polymorphisms were investigated by Shifman et al. (2002), and this study did too.

The dopamine transporter (DAT1 or SLC6A3) gene on chromosome 5p15.33 was investigated by focussing on the variation in the 3' untranslated region (Feng et al., 2005), suggesting that it may play a role in DAT1 expression. The current study examined two polymorphisms; the *MspI* polymorphism (rs27072) located 480 bp upstream of the VNTR, and SNP rs6347 on exon 9. These DAT1 SNPs and others were investigated by Feng et al. (2005). Faraone et al. (2001) found evidence of a genetic association of the 7-repeat allele of a 48 bp VNTR in the exon-III of the Dopamine D4 receptor (DRD4) found on 11p15.5. This study also included one SNP (rs3758653) of

the DRD4 gene (-906T>C) out of eleven identified common polymorphisms that were scanned, re-sequenced and reported by Nakajima et al. (2007).

The last group of SNPs included in this study are from the 6p22.2 region. Previous studies reported that this region may be susceptible to both ADHD and RD (Stevenson, Langley et al., 2005; Stevenson, 1993; Willcutt, DeFries et al., 2003; Willcutt et al., 2002). Cope et al. (2005) studied the 575 kb region of chromosome 6p22.2, which includes ten candidate genes implicated in Reading Disability. Out of those ten genes, this study has included four: the TTRAP gene represented by two SNPs (rs2143340, and rs6935076); the KIAA0319 gene represented by two SNPs (rs2179515, and rs4504469); the THEM2 gene represented by rs3777664 SNP; and MRS2L represented by rs2793422 SNP.

This study was designed to investigate those ADHD-RD candidate genes through their SNPs and some SNPs of the 6p22 region. The family-based association approach is a tool to determine this phenotype-genotype relationship, helping to determine if particular SNPs occur more frequently in twins with ADHD alone, RD alone, and ADHD-RD comorbidity.

CHAPTER 3: IDENTIFICATION OF PARTICIPANTS

3.1 Introduction

This chapter describes the participants' recruitment, including exclusionary criteria, how participants were identified and classified for ADHD based on *DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition)*, how they were diagnosed for Reading Disability, and the items used to measure Reading Disability. In addition, this description will cover the zygosity measure. As this study is a part of a new cohort of the fourth-wave Australian Twin Attention Deficit Hyperactivity Disorder Project (ATAP), I will start my description by giving a historical overview of ATAP.

3.2 Australian Twin ADHD Project

ATAP is one of the world's largest ADHD projects attempting to offer a better understanding of ADHD aetiology including phenotype, classification and comorbidity. The goals of ATAP are to understand the aetiological and developmental pattern of ADHD, to establish an informative ADHD database for performing quantitative and molecular genetic analyses, in order to determine the contributions of genetic and environmental factors with ADHD, and to understand the comorbidity of ADHD with other behavioural disorders such as Reading Disability (RD), Conduct Disorder (CD), Oppositional Defiant Disorder (ODD), and Generalized Anxiety Disorder (GAD) (Bennett et al., 2006).

In 1990, David Hay and Florence Levy established ATAP because there was a need to understand ADHD more clearly. Previous twin studies obtained inconsistent findings because they had only a small number of subjects, so there was a need to design large twin studies that would be compatible with the behaviour-genetic methodology (Hay et al., 2001; Hay et al., 2002). As a result, ATAP collected four waves of data over the last fifteen years from 1991 to 2006. The first three waves were longitudinal using the same twin families, whereas the fourth wave was a new cohort. In the first wave in 1991, a questionnaire was sent to 3215 Australian twin families, asking questions about diagnoses of ADHD generated from the *DSM-III-R* (APA, 1987). In the second wave (1994-1995),

1550 families responded to the Australian Twin Behaviour Rating Scale (ATBRS) created by Levy, Hay, McStephen, Wood and Waldman (1996), based on the *DSM-IV* diagnostic criteria (APA, 1994). In the third wave, 1515 twin families were approached in 2000-2001: adolescent twins completed a self-report questionnaire entitled ‘Behaviour Questionnaire for Young People’, and their parents filled in a self-report questionnaire entitled ‘Parent Behaviour Questionnaire’. ATAP has been comprehensively described by Hay et al. (2001; 2002) and by Bennett et al. (2006). Figure 3.1 outlines the previous three waves chronologically (Hay et al., 2002). One of the primary findings of ATAP was that ADD, based on *DSM-III-R* criteria, was found to be an inheritable ($h^2 = 0.75-0.95$) and continuous (as opposed to discrete) disorder (Levy et al., 1997).

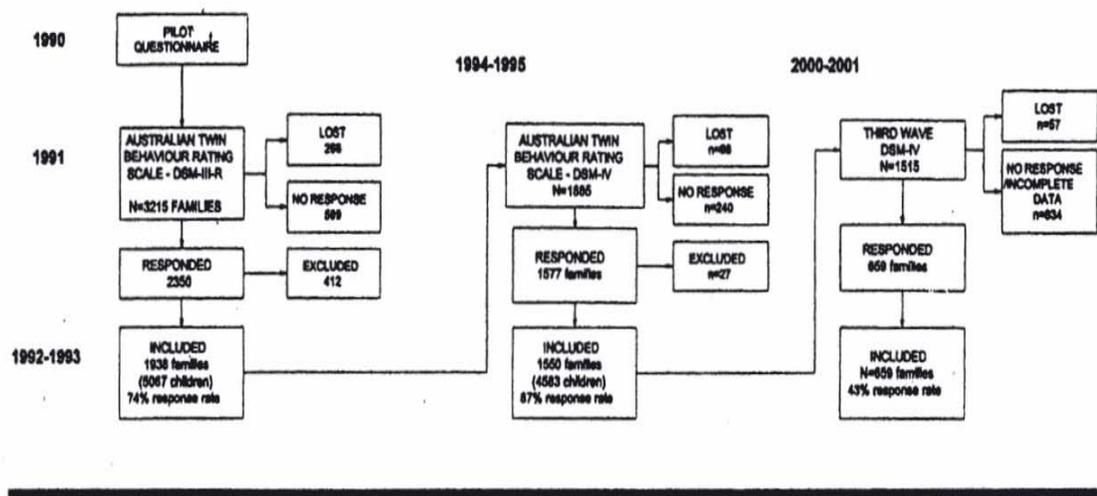


Figure 3.1 Waves of ATAP study. (From: Hay et al., 2002).

In the first stage of the fourth wave (2004-2006), the parents of about 3000 twin families completed a questionnaire named ‘Twin and Sibling Questionnaire’ (Hay & Levy, 2004), in order to perform further investigations on the genetic and developmental patterns of ADHD and its comorbidity with other behavioural disorders. Historically, this questionnaire was developed based on the Australian Twin Behaviour Rating Scale (ATBRS) (Levy et al., 1996).

3.3 *Participant Recruitment*

The participants of this study were twin families who participated in the current, ongoing, fourth wave ATAP study. These twin families were members of the Australian Twin Registry (ATR) (<http://www.twins.org.au>), which is a nationwide volunteer-based twin registry having approximately 30,000 pairs of twins from all over Australia. The sources of ATR recruitment are the Australian Multiple Birth Association (AMBA), schools, medical centres, posters, and media. The ATR is an important national and international resource for medical and scientific twin research, in the study of the human genome. In addition, advances in the knowledge of the human genome provide opportunities to identify variants associated with complex disorders and examine the gene-environment interaction (Hopper, 2002).

At the time of this study, ATAP had recruited 2610 twin families from all over Australia out of 3500 twin families approached (a response rate of 75%). The length of the family questionnaire could be the reason for obtaining this lower response rate (Bennett et al., 2006). These families constituted the participants of this study, including monozygotic (MZ), dizygotic (DZ) twins and their siblings. The 2610 twin families gave a total twins and siblings number of 7209. The total number of male children was 3681 (51.1%), and that of female children was 3528 (48.9%). There were 2262 (31.4 %) monozygotic twins and 2910 (40.4%) dizygotic twins (with 25 families excluded as they had no zygosity information). The total number of siblings 1 was 1609 (22.3 %) children, whereas the number of siblings 2 was 430 (6.0 %) children. Sibling 1 is older than sibling 2. The age range of the total sample was from 4 to 18 years old, with a mean age of 12.94, +/- 3.9 years.

This study applied the exclusionary criteria that Hay and his colleagues (2002) established on the four waves of ATAP. The criteria for exclusion involve any one of the following problems: if any of the twins or siblings suffers from any mental retardation, psychosis, autism, or a major medical or neurological illness including deafness, blindness, cerebral palsy, as well as major cardiac malformations. Children with obvious physical and health problems, such as retinopathy, were thus excluded (Hay et al., 2002). Other conditions

such as muscular dystrophy, leukaemia, Down's Syndrome, or rare genetic conditions or specific environmental disorders such as meningitis were also causes for exclusion. The exclusion extends to the whole family, if one child suffers from a disability or other identified developmental disorders other than ADHD and RD. Commonly in such cases, one of the twins is an affected family member, and less likely to be a sibling. The criteria also excluded families who participated previously in long-term behavioural studies. Another criterion for exclusion was multiples who were born both pre-term and with extremely low weight, due to the likelihood of these problems causing subsequent learning and ADHD difficulties. Furthermore, families were excluded if either one of the biological parents was unavailable for DNA collection.

3.4 Measures

3.4.1 The Australian Twin Behaviour Rating Scale (ATRBS)

The ATRBS was designed by Levy et al. (1996) and used with the first wave of the ATAP. It was originally based on *DSM-III-R*, and subsequently *DSM-IV*, and designed to measure the presence of childhood behavioural disorders such as ADHD, CD, ODD, GAD, Separation Anxiety (SA), Reading and Spelling Disorder, and Depression. Levy et al. (1996) stated that the use of the ATBRS based on parent ratings can be considered as a conservative sign of attendance of symptoms. Levy et al. (1996) found the criteria of *DSM-III-R* ADHD symptoms, Speech and Language problem symptoms, and Reading Disability symptoms, were highly reliable (0.86, 0.71, and 0.82 respectively) based on Cronbach alphas. As mentioned earlier, the ATBRS, which ATAP developed for measuring childhood behavioural problems, was refined and renamed the 'Twin and Sibling Questionnaire'.

The Twin and Sibling Questionnaire is a comprehensive questionnaire developed to obtain information on a wide range of childhood behavioural disorders. The questionnaire asked parents to report about their children's behaviour. In addition, the questionnaire seeks information about their children's birth history, zygosity, medication and substance history, movement ability, and personality.

Because this study sought to investigate *DSM-IV* ADHD subtypes and ADHD comorbidity with Reading Disability (RD), only certain information was used: the *DSM-IV* ADHD and Reading Disability symptoms of the twins and their siblings, plus demographic data such as sex, age, and zygosity. The study did not use the items for measuring other childhood behavioural disorders, as it did not aim to investigate them.

3.4.2 Measurement of Zygosity

Numerous twin studies depend widely on questionnaires for measuring zygosity. Hay et al. (2001) stated that one of the problems with determining zygosity is that drawing blood from children is too invasive; therefore, an alternative way to determine zygosity is the use of questionnaires. Rietveld, Posthuma, Dolan and Boomsma (2003) pointed out that measuring zygosity based on a mailed questionnaire still offers substantial accuracy (95%) in determining zygosity, compared to blood tests or DNA fingerprinting, although these are the best choice for determining zygosity. However, the limitations of cost, and ethical and practical challenges could render them more problematic in epidemiological research.

This study determined zygosity based on the ‘Twin and Sibling Questionnaire’. Hay and his colleagues (2001) designed fourteen questions for zygosity assessment (Table 3.1). Six questions look at similarity of features and six questions focus on confusion of the twins’ identities (Cohen et al., 1975; Nichols & Bilbro, 1966). They have merged these questions together in order to exploit the diagnosis sufficiency by the use of the Discriminant Function Analysis (DFA), a statistical approach that discriminates if twins are MZ or DZ (Abu Alhajja & Richardson, 2003), and the other two questions used to identify placentation and blood group polymorphisms in order to distinguish MZ twins. Determining the zygosity based on placentation alone is not 100% accurate, as about one third of MZ twin pairs can have two separate placentas as DZ twin pairs possess.

Table 3.1
Zygosity Measures. (Source: (Hay et al., 2001)

Question	Responses		
I believe the twins to be:	Genetically Identical (one egg)	Genetically Non-Identical (two eggs)	Not sure
Q. To what extent are the twins similar at this time for the following features?			
A. Height	Not at all similar	Somewhat similar	Exactly similar
B. Weight	Not at all similar	Somewhat similar	Exactly similar
C. Facial Appearance	Not at all similar	Somewhat similar	Exactly similar
D. Natural Hair Colour	Not at all similar	Somewhat similar	Exactly similar
E. Eye Colour	Not at all similar	Somewhat similar	Exactly similar
F. Complexion	Not at all similar	Somewhat similar	Exactly similar
G. Do they look as alike as two peas in a pod?		Yes	No
H. Does their mother ever confuse them in appearance?		Yes	No
I. Does their father ever confuse them in appearance?		Yes	No
J. Are they sometimes confused in appearance by other people in the family?		Yes	No
K. Is it hard for strangers to tell them apart?		Yes	No
L. Do they have very similar personalities?		Yes	No
M. Did they have the same placenta?	Yes	No	Don't Know
N. Do they have the same blood group?	Yes	No	Don't Know

In this study, zygosity was determined by asking parents the fourteen questions that Hay et al. (2001) designed (Table 3.1). They responded to the following prompts: “I believe the twins to be: Genetically identical (one egg, monozygotic)”, or “Genetically non-identical

(two eggs, dizygotic)”. If twins were not the same sex, we knew that they were dizygotic. If this was not the case, parents were asked if the twins had undergone a blood or DNA zygosity test. If the parents’ answer was ‘yes’ follow-up questions were: “The test found the twins to be: genetically identical or genetically non-identical?” and “What was the test used?”

3.4.3 DSM-IV ADHD items measure

The Twin and Sibling Questionnaire (Hay & Levy, 2004) used by the fourth wave ATAP study contained the 18 items of ADHD subtypes generated from DSM-IV manual, and was designed to be answered by a mother or father. The DSM-IV criteria for ADHD comprise 18 items for ADHD subtypes; nine items are for the Inattentive subtype (Table 3.2), six items for the Hyperactivity (Table 3.3), and three items for Impulsivity (Table 3.4). However, as can be seen from Table 3.2, the Twin and Sibling Questionnaire used ten items instead of nine, which were derived from the DSM-IV Inattention criteria. The questionnaire combined the scores of two items and put the new scores in one new variable to match the DSM-IV criteria with nine items. Those two items are: ‘Has trouble following through on instructions’ and ‘Completes schoolwork, chores, or duties’.

Table 3.2

The nine Inattention items

ADHD Inattentive criteria based on *DSM-IV*

1. Makes careless mistakes in schoolwork, work or other activities
 2. Has difficulty keeping attention on work or games
 3. Listens when spoken to directly
 - 4.a Has trouble following through on instructions
 - 4.b Completes schoolwork, chores, or duties
 5. Has difficulty organising tasks or activities
 6. Avoids, dislikes or is reluctant to engage in tasks that require prolonged concentration (e.g. schoolwork or homework)
 7. Loses things needed for tasks or activities at home or school (e.g. pencils, toys, or tools)
 8. Is easily distracted by things happening around him/her (e.g. noise or people talking)
 9. Forgets things in day to day activities
-

Table 3.3

The six Hyperactive items.

ADHD Hyperactive criteria based on *DSM-IV*

1. Fidgets with hands or feet or squirms in seat
 2. Finds it hard to stay seated in the classroom or other situations in which sitting is expected
 3. Runs around or climbs on things in situations where this is inappropriate
 4. Has difficulty playing or engaging in leisure activities quietly
 5. Is always 'on the go' or acts as if 'driven by a motor'
 6. Talks excessively
-

Table 3.4

The three Impulsive items.

ADHD Impulsive criteria based on *DSM-IV*

1. Blurts out answers to questions before they have been completed
 2. Has difficulty awaiting his/her turn
 3. Interrupts or intrudes on others (e.g. butts into conversations or games)
-

The reliability of the nine Inattentive items and the nine Hyperactive-Impulsive items was examined. The function of reliability tests is to examine the properties of measurement scales and the items that constitute them. The Cronbach's Alpha model of internal consistency was used to establish the reliability of items based on the average inter-item correlation. A Cronbach's Alpha of 0.80 or higher is considered acceptable. Both the nine Inattentive items and the nine Hyperactive-Impulsive items were found to be consistent as Cronbach's Alpha for the Inattentive items was 0.857 and for the Hyperactive-Impulsive items was 0.825, indicating that the items were a reliable measure of the presence of Inattentive symptoms and Hyperactive-Impulsive symptoms (Table 3.5 and Table 3.6).

Table 3.5
The reliability test for the nine Inattention items

The nine Inattention items	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
I1	0.616	0.838
I2	0.619	0.838
I3	0.249	0.878
I4	0.613	0.840
I5	0.666	0.833
I6	0.665	0.833
I7	0.624	0.837
I8	0.669	0.832
I9	0.596	0.841

Table 3.6
The reliability test for the nine Hyperactive-Impulsive items

The nine Hyp.-Imp. items	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
H1	0.563	0.803
H2	0.580	0.805
H3	0.521	0.811
H4	0.549	0.808
H5	0.414	0.828
H6	0.566	0.804
Im1	0.538	0.806
Im2	0.567	0.803
Im3	0.607	0.798

The identification of participants was based on *DSM-IV* ADHD diagnostic criteria. As known there are nine items for the Inattentive subtype (Table 3.2) and nine items for the Hyperactive-Impulsive subtype (Table 3.3 and Table 3.4). In order for a child to be diagnosed with Inattentive or Hyperactive-Impulsive ADHD, he or she should have six or more symptoms out of nine for either subtype respectively. For a child to be diagnosed with the Combined subtype, he or she should have six or more Inattentive symptoms, and six or more Hyperactive-Impulsive symptoms. To rate a twin or sibling in this study for *DSM-IV* ADHD symptoms, the Twin and Sibling Questionnaire used a 4-point scale coded as shown in Table 3.7. This method of establishing symptom endorsement is a valid

way of identifying children with subtypes of ADHD (Levy & Hay, 2001). A parent rating of 0 or 1 means that symptoms were absent, and a parent rating of 2 or 3 means that symptoms were present.

Table 3.7
The Four-Point Scale used by the Twin and Sibling Questionnaire

0=Not at all				
1=Just a little/Sometimes	Twin A	Twin B	Sibling 1	Sibling 2
2=Pretty much/Often				
3=Very much/Very Often	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3

Six or more out of nine responses for Inattention or Hyperactivity-Impulsivity were used as a cut-off to indicate if a child had either subtype. In addition, if a child had a cut-off score of six or more of the Inattentive symptoms and six or more Hyperactive-Impulsive, this child was diagnosed as having the Combined subtype.

In addition, the Inattentive and Hyperactive-Impulsive scores were calculated based on the sum of nine scaled items for Inattention and the sum of nine scaled items for Hyperactivity-Impulsivity, which gave a maximum score of 27 for each subtype, whereas the scores of the Combined subtype were calculated based on the sum of nine scaled items for both Inattention and Hyperactivity-Impulsivity, giving a maximum score of 54.

3.4.4 Reading Disability Measure

The Twin and Sibling Questionnaire contains seven items for measuring Reading Disability (RD). These seven RD items were originally created and designed by Erik Willcutt and his colleagues in the 'Learning and Behavior Questionnaire' for the Colorado Learning Disabilities Research Center (CLDRC) (Willcutt et al., 2003) (Table 3.8). In 2003 they tested the internal and external validity and reliability of the seven items, which showed high validity and reliability. The inter-rater reliability was 0.82 and the scale's alpha was 0.89 when tested by factor analysis and rated by three different groups: the child's neuropsychology clinic, the fathers and the mothers of twins. In addition, the correlations of reading ratings were 0.65, 0.61, 0.61 and 0.71 when tested

with the Peabody Individual Achievement Tests (PIAT) for Reading Recognition, Reading Comprehension, and Spelling and Reading Composite scores respectively. Those seven reading items showed correlations of 0.65, 0.65, and 0.42 with Woodcock-Johnson Letter Word Identification, Gray Oral Reading Test Passage, and Gray Oral Reading Test Comprehension scores respectively.

Table 3.8

The Reading Disability Items (Willcutt, Boada et al., 2003)

Reading Disability items
1. Does this child have difficulty with spelling
2. Did this child have difficulty learning letter names
3. Did this child have difficulty learning phonics (sounding out words)
4. Does this child read more slowly than other children of the same age
5. Does this child read below grade or expectancy level
6. Did this child have difficulty learning the days of the week or the months of the year
7. Has this child required extra help in school because of problems in reading or spelling

Willcutt et al. (under review) reassessed the validity and reliability of the seven reading items and found that only six loaded strongly on the first rotated factor when exploratory and confirmatory factor analyses were performed on four different groups. One item, ‘Difficulty learning days and months’, exhibited a weak loading in all four samples. As a result, Willcutt (2007) dropped this item from the other six, as it demonstrated lower inter-rater and test-retest reliability. The composite score for the other six items offered a continuous measure of early reading development, ranging from 6 to 30.

The internal consistency represented by Cronbach’s alpha showed a high alpha ranging from 0.89 to 0.94 of the six items’ composite score. The analyses also showed high correlations for inter-rater ($r=0.84$) and test-retest ($r=0.82$) reliability. The Learning and Behaviour Questionnaire’s (LBQ) results also exhibited significant correlations of single word reading measure ($r=0.61-0.71$), reading fluency ($r=0.41-0.55$), and reading comprehension ($r=0.42-0.58$). As expected, the correlations testing discriminate validity

were low ranging from 0.15-0.40 with intelligence, short and long term verbal memory, motor functioning, and math achievement.

Willcutt (2007) reported that the reading achievement score discriminated individuals with Reading Disability and without Reading Disability, based on a categorical cut-off score.

The assessment of participants with Reading Disability in this study was based on the earlier version with seven RD items (Willcutt, Boada et al., 2003), which was used from 2004 to 2006. Because the latest version that contains six RD items (Willcutt) was published only recently, both ATAP and this study had already completed the assessment of Reading Disability based on the 2003 version. Therefore, all of the analyses of RD were not based on the current version of the LBQ, which uses six RD items.

As this study used the seven RD items instead of six for identifying individuals with Reading Disability, reliability was examined on the study sample of 7209 individuals, for both seven and six RD item scales. Both the seven and six RD items were found to be consistent (Table 3.9 and Table 3.10). However, the Cronbach's Alpha of internal consistency for the seven RD items was higher (0.930) than the Cronbach's Alpha for the six RD items (0.928). Table 3.9 shows the seven RD items have high internal consistency with Cronbach's Alpha ranging from 0.913 to 0.928 and high inter-item correlations ranging from 0.688 to 0.840, whereas Table 3.10 showed the six RD items have internal consistency with Cronbach's Alpha ranged from 0.907 to 0.926 and high inter-item correlations ranged from 0.709 to 0.848.

Table 3.9
The reliability test for the seven RD items

The seven RD items	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
RD1	0.731	0.925
RD2	0.739	0.924
RD3	0.816	0.915
RD4	0.840	0.913
RD5	0.829	0.914
RD6	0.688	0.928
RD7	0.836	0.913

Table 3.10
The reliability test for the six RD items

The six RD items	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
RD1	0.737	0.924
RD2	0.709	0.926
RD3	0.811	0.912
RD4	0.848	0.907
RD5	0.833	0.910
RD7	0.835	0.909

The scores on each RD item were added together to produce a total RD score for each twin and sibling. The seven RD items offered a continuous measure, giving a maximum score of 21. Any child who gained a score of seven or more was defined as 'RD affected', whereas any child had a score of less than 7 was defined as 'RD unaffected'.

3.5 Procedure

The fourth wave of the Australian Twin ADHD project (ATAP) including this study, obtained ethics approval from both the Human Research Ethics Committee (HREC) of Curtin University of Technology and from the ethics committee of the Australian Twin Registry (ATR). Subsequently, participants were recruited by obtaining consent from

approximately 3500 Australian twin families nationwide, from 2004 to 2006. Each family was mailed a package containing an information sheet about the fourth-wave ATAP study, and a parent-report questionnaire entitled ‘Twin and Sibling Questionnaire’ (Hay & Levy, 2004) (See Appendix A.1). The completed questionnaire packages were mailed back to Curtin University in ‘Reply Paid’ envelopes. For privacy and confidentiality purposes, each returned questionnaire contained a detachable personal information sheet for each twin family; these contained the family name, address, contact details, and email. This sheet was detached from the questionnaire and kept in a secure cabinet, separate from the completed ATR-labelled questionnaire in order to ensure privacy and confidentiality.

CHAPTER 4: PREVALENCE AND TWIN-SIBLING DIFFERENCES FOR ADHD
AND READING DISABILITY

This chapter further investigates the prevalence of ADHD subtypes and Reading Disability (RD). It then examines the relationship between twins and siblings for prevalence, gender and age effects for ADHD and RD separately.

4.1 DSM-IV ADHD subtypes

4.1.1 Prevalence

All participants were recruited from the Australian Twin ADHD Project (ATAP) and consisted of 2610 twin families. Table 4.1 shows the prevalence of *DSM-IV*-defined ADHD subtypes in this study. Comparing these results with the *Child and Adolescent Component of the National Survey of Mental Health and Well-being: Mental Health of Young People in Australia* by Sawyer et al. (2000), it was found that the Inattentive subtype in both studies was the most prevalent; the Combined subtype is less prevalent and the least prevalent subtype was the Hyperactive-Impulsive. The total prevalence of ADHD in this sample was 4.9%.

Table 4.1
The frequencies and prevalence of DSM-IV ADHD subtypes

<i>DSM-IV</i> ADHD subtypes	Frequency	The current study prevalence	Australian National Mental Health Survey by Sawyer et al. (Sawyer et al., 2000)
No ADHD	6857	95.1 %	88.9 %
Inattentive	196	2.8 %	5.8 %
Hyperactive-Impulsive	71	1.0 %	2.0 %
Combined	85	1.1 %	3.3 %
Total	7209	100.0 %	100.0 %

4.1.2 Symptom overlap

Todd and his colleagues (Rasmussen, Neuman et al., 2002; Todd, Rasmussen et al., 2001) reported that using *DSM-IV* diagnostic criteria to diagnose children with a subtype of ADHD is unreliable, as there is an overlap between the nine Inattentive symptoms with

the nine Hyperactive-Impulsive symptoms. This section will show the nature and implications of this overlap in identifying individuals with ADHD subtypes.

Table 4.2 shows the frequencies of Hyperactive-Impulsive symptoms (0 to 5) in Inattentive individuals based on six cut-off symptoms and Table 4.3 shows the frequencies of Inattention symptoms (0 to 5) in Hyperactive-Impulsive individuals, based on six cut-off symptoms.

Table 4.2
The distribution of Hyperactive-Impulsive symptoms in Inattentive individuals

Inattentive symptoms in Inattentive individuals	Frequency	%
6	79	40.3
7	68	34.7
8	36	18.4
9	13	6.6
Total	196	100.0

Hyperactive Impulsive symptoms in Inattentive individuals	Frequency	%
0	29	14.8
1	48	24.5
2	27	13.8
3	35	17.9
4	30	15.3
5	27	13.8
Total	196	100.0

As can be seen from both tables, 15.3% and 13.8% of Inattentive individuals had four and five Hyperactive-Impulsive symptoms respectively while 19.7% and 31% of Hyperactive-Impulsive individuals had four and five Inattention symptoms respectively. This overlap of Hyperactive-Impulsive symptoms in Inattentive individuals and vice-versa can cause uncertainty in diagnosing ADHD individuals based on *DSM-IV*'s strict cut-off approach, causing potential problems for genetic analyses. Moreover, there is a real difference between the overlap of Hyperactive-Impulsive symptoms in Inattentive individuals, and the overlap of Inattentive symptoms in Hyperactive-Impulsive individuals. With the latter, many individuals may have developed Combined ADHD, whereas with the former, either subtype may have become dominant.

Table 4.3

The distribution of Inattentive symptoms in Hyperactive-Impulsive individuals

Hyperactivity-Impulsive symptoms in Hyperactive-Impulsive individuals		Frequency	%
6		40	56.3
7		17	23.9
8		11	15.5
9		3	4.2
Total		71	100.0

Inattentive symptoms in Hyperactive-Impulsive individuals		Frequency	%
0		6	8.5
1		6	8.5
2		10	14.1
3		13	18.3
4		14	19.7
5		22	31.0
Total		71	100.0

4.1.3 Gender

Table 4.4 shows the gender prevalence among *DSM-IV* ADHD subtypes. The prevalence of boys in all ADHD subtypes is higher than in girls by more than twofold. The total gender prevalence of boys-girls ratio among ADHD subtypes is 7.1%: 2.5%.

Table 4.4

Total number of Males and Females among DSM-IV ADHD subtypes

DSM-IV ADHD subtypes	Gender		
	Male	Female	Total
Unaffected	3419 (92.9 %)	3438 (97.4 %)	6857 (95.1 %)
Inattentive	146 (4.0 %)	50 (1.4 %)	196 (2.7 %)
Hyp-Imp	49 (1.3 %)	22 (0.6 %)	71 (1.0 %)
Combined	67 (1.8 %)	18 (0.5 %)	85 (1.2 %)
Total	3681 (100 %)	3528 (100 %)	7209 (100 %)

Table 4.5 shows the means and standard deviations of males and females, based on Inattentive and Hyperactive-Impulsive scores.

Table 4.5
Total number of Males and Females among DSM-IV ADHD categories

DSM-IV ADHD scores	Sex				Total 7209 (100%)
	Male N = 3681 (51.1%)		Female N = 3528 (48.9%)		
Inattentive	M = 6.40	StdD = 4.82	M = 4.64	StdD = 3.91	M = 5.54 StdD=4.49
Hyp.-Imp	M = 4.52	StdD = 4.45	M = 3.38	StdD = 3.46	M = 3.96 StdD=4.03
Combined	M = 10.93	StdD = 8.36	M = 8.02	StdD = 6.60	M = 9.50 StdD=7.69

M = Mean, StdD = Standard Deviation, N = Number, Hyp-Imp = Hyperactive-Impulsive

4.1.4 Age

The age differences among the ADHD subtypes showed that the Hyperactive-Impulsive subtype was the youngest age group (Table 4.6). Both unaffected individuals and those diagnosed with the Inattentive subtype were older than those with Combined ADHD.

Table 4.6
Descriptive of Age among DSM-IV ADHD subtypes

DSM-IV ADHD subtypes	N	M	Std.D
Unaffected	6857	12.9	3.4
Inattentive	196	13.2	2.9
Hyp-Imp	71	11.3	3.2
Combined	85	12.1	3.6
Total	7209	12.9	3.4

N = Number M = Mean, StdD = Standard Deviation

Barkley (Barkley, 1997a) stated that the symptoms of the Hyperactive-Impulsive subtype appeared earlier (3-4 years old) than the symptoms of the Inattentive subtype, which started at school age (5-7 years old). It was found that the Hyperactive-Impulsive symptoms gradually decrease with age and development. Hay et al (2001) believed that the presence of common-environment factors such as family, school, or medication intervention may eventually influence the symptoms of the Hyperactive-Impulsive ADHD. Cohen et al. (1993) stated that ADHD is less prevalent in the younger age groups, with the exception of the Inattentive subtype in females, which can increase as they get older (Levy et al., 2005).

The one-way analysis of variance showed significant age differences between the ADHD subtypes ($F(3, 265.179) = 7.702, p < 0.05$). According to the Scheffe homogeneity test (Table 4.8), group 1 showed no age significant difference of the individuals with the Combined subtype and Hyperactive-Impulsive subtype. Similarly, group 2 showed no age significant difference of the Combined subtype (homogenous) with the Inattentive subtype as well as with unaffected individuals. However, the age of group 1 was heterogeneous with group 2, demonstrating a significant difference between them (Table 4.7).

Table 4.7
Scheffe homogeneity test between age groups between ADHD subtypes

	DSM-IV Strict Diagnosis	N	Subset for alpha = .050	
			Group 1	Group 2
Scheffe Test	Hyp.-Imp.	71	11.31	
	Combined	85	12.06	12.06
	Unaffected	6857		12.95
	Inattentive	196		13.18
P- value			0.375	0.07

N = Number

4.2 Reading Disability

4.2.1 Prevalence

In order for an individual to meet the criteria for Reading Disability based on *DSM-IV* criteria, he or she must demonstrate reading achievement that falls significantly below that which would be expected given their chronological age, and age-appropriate education. This study used the seven RD items (Chapter 3; Table 3.6) (Willcutt, Boada et al., 2003) to identify individuals whose reading achievement is not in the standard level. The assessment of individuals with RD was based on continuous criteria. The seven parents' reported symptoms were based on a scale from 0 to 3. The seven scaled items gave a maximum score of 21. In order for an individual to be identified with RD, he or she should have a cut-off score of at least 7. Based on this criterion, the prevalence of individuals identified with RD was 14.8% (Table 4.8).

Table 4.8
The frequencies and prevalence of Reading Disability

Reading Disability	Frequency	%
No RD (-)	6145	85.2
Yes RD (+)	1064	14.8
Total	7209	100

4.2.2 Gender

Table 4.9 shows the prevalence of males and females with RD among the unaffected and affected individuals. RD is more prevalent in males than in females.

Table 4.9
Total number of males and females by Reading Disability

RD affected status	Sex		Total
	Male	Female	
No RD	N = 3012 (81.8%)	N = 3133 (88.8 %)	6145 (85.2%)
Yes RD	N = 669 (18.2%)	N = 395 (11.2%)	1064 (14.8%)
Total	N = 3681 (100%)	N = 3528 (100%)	7209 (100%)

N = Number, RD= Reading Disability

In addition, Table 4.10 shows the means and standard deviations of Reading Disability score among males and females.

Table 4.10
The score mean of Reading Disability among male and females

RD affected status	Sex				Total
	Male		Female		
RD score	M = 3.31	StdD = 4.975	M = 2.23	StdD = 3.964	M = 2.78 StdD = 4.450

M = Mean, StdD = Standard Deviation, RD = Reading Disability

4.2.3 Age

The mean age among ‘No RD’ group (n= 6154) was 13.03 years old +/- 3.41, whereas among ‘Yes RD’ group (n= 1064) it was 12.42 years old +/- 3.28 years. The F- test showed significant differences between ‘No’ and ‘Yes’ RD groups ($F(1, 325.276) = 18.261$; $p < 0.01$). The Mann-Whitney test revealed age significant differences with Reading Disability scores (Table 4.11).

Table 4.11
Age differences among unaffected and affected Reading Disability individuals

Tset	RD scores with age
Mann-Whitney U	2868241.000
P-value	0.000

4.3 Twin-sibling differences

As this is a genetic study performed on Twin families, it is important to describe the Monozygotic twins (MZ), Dizygotic twins (DZ), and their siblings. Prior to examining twin-sibling differences among ADHD and RD, the next two tables will describe the gender prevalence and age means among MZ, DZ, Sibling1, and Sibling2 in the whole sample. Table 4.12 shows that the prevalence of twins and siblings is almost equal.

However, the data between participants is not independent: twins are also siblings. So when using descriptive statistics one must be cautious in analysing results.

Table 4.12

Gender among twins and siblings

Child Relationship	Gender		Total
	Male	Female	
MZ	1123 (30.5%)	1137 (32.2%)	2260 (31.3%)
DZ	1503 (40.8%)	1407 (39.9%)	2910 (40.4%)
Sibling 1	835 (22.7%)	774 (21.9%)	1609 (22.3%)
Sibling 2	220 (6.0%)	210 (6.0%)	430 (6.0%)
Total	3681 (100%)	3528 (100%)	7209 (100%)

The age range of the sample was from 4 years to 18 years old, with an average age of 12.9 years +/- 3.39 years. Table 4.13 shows the means of ages of MZ and DZ twins, which is similar, whereas the oldest mean age was found for sibling1.

Table 4.13

The means of ages of twins and siblings

Child Relationship	Age Mean	Standard Deviation	Range
MZ	12.70	3.25	13.86
DZ	12.60	3.31	13.81
Sibling 1	14.04	3.45	14.55
Sibling 2	12.33	3.57	14.38

4.3.1 ADHD Twin-sibling differences

4.3.1.1 Prevalence

Table 4.14 shows the prevalence of ADHD subtypes in MZ and DZ twins, sibling 1 and sibling 2. Prevalence appears roughly equivalent across all four groups.

Table 4.14

Total number of MZ, DZ twins and siblings among DSM-IV ADHD subtypes

DSM-IV ADHD Diagnostic criteria	Child Relationship				Total
	MZ	DZ	Sib1	Sib2	
Unaffected	2164 (95.8%)	2752 (94.6%)	1531 (95.2%)	410 (95.3%)	6857 (95.1%)
Inattentive	50 (2.2%)	90 (3.1%)	46 (2.9%)	10 (2.3%)	196 (2.7%)
Hyp.-Imp.	22 (1.0%)	32 (1.1%)	14 (0.9%)	3 (0.7%)	71 (1.0%)
Combined	24 (1.1%)	36 (1.2%)	18 (1.1%)	7 (1.6%)	85 (1.2%)
Total	2260 (100%)	2910 (100%)	1609 (100%)	430 (100%)	7209 (100%)

MZ = Monozygote, DZ= Dizygote, Sib1= Sibling 1, Sib2= Sibling 2, Hyp-Imp= Hyperactivity-Impulsivity

Table 4.15 demonstrates the distribution of the three ADHD subtypes among twins and siblings. The distributions of all ADHD diagnostic criteria were similar between twins and siblings. The Pearson Chi-Square test showed no significant difference ($\chi^2 = 1.160$, d.f=3, $P > 0.05$) of ADHD distribution among twins and siblings.

Table 4.15

The distribution of DSM-IV ADHD subtypes among twins and siblings

DSM-IV ADHD subtypes	Twin or sibling		Total
	Twins	Siblings	
Unaffected	4916 (95%)	1941 (95.2%)	6857 (95.1%)
Inattentive	140 (2.7%)	56 (2.7%)	196 (2.7%)
Hyperactive- Impulsive	54 (1.1%)	17 (0.8%)	71 (1.0%)
Combined	60 (1.2%)	25 (1.2%)	85 (1.2%)
Total	5170 (100%)	2039 (100%)	7209 (100%)

Moreover, Table 4.16 shows the mean scores of Inattentive and Hyperactive-Impulsive symptoms among MZ, DZ, sibling 1 and sibling 2 individuals. It was expected that mean scores of ADHD subtypes in both twins and siblings would be significantly low, as the majority of individuals in the sample (95.1%) are classed as ‘unaffected’ on both scales.

Table 4.16

The mean scores for Inattentive and Hyperactive-Impulsive symptoms for all twins and siblings

<i>DSM-IV</i> ADHD subtype scores	Child Relationship	N	Mean	Std. Deviation
<i>DSM-IV</i> Inattentive Score	MZ	2260	5.37	4.284
	DZ	2910	5.76	4.557
	Sibling 1	1609	5.35	4.543
	Sibling 2	430	5.60	4.849
<i>DSM-IV</i> Hyp.Imp- Score	MZ	2260	4.00	3.896
	DZ	2910	4.15	4.187
	Sibling 1	1609	3.57	3.813
	Sibling 2	430	3.99	4.373

M = Mean, StdD = Standard Deviation, N = Number, Hyp-Imp = Hyperactive-Impulsive

Because the test of normality showed that the data were not normally distributed and assumption testing showed the data had unequal variances, I treated the data as non-parametric. In order to examine whether the mean scores of Inattention and Hyperactivity-Impulsivity were significantly different between all MZ and all DZ twins and also between all siblings 1 and all siblings 2, a Mann-Whitney test was performed for assessing whether all MZ with DZ twins and all sibling 1 with sibling 2 individuals come from the same distribution. It is one of the best-known non-parametric significance tests (Field, 2000). The Mann-Whitney U test works by looking at the differences in the ranked positions of the Inattentive and Hyperactive-Impulsive scores between MZ and DZ groups and between sibling 1 and sibling 2 groups.

Table 4.17

The results of Mann-Whitney U-test on MZ versus DZ twins and sibling 1 versus sibling 2 individuals on Inattentive and Hyperactive-Impulsive scores

Test	Inattentive Score MZ vs. DZ	Inattentive Score sib1 vs. sib2	Hyp-Imp Score MZvs. DZ	Hyp-Imp Score sib1 vs. sib2
Mann-Whitney U	3136147.500	336892.000	3282217.000	330352.500
P - value	0.003	0.538	0.848	0.218

Mann-Whitney U- test results (Table 4.17) demonstrated significant differences between MZ and DZ twins on the Inattentive scores, whereas the results of the sibling 1 versus sibling 2 individuals on Inattentive scores and the MZ versus DZ twins and sibling 1

versus sibling 2 individuals on the Hyperactivity-Impulsivity scores did not show significant differences.

Table 4.18

The results of a Mann-Whitney U-test on twins versus siblings on Inattentive and Hyperactive-Impulsive scores

Test	DSMIV Inattentive Score twins versus siblings	DSMIV Hyp-Imp Score twins versus siblings
Mann-Whitney U	4805901.000	4607113.500
P - value	0.112	0.000

The same test when applied to the Inattention and Hyperactive-Impulsive scores of twins versus siblings gave contrasting results. The Inattentive scores of twin versus sibling group showed no significant difference, whereas the Hyperactive-Impulsive scores of twins versus siblings showed significant differences (Table 4.18). Hay et al. (2001) stated that Hyperactive-Impulsive symptoms gradually decrease with increasing age and development. Therefore, this could be the reason for a significant difference between twins and sibling as, siblings were older than twins.

4.3.1.2 Gender

Table 4.19 shows the means of the Inattentive and Hyperactive-Impulsive scores between genders with twin-sibling individuals represented by four variables: ‘Male Twins’, ‘Female Twins’, ‘Male Siblings’, and ‘Female Siblings’.

Table 4.19

The means of the Inattentive and Hyperactive-Impulsivity scores between gender with twin-sibling individuals

Child Relationship		DSM-IV Inattentive Score	DSM-IV Hyp-Imp Score
Male twins	M	6.46	4.70
	N	2628	2628
	StdD	4.770	4.459
Female twins	M	4.69	3.45
	N	2545	2545
	StdD	3.882	3.496
Male Siblings	M	6.26	4.09
	N	1053	1053
	StdD	4.953	4.382
Female Siblings	M	4.49	3.21
	N	983	983
	StdD	4.014	3.346
Total	M	5.54	3.96
	N	7209	7209
	StdD	4.491	2.706

M = Mean, StdD = Standard Deviation, N = Number, Hyp-Imp = Hyperactive-Impulsive

In order to examine whether there was a statistically significant difference among gender with twin-sibling families, the Kruskal-Wallis test was applied. This test works by looking at the differences in the ranked positions of the Inattentive and Hyperactive-Impulsivity scores between the four above groups. The Kruskal Wallis test showed significant differences between gender with twins and siblings in both means of the Inattentive and Hyperactive-Impulsive subtypes (Table 4.20). These results may indicate that gender has a large effect in causing these differences.

Table 4.20

Results of Kruskal Wallis Test on the means of the Inattention and Hyperactive-Impulsive scores between gender with twin-sibling individuals

Kruskal Wallis Test	<i>DSM-IV</i> Inattentive Score between gender with twin-sibling	<i>DSM-IV</i> Hyp-Imp Score between gender with twin-sibling
Chi-Square	282.654	149.808
df	3	3
P- value	0.000	0.000

df= degree of freedom

Because the test produced an unequal mean rank for each group in both Inattentive and Hyperactive-Impulsive, a one-way ANOVA test of homogeneity of variances (Levene's test) was performed, and results showed a statistically significant difference between both scores ($p < 0.05$), indicating that both scores had unequal variances. The ANOVA tables indicated significant differences for gender with twin-sibling groups for both Inattentive ($F(3, 1885.25) = 97.21, p < 0.05$) and Hyperactive-Impulsive scores ($F(3, 895.18) = 56.32, P < 0.05$). Those unequal variances bias the F-ratio to be conservative, as the homogeneity of variances was violated. Therefore, the robust tests of equality of means were represented by the robust Welch and Brown-Forsythe tests. These tests were performed to overcome this problem by weighting the group variances, not testing them by their sample size. The results were the same as that of the one-way ANOVA test (both tests have significance values of less than 0.05).

However, it is still not known what the effects of gender with twin-sibling groups are, and which groups are different; therefore, the post hoc tests by the Games-Howell test (because variances were unequal) was applied. According to Table 4.21, the post hoc comparison results revealed significant differences between the groups ($p < 0.05$) for all tests, except between the male twin group with the male sibling group for the Inattentive scores, and between the female twin group with the female sibling group for both the Inattentive and Hyperactive-Impulsive scores.

Table 4.21

The post hoc multiple comparison tests on gender with twin-sibling groups (Games-Howell) by Inattentive and Hyperactive-Impulsive scores

Multiple comparisons by <i>DSM IV</i> Inattentive score and by <i>DSM IV</i> Hyp-Imp score			
Tested group	gender with twin-sibling groups	<i>p</i> -values for Inattention	<i>p</i> -values for Hyp-Imp
Male twin	Female twin	0.000	0.000
	Male sibling	0.69	0.001
	Female sibling	0.000	0.000
Female twin	Male twin	0.000	0.000
	Male sibling	0.000	0.000
	Female sibling	0.53	0.227
Male sibling	Male twin	0.69	0.001
	Female twin	0.000	0.000
	Female sibling	0.000	0.000
Female sibling	Male twin	0.000	0.000
	Female twin	0.53	0.23
	Male sibling	0.000	0.000

4.3.1.3 Age

Based on Table 4.13, an independent sample T-test was conducted to reveal age differences between MZ versus DZ twins, sibling1 versus sibling2, and twins versus siblings. Results showed no significant difference between MZ and DZ: $t(5145) = 0.881$, $p > 0.05$; whereas there was such a difference between sibling1 versus sibling2: $t(1995) = 8.942$, $p < 0.01$, and also between twins versus siblings: $t(7142) = -11.352$, $p < 0.01$.

Consequently, a non-parametric correlation test was performed based on Spearman's test to find out if the ages of twins and siblings were related to the Inattentive and Hyperactive-impulsive scores. Table 4.22 shows there was a significantly low correlation between age and Inattentive scores, indicating there is no relationship between age and Inattention. The correlation between age and Hyperactive-Impulsive scores was significant, indicating a relationship between age and this ADHD subtype.

Table 4.22

Non-parametric correlation tests between age and Inattentive scores and age and Hyperactive-Impulsive scores for MZ versus DZ twins and sibling1 versus sibling 2 individuals

<i>DSM-IV ADHD subtype scores</i>	<i>MZ and DZ</i>	<i>Sibling 1 and Sibling 2</i>
<i>Inattentive scores</i>	<i>-0.046</i>	<i>-0.099</i>
<i>Hyperactivity-Impulsive scores</i>	<i>-0.202</i>	<i>-0.267</i>

4.3.2 RD twin-sibling differences

4.3.2.1 Prevalence

Table 4.23 gives statistical information about the prevalence of MZ and DZ twins and Siblings 1 and 2 among the ‘No’ and ‘Yes’ categories of RD. In addition, Table 4.24 demonstrates the distribution of RD status among twins and siblings, and showed significant difference ($\chi^2= 13.558$, d.f=1, $p < 0.05$) of RD distribution among twins and siblings.

Table 4.23

Total number of a RD among MZ, DZ twins and their siblings

<i>RD affected status</i>	<i>Child Relationship</i>				<i>Total</i>
	<i>MZ</i>	<i>DZ</i>	<i>Sibling 1</i>	<i>Sibling 2</i>	
<i>No RD</i>	<i>1930 (85.4%)</i>	<i>2427 (83.4%)</i>	<i>1428 (88.8%)</i>	<i>360 (83.7%)</i>	<i>6145 (85.2%)</i>
<i>Yes RD</i>	<i>330 (14.6%)</i>	<i>483 (16.6%)</i>	<i>181 (11.2%)</i>	<i>70 (16.3%)</i>	<i>1064 (14.8%)</i>
<i>Total</i>	<i>2260 (100%)</i>	<i>2910 (100%)</i>	<i>1609 (100%)</i>	<i>430 (100%)</i>	<i>7209 (100.0%)</i>

Table 4.24
The distribution of RD among twins and siblings

RD affected status	Twin or sibling		Total
	Twin	Sibling	
No RD	4357 (84.3%)	1788 (87.7%)	6145 (85.2%)
Yes RD	813 (15.7%)	251 (12.3%)	1064 (14.8%)
Total	5170 (100%)	2039 (100%)	7209 (100%)

As mentioned earlier, the data are not normally distributed. This also includes the RD status variable, which was expected as the number of unaffected individuals was considerably higher than that of affected individuals. Therefore, all of the following twin-sibling statistical tests were performed using non-parametric tests, in order to reveal the statistical differences between MZ and DZ twins, between sibling 1 and sibling 2 individuals, and between twins and siblings. For this purpose the Mann-Whitney U-test was utilised. It looked for the differences of RD scores between all MZ and all DZ groups and between all sibling 1 and all sibling 2 groups, and all twins versus siblings (Table 25).

Table 4.25
The results of Mann-Whitney U test on twins versus siblings for RD scores

Test	RD Score		
	RD Score between MZ and DZ twins	RD Score between sib1 and sib2	RD Score between twins and sibs
Mann-Whitney U	3203242.000	319257.500	4768705.000
P- value	0.101	0.017	0.000

RD= Reading Disability, MZ = Monozygote, DZ = Dizygote, Sib1= Sibling 1, Sib2= Sibling 2

The results of RD scores showed no significant differences between MZ and DZ twins, whereas the results for sibling 1 versus sibling 2 individuals, and twins versus siblings showed significant differences.

4.3.2.2 Gender

In addition, Table 4.26 shows the score means of the unaffected and affected RD scores between genders with the four twin-sibling groups; male twins, female twins, male siblings, and female siblings. The Kruskal-Wallis test was performed in turn to examine

whether there is statistical significant difference between gender with twin-sibling groups, by looking at the differences in the ranked positions of the RD-affected status scores between the four groups

Table 4.26
The score means of *Reading Disability among gender with twin-sibling individuals*

Child Relationship	D.S.	RD Score
Male twin	M	3.50
	N	2628
	StdD	5.114
Female twin	M	2.35
	N	2545
	StdD	4.068
Male sibling	M	2.83
	N	1053
	StdD	4.577
Female sibling	M	1.92
	N	983
	StdD	3.663

M = Mean, StdD = Standard Deviation, N = Number, RD= Reading Disability

The Kruskal Wallis test showed significant differences between gender with twins and siblings by the mean scores RD (Table 4.27), which might imply that gender has a major effect in causing these differences. The one-way ANOVA test of homogeneity of variances (Levene's test) showed a statistically significant difference ($p < 0.05$), indicating that RD and gender with the twin-sibling variable have unequal variances.

Table 4.27
Results of Kruskal Wallis Test on the mean score of Reading Disability between gender

Kruskal Wallis Test	Reading Disability Score
Chi-Square	125.741
df	3
P- value	0.000

df= degree of freedom

The ANOVA table indicated significant differences of gender with twin-sibling groups by RD ($F(3, 856.679) = 42.272, p < 0.05$). Tests of equality on the means of RD and gender with twin-sibling variable were performed (Welch and Brown-Forsythe tests) to avoid the problem of the violation of homogeneity of variances. Both Welch and Brown-Forsythe results showed same results as the one-way ANOVA test (both tests have significance values less than 0.05), indicating significant difference of RD scores between gender on the twin-sibling variable. In addition, the post hoc (Games-Howell) test showed significant differences between the gender twin-sibling groups for all tests ($p < 0.05$) (Table 4.28).

Table 4.28
The post hoc multiple comparison test on gender with twin-sibling groups (Games-Howell) by Reading Disability scores

Multiple comparisons by RD		
Tested group	gender with twin-sibling groups	<i>p</i> -values for RD
Male twin	Female twin	0.000
	Male sibling	0.001
	Female sibling	0.000
Female twin	Male twin	0.000
	Male sibling	0.016
	Female sibling	0.015
Male sibling	Male twin	0.001
	Female twin	0.016
	Female sibling	0.000
Female sibling	Male twin	0.000
	Female twin	0.015
	Male sibling	0.000

4.3.2.3 Age

The non-parametric correlation test was performed based on Spearman's test to find out if age of twins and age of siblings are correlated with RD scores. Table 4.29 showed low correlations between age of twins and age of siblings with RD scores.

Table 4.29

Non-parametric correlation tests between age and Reading Disability scores for MZ and DZ twins and sibling 1 and sibling 2 individuals

Spearman correlation	MZ and DZ	Sibling 1 and Sibling 2
Reading Disability scores	0.099	0.055

4.3.3 The Prevalence of DSM-IV ADHD-RD comorbidity

As this study investigated the comorbidity of ADHD subtypes with RD, it is important to highlight RD prevalence within *DSM-IV* ADHD subtypes and also the ADHD subtypes' prevalence with Reading Disability. Table 4.30 showed the total comorbidity prevalence of the three *DSM-IV* ADHD subtypes within RD was 14.8%.

Table 4.30

The total number of the comorbidity between DSM-IV ADHD and Reading Disability

DSM-IV ADHD Subtypes	Reading Disability		Total
	RD -	RD +	
% within No <i>DSM-IV</i> ADHD	5977 87.2%	880 12.8%	6857 100%
% within Inattentive <i>DSM-IV</i> subtype	83 42.3%	113 57.7%	196 100%
% within Hyperactivity-Impulsivity <i>DSM-IV</i> ADHD subtypes	49 69%	22 31%	71 100%
% within Combined <i>DSM-IV</i> ADHD subtypes	36 42.4%	49 57.6%	85 100%
% within total <i>DSM-IV</i> ADHD subtypes	6145 85.2%	1064 14.8%	7209 100%

RD - = RD unaffected; RD+ = RD affected

Moreover, the highest comorbid *DSM-IV* ADHD subtype with RD was the Inattentive subtype (57.7%), followed by the Combined subtype (57.6%), whilst the Hyperactive-Impulsive was least comorbid with RD (31%).

4.3.3.2 The Prevalence of DSM-IV ADHD-RD comorbidity among twins and siblings

Table 4.31 shows the comparison between twins and siblings in terms of the prevalence of RD among the three *DSM-IV* ADHD subtypes.

Table 4.31

The prevalence of comorbidity between DSM-IV ADHD and Reading Disability among twins and siblings

<i>DSM-IV</i> ADHD subtypes	Reading Disability		Total
	RD -	RD +	
<u>Twins No ADHD</u>	4251	665	4916
% within <i>DSM-IV</i> ADHD subtypes	86.5%	13.5%	100%
<u>Siblings No ADHD</u>	1726	215	1941
% within <i>DSM-IV</i> ADHD subtypes	88.9%	11.1%	100%
<u>Twins Inattention</u>	53	87	140
% within <i>DSM-IV</i> ADHD subtypes	37.9%	62.1%	100%
<u>Siblings Inattention</u>	30	26	56
% within <i>DSM-IV</i> ADHD subtypes	53.6%	46.4%	100%
<u>Twins Hyperactivity-Impulsivity</u>	34	20	54
% within <i>DSM-IV</i> ADHD subtypes	63%	37%	100%
<u>Siblings Hyperactivity-Impulsivity</u>	15	2	17
% within <i>DSM-IV</i> ADHD subtypes	88.2%	11.8%	100%
<u>Twins Combined</u>	19	41	60
% within <i>DSM-IV</i> ADHD subtypes	31.7%	68.3%	100%
<u>Siblings Combined</u>	17	8	25
% within <i>DSM-IV</i> ADHD subtypes	68%	32%	100%
<u>Twins Total</u>	4357	813	5170
% within <i>DSM-IV</i> ADHD subtypes	84.3%	15.7%	100%
<u>Siblings Total</u>	1788	251	2039
% within <i>DSM-IV</i> ADHD subtypes	78.7%	12.3%	100%

RD - =RD unaffected; RD+ = RD affected

The total twin prevalence of affected RD among the three *DSM-IV* ADHD subtypes was (15.7%), higher than sibling prevalence (12.3%). The prevalence of the twins' Inattentive subtype comorbid with RD (62.1%) is higher than the prevalence of the siblings' Inattentive subtype comorbid with RD (46.4%). The prevalence of the twins' Hyperactive-Impulsive ADHD comorbid with RD (37%) is considerably higher than the siblings' (11.8%). This is expected as siblings were older than twins, whereas Hyperactivity-Impulsivity is usually impaired in young children. The prevalence of RD comorbid with the Combined subtype (68.3%) is higher than that for siblings (32%).

4.4 Conclusion

The 2610 twin families from ATAP gave a total of 7209 individuals, 31.3% MZ twins, 40.4% DZ twins, 22.3% sibling1, and 6% sibling 2. The prevalence of males was 51.1%

and the prevalence of females was 48.9%. Age in the total sample ranged from 4 to 18 years old, with an average age of 12.93 years old (sd= +/-3.39). The twin-sibling analyses showed age-significant differences between sibling 1 and sibling 2, and twins versus siblings, but this was not so between MZ and DZ twins.

For *DSM-IV* ADHD, the diagnostic criteria was based on cut-off six symptoms, giving 2.8% for the Inattentive subtype, 1.0% for the Hyperactive-Impulsive subtype, and 1.1% of the Combined subtype out of the total sample. The analysis found a 29.1% overlap for four and five symptoms of Hyperactive-Impulsive in the individuals identified as Inattentive. On the other hand, there was a 50.7% overlap for four and five symptoms of Inattention symptoms in individuals identified as Hyperactive-Impulsive. This indicates the uncertainty of identifying Hyperactive-Impulsive individuals based on *DSM-IV*'s strict cut-off approach. The twin-sibling difference analyses with ADHD revealed no Inattentive or Hyperactive-Impulsive significant differences between MZ and DZ twin with ADHD, and sibling 1 and sibling 2, and twins versus siblings. The only significant differences found were between MZ versus DZ for Inattentive ADHD, and twins versus siblings for the Hyperactive Impulsive ADHD. These results were also obtained for age differences between twins and siblings, as there was no relationship detected between age and ADHD subtype scores. Among ADHD subtype individuals, boys were more prevalent than females by more than two fold. This is consistent with past research. Sawyer et al's (2000), report showed this prevalence of ADHD in males (15.4%) was higher than females (6.8%). Levy, Hay, Bennett, and McStephen (2005) also found the male-female ratio of ADHD prevalence was higher in males for all subtypes: Inattentive = 9.9%: 4.2%, Hyperactive-Impulsive = 3.0% : 1.7%, Combined = 5.8% : 2.0%. Several studies attributed the over-representation of ADHD in males to rater or referral bias. Heptinstall and Taylor (2002), challenged these conclusions by declaring that if this were true, one would expect to see substantial differences between both girls and boys meeting ADHD diagnostic criteria; however, their research has shown that these expected differences do not exist. The same study went on to suggest that ADHD sex prevalence ratios may be affected by age; a hypothesis supported by Gaub and Carlson (1997), who proposed that age may also affect the course of the disorder in boys and girls.

There were also gender and twin-sibling differences among the Inattentive and Hyperactive Impulsive subtypes. The exceptions were between male twins with male siblings, and between female twins with female siblings for both Inattentive and Hyperactive-Impulsive scores. This leads to the conclusion that there are differences in gender, but there were no twin-sibling differences.

The prevalence of individuals identified with RD was 14.8%. Willcutt and Pennington (2000b) reported that the prevalence of Reading Disability in school-aged children ranged from 3-10%; however, Shaywitz & Shaywitz (2005) reported a prevalence of 5%-17.5%. The twin-sibling difference analyses with RD showed no significant RD differences between MZ and DZ twins, whereas there were significant differences between sibling 1 and sibling 2, and twins versus twins. Among RD-affected individuals, boys again were more prevalent than girls. The gender and twin-sibling differences among RD scores showed significant differences between all groups.

Flannery, Liederman, Daly and Schultz (2000) found that RD was significantly more prevalent in boys than in girls: the prevalence of males with RD identified was 9.3% compared to 5.5% of females. However, Willcutt and Pennington (2000b) found that RD was relatively equal between boys and girls (1.3: 1).

These authors argued that the reason for the presence of this inconsistency in RD gender ratios could be based on the kind of sample, whether it is a referred (e.g. RD male-female ratio of 3:1) or a population sample (e.g. RD male-female ratio of 1.5:1). The authors proposed two contrasting hypotheses that may account for the latter result. They proposed that since boys with RD are more likely to display increased externalizing behaviour such as behaving disruptively, parents and teachers may identify more boys than girls with Reading Disability requiring clinical attention. Conversely, as girls with RD predominantly show internalizing behaviour, parents and teachers may find it harder to detect RD symptoms in girls. The second and contrasting hypothesis proposed that parents and teachers may go to greater lengths to remedy reading difficulties in boys due to the greater emphasis placed on the intellectual achievement of males than females (Willcutt & Pennington, 2000b). The mean age of RD children was 12.42 years old. Age was

significantly different with RD scores; however, there were no age and twin or sibling relationships with RD scores.

The total prevalence of *DSM-IV* ADHD comorbid with RD was 17.3 %, whereas the prevalence of RD with both the Inattentive (57.7%) and Combined (57.6%) subtypes was higher than with the Hyperactive-Impulsive subtype (31%), indicating that the comorbidity of RD among ADHD subtypes was higher with both Inattentive and Combined subtypes. Willcutt et al. (2000) stated that RD comorbidity with a sample chosen for ADHD ranges from 25-40%; vice-versa, the prevalence of ADHD comorbidity for a sample chosen for RD ranges from 15-40%. As his study was designed to investigate the comorbidity of Reading Disability within an ADHD sample, the prevalence of RD comorbid with Inattentive and Combined subtypes was higher than (57.7% and 57.6%) Willcutt's range, except RD comorbid with the Hyperactive-Impulsive subtype, which was within the Willcutt's range. In addition, ADHD-RD comorbidity was higher in twins than in siblings.

As this is a genetic study, the statistical analyses have important and beneficial implications as they provide a means to analyse data and to draw genetic assumption. Most importantly, they also assist to evaluate the description of the data being used in the genetic analysis, to measure confidence in genetic assumption, and to make the genetic data more biologically meaningful.

CHAPTER 5: LATENT CLASS ANALYSIS

5.1 Introduction

The previous chapter showed how the diagnostic criteria of *DSM-IV* was ambiguous in classifying the Inattentive children as there were 29.1% with four or five Hyperactive-Impulsive symptoms although classified as Inattentive. Also, 50.7% of the individuals classified as Hyperactive-Impulsive with four or five Inattentive symptoms. Todd (2005) argued that an individual can have ten symptoms of *DSM-IV* ADHD but if five are Inattentive and five are Hyperactive-Impulsive, the individual is classified as unaffected. Another person with ten symptoms of ADHD, with six Inattentive and four Hyperactive-Impulsive, would be categorized as Inattentive while another individual with ten symptoms of ADHD, four Inattentive and six Hyperactive-Impulsive, would be categorized as Hyperactive-Impulsive.

Therefore, Todd and his collaborative research team demonstrated in several studies (Hudziak et al., 1998; Neuman et al., 1999; Rasmussen, Neuman et al., 2002; Rasmussen et al., 2004; Todd et al., 2005) how the Latent Class Analysis can overcome the above problem (classifying and identifying ADHD subtypes) in order to obtain genetically distinctive groups. This is because LCA has the ability to re-subtype ADHD (or any other psychiatric disorder) based on the clustering of symptoms in a general population sample (Todd et al, 2005).

LCA is an efficient tool for refining the ADHD subtypes and RD of related groups. It produces homogenous phenotypic classes, whereas the *DSM-IV* diagnostic criteria makes the selection of ADHD and RD arbitrary because *DSM-IV* can potentially classify a child who has some major symptoms of a particular *DSM-IV* ADHD subtype and of RD.

This study used Latent Class Analysis in order to refine the phenotypes of ADHD and RD to obtain informative phenotype latent classes for performing genetic association studies. This will help to investigate the genes that contribute to ADHD subtypes alone, to RD alone, and whether these genes are the same or different for the ADHD-RD comorbid subtype. LCA can identify natural clusters of comorbid symptoms (Volk et al., 2006). As this study aimed to investigate some of the genetic aspects of ADHD

alone, RD alone, and comorbid ADHD-RD, it first attempted to refine the phenotypes of the individual disorders by using LCA.

5.2 Methodology

The data for Latent Class Analysis were based on parents' responses about their offspring from the questionnaire using 18 *DSM-IV* defined ADHD items and seven RD items (Willcutt, Boada et al., 2003). Participants' descriptions and the measures for *DSM-IV* ADHD and for the RD items were previously described in Chapter three.

5.2.1 Implementation of Latent Class Analysis

The latent class analysis was applied in this study by the Latent Class Analysis Program (LCAP) Version 2.34 (Neuman et al., 1999). LCAP is a computer software application that uses a statistical methodology to investigate an observed association among a group of discrete variables. Accordingly, the form of data used in LCA is often categorical, resulting in identifying distinct diagnostic subtypes that can then be used as a classification tool. The maximum likelihood algorithm (EM algorithms) was utilised (Dempster, Laird, & Rubin, 1977) in the Latent Class Analysis Program (LCAP).

As the parents' endorsement of the *DSM-IV* ADHD and RD items (Willcutt, Boada et al., 2003) for their children was based on a 4-point scale, this data was implemented in LCAP in a categorical form; 'zero' and 'one' coded as 'zero' meaning "unaffected", and 'two' and 'three' were coded as 'one' meaning 'affected'. This categorical data was fitted to latent class solutions from one to sixteen in LCAP (Neuman et al., 1999) by a maximum likelihood algorithm (EM algorithm) (Dempster et al., 1977). In addition, three elements were considered for estimating the best fitting model: a Bayesian Information Criterion (BIC), the likelihood ratio chi square (χ^2), and class membership criteria stability. As the number of those 16 classes increased (1 to 16), the chi square goodness-of-fit gradually improved which can be indicated by a decline in the BIC. The lowest BIC among the 16 latent classes, the best-fitting solution was selected as the best representative of the sample.

The subtypes of the best latent class solutions were marked by a line chart based on the pattern of symptom endorsements, which represented probabilities for each class in the LCA output file. The line chart was used to show the strength and/or weakness for each ADHD and RD symptom amongst the sample.

This chapter will describe characteristics of the ADHD/RD Latent Classes, *DSM-IV* ADHD subtypes, and RD categories. These characteristics include prevalence, sex, and age differences for each criterion. In addition, this description will also contain the overlap between the ADHD-RD Latent Classes and *DSM-IV* ADHD subtypes in the total sample, and in the male and female samples. The extent to which both ADHD-RD latent class criteria and *DSM-IV* ADHD subtypes are compatible is determined by examining the degree of overlap to see if individuals are assigned to the same or different phenotype using both classification criteria. The characterisation of ADHD-RD comorbidity using both sets of criteria is also revealed, detecting the prevalence and sex differences between the two sets of criteria. Furthermore, this analysis highlights the prevalence of MZ and DZ twins assigned to each ADHD-RD latent class, compares and estimates the expression of *DSM-IV* ADHD and RD item endorsements in *DSM-IV* ADHD subtypes, the RD category, and ADHD-RD latent classes. All of the above investigations examine the validity of the ADHD-RD latent classes.

5.3. Results

5.3.1 Best-Fitting Model

The LCA results showed that there were two best-fitting models, one with eight latent classes (LC-8) and one with nine (LC-9). This finding was based on selecting the lowest Bayes Information Criteria (BIC) and improved likelihood ratio chi square. In this instance, it was found that the BIC for both LC-8 and LC-9 were the lowest. The BIC for LC-8 was 77887.94269, whereas the BIC for LC-9 was 77858.02140. Figures 5.1 and 5.2 show the eight and nine latent classes respectively.

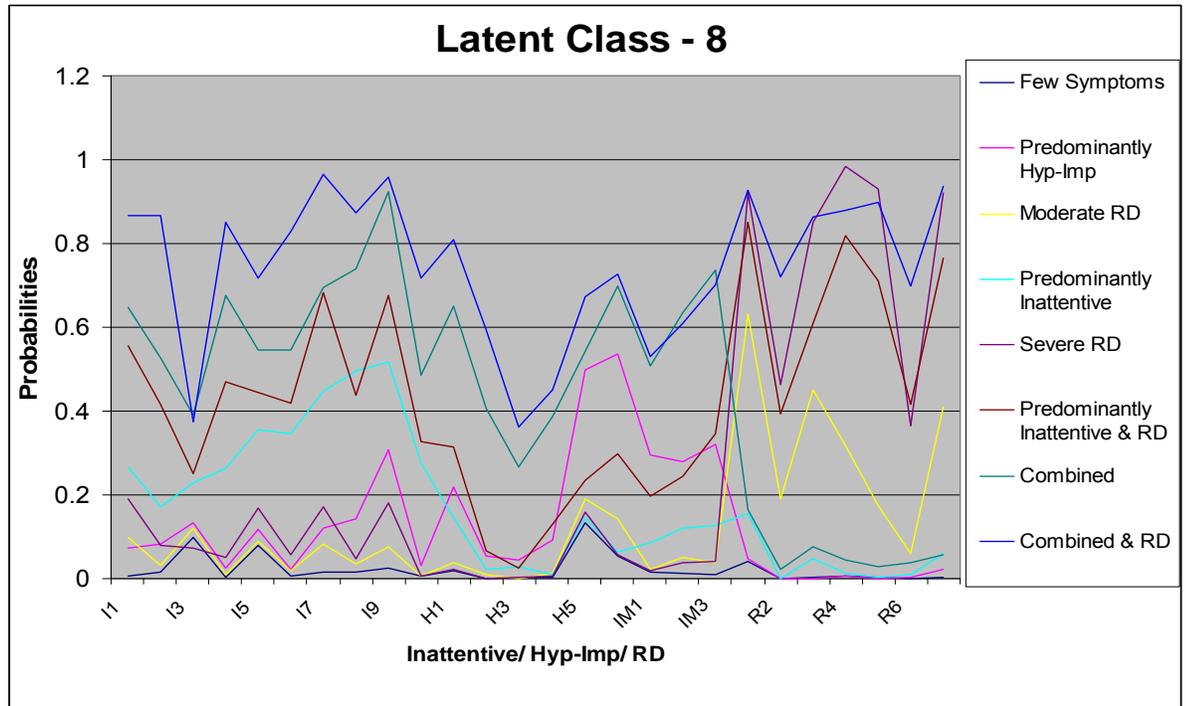


Figure 5.1 The eight latent classes

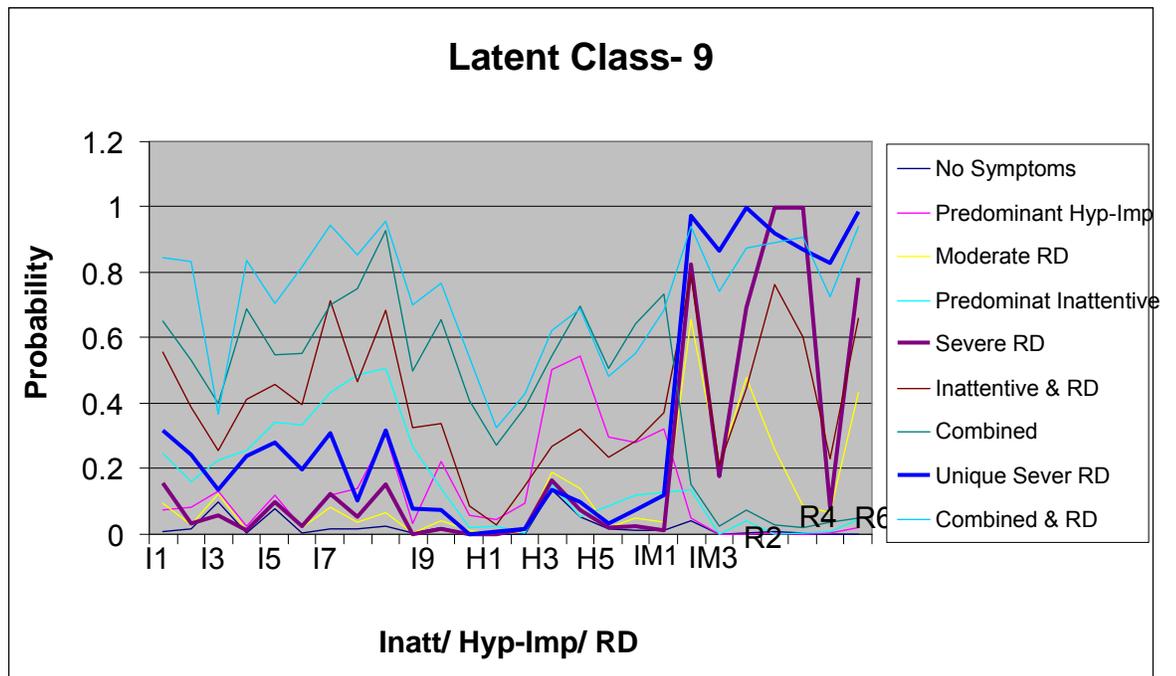


Figure 5.2 The nine latent classes. The bolded lines indicate both latent classes 'Severe RD' and 'Unique Severe RD'.

As can be seen from Figures 5.1 and 5.2, LC-9 has an extra class called “Unique Severe RD”. A comparison of “Severe RD” and “Unique Severe RD” in Figure 5.2 shows a major difference between these two classes. Despite both classes involving severe RD, parents’ endorsement for two RD items out of the seven in the “Severe RD” are markedly lower compared to the five RD items on the same class, and also compared to the parents’ endorsement for the seven items for the “Unique Severe RD” latent class. These two lower RD items are “Difficulty learning letter names” and “Difficulty learning the days or months”. This result for the latter item may confirm Willcutt’s advice to drop this item from the other six RD items, as it loaded weakly in the confirmatory factor analysis (Willcutt, 2007). Unfortunately, this analysis of our study was completed before we were aware of this fact. A decision had been made to consider Latent Class-9 as the best-fitting model to represent the 2611 twin families. This was because it has the lowest BIC, and also it has another distinctive RD latent class “Unique Severe RD”. This result could lead to new findings related to RD and to ADHD/RD comorbidity.

5.3.2 Characteristics of the Nine ADHD/RD Latent Classes

5.3.2.1 The prevalence of the nine ADHD/RD latent classes

The Latent Class-9 models expressed a presence of nine ADHD-RD latent classes (Table 5.1). One class was unaffected, three classes demonstrating the three subtypes of ADHD, three subtypes showing different severities of Reading Disability, and two classes expressing comorbidity of RD with two different ADHD subtypes. The analysis was performed on 6535 individuals.

Table 5.1
The frequencies and percentages for each criteria of the Latent class-9 model

	Total Latent Class-9	Frequency	Percent
1.	Few Symptoms	4422	67.7
2.	Predominantly Hyperactive-Impulsive	502	7.7
3.	Moderate Reading Disability	362	5.5
4.	Predominantly Inattentive	389	6.0
5.	Severe Reading Disability	233	3.6
6.	Predominantly Inattentive & RD	182	2.8
7.	Combined	180	2.8
8.	Unique Severe Reading Disability	147	2.2
9.	Combined & Reading Disability	118	1.8
	Total	6535	100.0

5.3.2.2 Sex differences among the nine ADHD-RD latent classes

The prevalence of the ‘Few Symptoms’ latent class for females (36.5%) indicates that females were less affected by the ADHD-RD phenotypes; all the other eight ADHD-RD latent classes were more prevalent in boys than in girls (Table 5.2). The total percentage of both male ‘Predominantly Inattentive’ and ‘Predominantly Inattentive & RD’ latent classes was 5.6%, indicating that the Inattentive latent classes were more prevalent than male ‘Predominantly Hyperactive-Impulsive’ latent class (4.3%).

Nevertheless, this was not the case with the total female Inattentive latent classes (3.1%), which were slightly less prevalent than the female ‘Predominantly Hyperactive-Impulsive’ latent class (3.4%). The total male RD latent classes were more prevalent (6.9%), than female RD latent classes (4.5%). The same scenario was found with the male Combined latent classes (3.3%), whilst the female combined latent class was less (1.3%). The above prevalence rates indicated that ADHD/RD latent classes are more prevalent in boys than in girls.

Table 5.2
Sex differences among the nine ADHD-RD latent classes

Total Latent Class-9	Total Sex		Total
	Male	Female	
1. Few Symptoms	2038 (31.2%)	2384 (36.5%)	4422 (67.7%)
2. Predominantly Hyp-Imp	281 (4.3%)	221(3.4%)	502 (7.7 %)
3. Moderate RD	215 (3.3%)	147 (2.2 %)	362 (5.5 %)
4. Predominantly Inattentive	243 (3.7 %)	146 (2.2 %)	389 (6.0 %)
5. Severe RD	134 (2.1 %)	99 (1.5 %)	233 (3.6 %)
6. Predominantly Inattentive & RD	126 (1.9 %)	56 (0.9%)	182 (2.8 %)
7. Combined	128 (2.0 %)	52 (0.8 %)	180 (2.8 %)
8. Unique Severe RD	97 (1.5 %)	50 (0.8 %)	147 (2.2 %)
9. Combined & RD	87 (1.3 %)	31 (0.5 %)	118 (1.8 %)
Total	3349 (51.2 %)	3186 (48.8 %)	6535 (100 %)

$\chi^2 = 173.12, df = 8, P = 0.000$

5.3.2.3 Age differences among the nine ADHD-RD latent classes

According to Table 5.3, the oldest age groups were the ‘Few Symptoms’, ‘Predominantly Inattentive’, and ‘Predominantly Inattentive & RD’ latent classes (13.04, 13.01, and

13.11 yrs old respectively). On the other hand, the youngest age groups were the ‘Predominantly Hyperactive-Impulsive’ and ‘Combined RD’ latent classes (11.94 yrs old). The intermediate age groups were all RD latent classes; ‘Moderate RD’ aged 12.96 yrs old, ‘Severe RD’ aged 12.51 yrs old, ‘Unique Severe RD’ aged 12.84 yrs old, and the ‘Combined’ latent class aged 12.25 yrs old. The univariate test showed a significant age grouping among the nine Latent Classes ($F(8, 6526) = 8.461; P < 0.05$) as well as with unaffected individuals among the ADHD and RD latent classes regardless of the ‘Few Symptom’ latent class ($F(7, 2096) = 5.680; P < 0.05$).

Table 5.3

Age significant test with LC-9

Total Latent Class-9	Descriptive Statistics		
	Age Mean	Std. Deviation	N
Few Symptoms	13.04	3.3	4422
Predominantly Hyp-Imp	11.94	3.5	502
Moderate RD	12.96	3.4	362
Predominantly Inattentive	13.01	3.5	389
Severe RD	12.51	3.3	233
Predominantly Inattentive & RD	13.11	3.5	182
Combined	12.25	3.5	180
Unique Severe RD	12.84	3.4	147
Combined & RD	11.94	3.3	118
Total	12.88	3.4	6535

5.3.2.4 Endorsement of the 18 DSM-IV ADHD items among the ADHD latent classes

Table 5.4 shows the 18 *DSM-IV* ADHD symptom endorsements that fit with the ADHD latent class subtypes. These endorsement probabilities represent the class assignment parameters used to allocate individuals from *DSM-IV* ADHD subtypes to latent classes. The following outlines the characteristics of each ADHD latent class.

5.3.2.4.1 Few symptoms

In table 5.4, the ‘Few Symptom’ latent class is the most prevalent (67.7%), and is associated with very few symptom endorsements, with proportions ranging from 0.001 to 0.14. All of the 18 *DSM-IV* ADHD symptom endorsements are distinctly low across the population. The lowest endorsed *DSM-IV* criterion ADHD symptom with the ADHD latent class is ‘No quiet play’ (0.001), while the highest endorsed probability is ‘Always on the go’ (0.14).

Table 5.4

The frequency of latent class subtype endorsements and subtype prevalence

ADHD Latent Class Conditional item endorsement probabilities with the 18 DSM-IV 18 criterion ADHD symptoms						
	Few Symptoms	Predominantly Hyp-Imp	Predominantly Inattentive	Predominantly Inattentive & RD	Combined	Combined & RD
DSM-IV Criterion symptoms	ADHD (n=4422) 67.7%	(n=502) 7.7%	(n= 389) 6.0 %	(n= 182) 2.8 %	(n= 180) 2.8 %	(n= 118) 1.8 %
1. Careless mistakes	0.006	0.08	0.26	0.56	0.65	0.86
2. Sustaining attention	0.02	0.10	0.16	0.40	0.54	0.83
3. Does not listen	0.10	0.13	0.23	0.26	0.40	0.37
4. Fails to follow instructions	0.04	0.08	0.30	0.44	0.62	0.78
5. Can not organise	0.005	0.02	0.36	0.40	0.55	0.82
6. Concentration	0.017	0.13	0.45	0.73	0.70	0.94
7. Loses things	0.017	0.14	0.51	0.47	0.76	0.86
8. Easily distracted	0.02	0.34	0.55	0.70	0.94	0.96
9. Forgetful	0.005	0.03	0.30	0.33	0.50	0.70
10. Fidgets	0.02	0.26	0.13	0.35	0.66	0.78
11. Leaves seat	0.001	0.07	0.01	0.09	0.42	0.54
12. Runs or climbs on things	0.002	0.05	0.02	0.02	0.27	0.33
13. No quiet play	0.001	0.11	0.002	0.16	0.40	0.43
14. Always on go	0.14	0.50	0.13	0.26	0.55	0.64
15. Talks excessively	0.06	0.63	0.03	0.32	0.72	0.70
16. Blurts out	0.02	0.40	0.08	0.23	0.52	0.48
17. Can not wait turn	0.01	0.33	0.11	0.29	0.64	0.56
18. Interrupts	0.01	0.40	0.11	0.40	0.75	0.70

5.3.2.4.2 Predominantly Hyperactive-Impulsive

The prevalence of the ‘Predominantly Hyperactive-Impulsive’ latent class (7.7%) was the highest among the other latent classes. The endorsement probabilities for the nine *DSM-IV* Hyperactive-Impulsive symptoms across the Predominantly Hyperactive-Impulsive latent class sample are relatively higher than for the nine Inattentive symptoms, ranging from 0.63 to 0.05 for the former and from 0.02 to 0.34 for the latter. The highest endorsed *DSM-IV* criterion ADHD symptom with the ADHD latent class is ‘Talks excessively’ (0.63), while the lowest endorsed criteria ‘Can not organise’ (0.02).

Hay et al. (2001) stated the symptoms of the Hyperactive-Impulsive subtype appeared at a younger age (3-4 years old), than the symptoms of Inattentiveness which start at school age (5-7 years old). It has been found that the Hyperactivity-Impulsivity symptoms gradually decrease with the increase in the child’s age and development. Hay et al (2001) believed that the presence of the common environmental factors such as

family, school, or medication intervention may gradually influence the symptoms of the Hyperactivity-Impulsivity.

5.3.2.4.3 Predominantly Inattentive

The prevalence of this latent class is smaller (6.0%) than the Predominantly Hyperactive-Impulsive latent class (7.7%), with moderate endorsement probabilities for the 18 *DSM-IV* ADHD symptoms, ranging from 0.002 for the ‘No quiet play’ item to 0.55 for the ‘Easily distracted’ item (Table 5.4). The endorsement probabilities for the nine Inattentive symptoms across the Predominantly Inattentive latent class are mostly higher than those for the nine Hyperactive-Impulsive symptoms on the same latent class.

5.3.2.4.4 Predominantly Inattentive with Reading Disability

The prevalence of the Predominantly Inattentive with Reading Disability latent class (2.8%) was lower than the Predominantly Inattentive class (6.0%). However, the endorsement probabilities of the 18 *DSM-IV* ADHD criteria with this class are relatively higher than those for the Predominantly Inattentive latent class. Moreover, the endorsement probabilities for the nine Inattentive criteria are higher, ranging from 0.26 to 0.73, compared to the endorsement probabilities for the nine Hyperactive-Impulsive symptoms, which ranged from 0.02 to 0.40. The highest endorsed *DSM-IV* ADHD symptom with this latent class is ‘Concentration’ (0.73), while the lowest endorsed probability is ‘Runs or climbs on things’ (0.02). The chi-square test showed a significant difference between the ‘Predominantly Inattentive’ and the ‘Predominantly Inattentive & RD’ latent classes ($\chi^2=65.315$, d.f=1, $p < 0.01$).

5.3.2.4.5 Combined Latent Class

Table 5.4 shows the prevalence of the Combined latent class (2.8%), which is the same as Predominantly Inattentive with Reading Disability (2.8%). The endorsement probabilities for the nine Inattentive symptoms are higher, ranging from 0.26 to 0.94, compared to the nine Hyperactive-Impulsive symptoms, which ranged from 0.02 to 0.75. The highest endorsed *DSM-IV* ADHD symptom within this latent class is ‘Easily distracted’ (0.94), while the lowest endorsed probability is ‘Runs or climbs on things’ (0.27).

5.3.2.4.6 Combined with Reading Disability

The prevalence of the ‘Combined/Reading Disability’ latent class is the lowest (1.8%) compared to the previous ADHD-RD latent classes (Table 5.1), with distinctive high endorsement probabilities for the 18 *DSM-IV* ADHD symptoms ranging from 0.33 for the ‘Runs or climbs on things’ item to 0.96 for the ‘Easily distracted’ item. The endorsement probabilities for the nine inattention symptoms across the Combined with Reading Disability latent class (from 0.37 to 0.96) are mostly higher than the endorsement probabilities ranging nine Hyperactive-Impulsive symptoms which ranged from 0.33 to 0.78 in the same latent class. The chi-square test exhibited a significant difference between the ‘Combined’ and the ‘Combined/RD’ latent classes ($\chi^2=16.016$, $d.f=1$, $p < 0.01$).

5.3.2.5 Endorsements of the 18 DSM-IV ADHD items among the DSM-IV ADHD subtypes

Table 5.5 represents the frequencies of the *DSM-IV* Inattentive, Hyperactivity-Impulsivity, and Combined symptoms that parents endorsed for their children. The frequency of the 18 ADHD symptoms was calculated by dividing the prevalence of parents’ response for each symptom relative to the prevalence for each ADHD subtype. The parents’ endorsements for the combined subtype were higher than for the Inattentive and Hyperactive-Impulsive subtypes. The average of parents’ endorsements for the nine Hyperactive-Impulsive symptoms among children diagnosed with the combined subtype (0.63) was higher than for the *DSM-IV* Hyperactive-Impulsive subtype (0.45), whereas with children diagnosed with Inattentive ADHD the level of endorsement was lower (0.13). The endorsement of the 18 *DSM-IV* ADHD symptoms in the ‘No ADHD’ latent class was severely low, therefore prevalence of this latent class was high (88.5%).

In general, although the prevalence of the Inattentive subtype (5.9%) was higher than Hyperactive-Impulsive subtype (2.0%) and the combined subtype (3.6%), the endorsements of the 18 *DSM-IV* ADHD symptoms were found to be high in the combined subtype, and were low in the ‘No ADHD’ latent class.

Table 5.5
The frequency of DSM-IV ADHD subtypes endorsement and subtype prevalence

	Frequency (%) of symptom endorsement			
	No ADHD (n=6250) 88.5%	Inattentive (n=418) 5.9%	Hyperactive- Impulsive (n=138) 2.0%	Combined (n= 253) 3.6 %
DSM-IV Criterion ADHD symptoms				
1. Careless mistakes	0.04	0.50	0.10	0.70
2. Sustaining attention	0.03	0.41	0.10	0.57
3. Does not listen	0.10	0.32	0.12	0.40
4. Fails to follow instructions	0.06	0.43	0.14	0.62
5. Can not organise	0.02	0.50	0.05	0.57
6. Concentration	0.05	0.65	0.22	0.77
7. Loses things	0.04	0.60	0.14	0.70
8. Easily distracted	0.07	0.67	0.42	0.85
9. Forgetful	0.01	0.42	0.05	0.47
10. Fidgets	0.04	0.21	0.51	0.70
11. Leaves seat	0.005	0.06	0.21	0.50
12. Runs or climbs on things	0.004	0.03	0.16	0.31
13. No quiet play	0.01	0.05	0.22	0.44
14. Always on the go	0.15	0.17	0.75	0.60
15. Talks excessively	0.10	0.17	0.76	0.71
16. Blurts out	0.04	0.11	0.42	0.57
17. Can not wait turn	0.03	0.14	0.50	0.70
18. Interrupts	0.04	0.20	0.56	0.75

5.3.3 The overlap between ADHD/RD Latent Class-9 and DSM-IV ADHD subtypes

Table 5.6 shows the overlap of the nine ADHD RD latent classes with *DSM-IV* ADHD subtypes across the sample. The overlap indicates that in some cases participants were not assigned to the same phenotype defined by the two diagnostic criteria. For example, cases of 95.5% of the *DSM-IV* ‘No ADHD’, 1.4% of the ‘Inattentive’, and 0.8% of the ‘Combined’ subtypes were not assigned to the ‘Predominantly Hyperactive-Impulsive’ latent class representing 97.4% of the cases. Similarly, 92.8% of the ‘Predominantly Inattentive’, and 86.3% of the ‘Predominantly Inattentive RD’ latent classes were not assigned to the *DSM-IV* ‘No Inattentive subtype’.

Table 5.6

Cross-tabulation between total latent class-9 and the DSM-IV ADHD subtypes

9- ADHD/RD Latent Classes	DSM-IV ADHD Subtypes				Total
	No ADHD	Inattentive	Hyperactive-Impulsive	Combined	
1 Few Symptoms	4375 (98.9%)	31 (0.7%)	9 (0.2%)	7 (0.2%)	4422 (100%)
2 Predominantly Hyp-Imp	478 (95.2%)	7 (1.4%)	13 (2.6%)	4 (0.8%)	502 (100%)
3 Moderate RD	354 (97.8%)	4(1.1%)	3 (0.8%)	1 (0.3%)	362 (100%)
4 Predominantly Inattentive	351 (90.2%)	28 (7.2%)	8 (2.1%)	2 (0.5%)	389 (100%)
5 Severe RD	227 (97.4%)	3 (1.3%)	2 (0.9%)	1 (0.4%)	233 (100%)
6 Predominantly Inattentive & RD	147 (80.8%)	25 (13.7%)	4 (2.2%)	6 (3.3%)	182 (100%)
7 Combined	100 (55.6%)	34 (18.9%)	20 (11.1%)	26 (14.4%)	180 (100%)
8 Unique Severe RD	141 (95.9%)	5 (3.4%)	0 (0.0%)	1 (0.7%)	147 (100%)
9 Combined & RD	40 (33.9%)	41 (34.7%)	8 (6.8%)	29 (24.6%)	118 (100%)
Total	6213 (95.1%)	178 (2.7 %)	67 (1.0 %)	77 (1.2%)	6535 (100 %)

5.3.3.1 Male and female overlaps

Out of 6535 individuals in the study, there were 51.2 % (3349) males, and 48.8 % (3186) females. Comparing Tables 5.7 and 5.8, the prevalence of ADHD-RD subtypes, when Latent Class–9 criteria was crosstabulated with the *DSM-IV* ADHD criteria was predominant in males. All nine latent classes of males assigned to *DSM-IV* Inattentive, Hyperactive-Impulsive, and combined subtypes were found to be greater than in female assignment. For instance, the total percentage of male assignments for *DSM-IV* Inattentive, Hyperactive-Impulsive, and Combined subtypes with the nine latent classes were 4.0%, 1.3%, and 1.9% respectively compared to only 1.3%, 0.7%, and 0.5% for the same total female assignments. Not surprisingly, the percentage of female *DSM-IV* ‘No ADHD’ subtypes assigned to the nine latent classes (97.5%) was higher than the male *DSM-IV* ‘No ADHD’ subtypes (92.2.2%).

Table 5.7
The male overlap between then nine ADHD-RD latent classes and the DSM-IV ADHD subtypes

Male Latent Class - 9	Male DSM-IV ADHD Subtypes				Total
	No ADHD	Inattentive	Hyperactive		
			-Impulsive	Combined	
1 Few Symptoms	2005 (98.4%)	24 (1.2%)	4 (0.2%)	5 (0.2%)	2038 (100%)
2 Predominantly Hyp-Imp	265 (94.3%)	6 (2.1%)	9 (3.2%)	14 (0.4%)	281 (100%)
3 Moderate RD	211 (98.1%)	2 (0.9%)	1 (0.5%)	1 (0.5%)	215 (100%)
4 Predominantly Inattentive	212 (87.2%)	22 (9.1 %)	7 (2.9%)	2 (0.8%)	243 (100%)
5 Severe RD	130 (97.0%)	1 (0.7%)	2 (1.5%)	1 (0.7%)	134 (100%)
6 Predominantly Inattentive & RD	99 (78.6%)	19 (15.1%)	4 (3.2%)	4 (3.2%)	126 (100%)
7 Combined	69 (53.9%)	25 (19.5%)	12 (9.4%)	22 (17.2%)	128 (100%)
8 Unique Severe RD	91 (93.8%)	5 (5.2%)	0 (0.0%)	1 (1.0%)	97 (100%)
9 Combined & RD	26 (29.9%)	31 (35.6%)	5 (5.7%)	25 (28.7%)	87 (100%)
Total	3108 (92.8 %)	135 (4.0%)	44 (1.3%)	62 (1.9%)	3349 (100 %)
<i>Rasmussen's et al. (2002) work</i>	<i>1294 (91.4 %)</i>	<i>45 (3.2 %)</i>	<i>16 (1.1 %)</i>	<i>61 (4.3 %)</i>	<i>1416 (100 %)</i>

Previously Rasmussen and his colleagues (2002) used the data from the Australian ADHD Twin Project (ATAP) and compared the data to the Missouri Adolescent Female Twin Study (MOAFTS) sample. The results observed were similar, which supported the hypothesis that LCA of ADHD classification was a robust method. In this instance, a comparison between the 2nd wave (Rasmussen, Neuman et al., 2002) and the 4th wave of ATAP shows that the prevalence of the female *DSM-IV* Inattentive subtype in both studies was relatively similar (1.1% : 1.3%) than the Hyperactive-Impulsive (0.6% : 0.7%) and Combined subtypes (1.0% : 0.5%) (Table 5.8). The comparison for both waves with male *DSM-IV* ADHD subtypes shows that the highest subtype prevalent in the 2nd wave was the combined subtype (4.3%), while in the 4th wave the highest subtype was the Inattentive subtype (4.0%) (Table 5.7). The nine latent classes of males assigned to the *DSM-IV* Inattentive subtype were found to be higher (4.0 %) compared to Rasmussen et al. (2002) study for the *DSM-IV* Inattentive subtype that used eight latent classes (3.2%), whereas the male combined subtype in Rasmussen's et al. study was found to be the highest (4.3%) compared to this study's result (1.9%) (Table 5.7).

Table 5.8
The female overlap between the nine ADHD-RD latent classes and the DSM-IV ADHD subtypes

Female Latent Class - 9	Female DSM-IV ADHD Subtypes				Total
	No ADHD	Inattentive	Hyperactive - Impulsive	Combined	
1 Few Symptoms	2370 (99.4%)	7 (0.3%)	5 (0.2%)	2 (0.1%)	2384 (100%)
2 Predominantly Hyp-Imp	213 (96.4%)	1 (0.5%)	4 (1.8%)	3 (1.4%)	221 (100%)
3 Moderate RD	143 (97.3%)	2 (1.4%)	2 (1.4%)	0 (0.0%)	147 (100%)
4 Predominantly Inattentive	139 (95.2%)	6 (4.1%)	1 (0.7%)	0 (0.0%)	146 (100%)
5 Severe RD	97 (98.0%)	2 (2.0%)	0 (0.0%)	0 (0.0%)	99 (100%)
6 Predominantly Inattentive & RD	48 (85.7%)	6 (10.7%)	0 (0.0%)	2 (3.6%)	56 (100%)
7 Combined	31 (59.6%)	9 (17.3%)	8 (15.4%)	4 (7.7%)	52 (100%)
8 Unique Severe RD	50 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	50 (100%)
9 Combined & RD	14 (45.2%)	10 (32.3%)	3 (9.7%)	4 (12.9%)	31 (100%)
Total	3105 (97.5%)	43 (1.3%)	23 (0.7%)	15 (0.5%)	3186 (100%)
<i>Rasmussen's et al. (2002) work</i>	<i>1393 (97.3%)</i>	<i>16 (1.1%)</i>	<i>8 (0.6%)</i>	<i>15 (1.0%)</i>	<i>1432 (100%)</i>

5.3.3.2 Endorsement of RD items among 'No RD' and 'Yes RD' groups

Table 5.9 shows the parents' endorsement of these seven RD items. As would be expected they were higher with children who had RD compared with children without RD.

Table 5.9
The prevalence of reading disability items endorsements

Reading Disability items (Willcutt et al., 2003)	RD item endorsement probabilities	
	Yes RD (yes=1029) 14.9%	No RD (no=5892) 85.1
1. Difficulty with spelling	0.75	0.06
2. Difficulty learning letter names	0.36	0.002
3. Difficulty learning phonics	0.62	0.015
4. Slow reading more than other children of the same age	0.66	0.007
5. Reading below expectancy level	0.56	0.006
6. Difficulty learning the days or months	0.30	0.002
7. Extra help in school with problems in reading or spelling.	0.70	0.014

5.3.3.3 Endorsement of RD items among the RD Latent Classes

Out of the nine latent classes (Figure 5.2 and Table 5.1), only three latent classes included individuals with pure RD. These three latent classes were: 'Moderate RD' with

prevalence of 5.5%, 'Severe RD' with prevalence of 3.6%, and 'Unique Severe RD' with a prevalence of 2.2%. The chi-square test of the 'Moderate RD' cross-tabulated with the 'Severe RD' showed significant differences ($\chi^2=78.949$, d.f=1, $p < 0.05$), as well as of the 'Moderate RD' with the 'Unique Severe RD' latent classes ($\chi^2=46.883$, d.f=1, $p < 0.05$).

As can be seen from table 5.10, the average endorsement probabilities of the seven RD items (Willcutt et al., 2003) with the Moderate RD (0.33) were the lowest, compared to the 'Severe RD' (0.65) and 'Unique Severe RD'. The lowest endorsement probabilities for RD items assigned to 'Moderate RD' latent class were 'Reading below expectancy level' and 'Difficulty learning the days or months' (0.07), whereas the highest endorsement probability was 'Difficulty with spelling' (0.70). The parents' endorsements for the seven RD items for children diagnosed with Severe RD latent classes were higher, in this instance, than the parents' endorsements for the seven RD items for children diagnosed with Severe RD latent class; ranging from 0.10 for 'Difficulty learning the days or months' to 1.00 for both 'Slow reading more than other children of the same age' and 'Reading below expectancy level'. The RD items that were least prevalent among the seven RD items were the 'Difficulty learning letter names' and 'Difficulty learning the days or months' items.

The seven RD items received the highest endorsements for children assigned to the 'Unique Severe RD' latent class. Scores ranged from 0.87 for 'Difficulty learning the days or months' to 1.00 for both 'Difficulty learning phonics' and 'Extra help in school with problems in reading or spelling'. This led to the conclusion that RD items were strongly related with the 'Unique Severe RD', and this relation reduced according to the kind of RD severity, as can be observed from Table 5.10. The chi-square test found a significant difference between the 'Severe RD' and the 'Unique Severe RD' latent classes ($\chi^2=27.351$, d.f=1, $p < 0.05$).

Table 5.10
The prevalence and the frequency of reading disability latent class endorsement with RD items

	RD Latent Class item endorsement probabilities		
	Moderate RD	Severe RD	Unique Severe RD
Reading Disability items (Willcutt et al., 2003)	(n=362) 5.5%	(n= 233) 3.6 %	(n= 147) 2.2 %
1. Difficulty with spelling	0.70	0.81	0.97
2. Difficulty learning letter names	0.25	0.17	0.90
3. Difficulty learning phonics	0.45	0.70	1.00
4. Slow reading more than other children of the same age	0.27	1.00	0.92
5. Reading below expectancy level	0.07	1.00	0.88
6. Difficulty learning the days or months	0.07	0.10	0.87
7. Extra help in school with problems in reading or spelling.	0.52	0.76	1.00
Mean	0.33	0.65	0.93

5.3.3.4 Endorsement of RD items among the ADHD latent classes

The endorsement ratings of the seven RD items were expected to be lower with ADHD latent classes that are not comorbid with RD (Table 5.11). This can be indicated by the lower total mean of the seven RD items endorsed in the ‘Few Symptoms’ (0.01), the ‘Predominantly Hyperactive-Impulsive’ (0.012), the ‘Predominantly Inattentive’ (0.033), the ‘Combined’ (0.05) latent classes. The ADHD latent classes’ comorbidity with RD exhibits a strong endorsement of the choice of the seven RD items as an indication of RD, especially for the ‘Predominantly Inattentive’ and ‘Predominantly Hyperactive-Impulsive’ latent classes. The mean of the total seven RD items endorsed in the ‘Predominantly Inattentive’ latent class was considerably higher (0.53) than the means of the other latent classes. The highest endorsement ratings for this latent class were found on both ‘Difficulty with spelling’ (0.80) and ‘Slow reading more than other children of the same age’ (0.77); the lowest- endorsed RD items were ‘Difficulty learning letter names’ (0.21), and the ‘Difficulty learning the days or months’ (0.23) items. The other comorbid latent class that exhibited robust correlation with the seven RD items was the ‘Combined RD’ latent class. The mean of the total seven RD items endorsed in this latent class was high (0.86). The endorsements of all of the RD items were high, ranging from 0.73 for ‘Difficulty learning the days or months’ to 0.95 for ‘Extra help in school with problems in reading or spelling’, similar to that found in the ‘Unique Severe RD’ group.

Table 5.11

The endorsement of the seven RD items among the ADHD latent classes

	ADHD Latent Class item endorsement probabilities with the Seven RD items (Willcutt et al., 2003)					
	Few Symptoms	Predominantly Hyp-Imp	Predominantly Inattentive	Predominantly Inattentive & RD	Combined	Combined & RD
Reading Disability items (Willcutt et al., 2003)	(n=4422)	(n=502)	(n= 398)	(n= 182)	(n= 180)	(n= 118)
	67.7%	7.7%	6.0 %	2.8 %	2.8 %	1.8 %
1. Difficulty with spelling	0.05	0.05	0.13	0.80	0.15	0.94
2. Difficulty learning letter names	0.00	0.00	0.00	0.21	0.02	0.75
3. Difficulty learning phonics	0.005	0.00	0.04	0.45	0.06	0.86
4. Slow reading more than other children of the same age	0.008	0.005	0.002	0.77	0.02	0.89
5. Reading below expectancy level	0.002	0.00	0.005	0.60	0.01	0.91
6. Difficulty learning the days or months	0.002	0.002	0.007	0.23	0.04	0.73
7. Extra help in school with problems in reading or spelling.	0.00	0.03	0.05	0.67	0.05	0.95
Mean	0.01	0.012	0.033	0.53	0.05	0.86

5.3.3.5 Endorsement of RD items among the DSM-IV ADHD subtypes

Table 5.12 shows the parents' endorsement of the seven RD items with the *DSM-IV* ADHD subtypes. According to this table, the most highly endorsed subtype with the Reading Disability items was the combined subtype. The least-endorsed subtype in this situation was the Hyperactive-Impulsive subtypes with the 'Inattention' subtype falling between the other two. The 'No ADHD' exhibited the lowest RD endorsement rating with an average of 0.07.

Table 5.12
The prevalence and the frequency of DSM-IV ADHD endorsements with Reading Disability items

	RD item endorsement probabilities with DSM-IV ADHD Subtypes			
	No ADHD	Inattentive	Hyp-mp	Combined
Reading Disability items (Willcutt et al., 2003)				
1. Difficulty with spelling	0.13	0.40	0.21	0.50
2. Difficulty learning letter names	0.04	0.17	0.10	0.30
3. Difficulty learning phonics	0.08	0.27	0.10	0.40
4. Slow reading more than other children of the same age	0.08	0.32	0.12	0.40
5. Reading below expectancy level	0.06	0.27	0.12	0.41
6. Difficulty learning the days or months	0.03	0.18	0.04	0.30
7. Extra help in school with problems in reading or spelling.	0.08	0.31	0.16	0.45
Mean	0.07	0.27	0.12	0.39

5.3.4 Characteristics of ADHD/ Reading Disability Comorbidity

5.3.4.1 Prevalence of the nine ADHD-RD latent class comorbidity

Table 5.13 displays the prevalence of the comorbidity of the nine ADHD-RD latent classes with the RD category. The total comorbidity of the nine ADHD-RD latent classes with the 'yes RD' was 14.3% (the comorbid 'Few Symptom' latent class with 'yes RD' was excluded). Furthermore, the comorbidity of the 'Moderate RD', the 'Severe RD', and the 'Predominantly Inattentive/RD' latent classes with 'no RD' category was 1.9%, 0.6% and 0.2% respectively. These rates revealed the efficiency of LCA to group the related phenotypes together.

Table 5.13

The prevalence of the comorbidity between the nine ADHD-RD latent classes with RD diagnosis

9- ADHD/RD Latent Classes	Reading Disability		Total
	no RD	yes RD	
1. Few Symptoms	4385 (67.1%)	37 (0.6%)	4422 (67.7%)
2. Predominantly Hyp-Imp	490 (7.5%)	12 (0.2%)	502 (7.7%)
3. Moderate RD	124 (1.9%)	238 (3.6%)	362 (5.5%)
4. Predominantly Inattentive	376 (5.8%)	13 (0.2%)	389 (6.0%)
5. Severe RD	6 (0.1%)	227 (3.5%)	233 (3.6%)
6. Predominantly Inattentive/RD	13 (0.2%)	169 (2.6%)	182 (2.8%)
7. Combined	166 (2.5%)	14 (0.2%)	180 (2.8%)
8. Unique Severe RD	0 (0.0%)	147 (2.2%)	147 (2.2%)
9. Combined/RD	0 (0.0%)	118 (1.8%)	118 (1.8%)
Total	5560 (85.1%)	975 (14.9%)	6535 (100%)

The LCA showed both the ‘Predominantly Hyperactive-Impulsive’ (0.2%) and ‘Combined’ (0.2%) latent classes were the lowest classes comorbid with RD. On the other hand, the ‘Combined-RD’ latent class (1.8%) was moderately high, but not as high as the ‘Predominantly Inattentive-RD’ (3.6%) class.

5.3.4.1 Sex differences among the nine ADHD-RD latent classes and RD diagnosis

Table 5.14 describes the prevalence of ‘no RD’ and ‘yes RD’ among the comorbid latent classes. The total comorbidity for males is 17.6% between the eight ADHD-RD latent classes with ‘yes RD’ category compared to girls at 11.0%. The highest comorbid male and female latent classes with ‘no RD’ were the ‘Predominantly Hyperactive-Impulsive’ (8.2% and 6.8%) and the ‘Predominantly Inattentive’ (7.2% and 4.3%). In the ‘yes RD’ category, the highest comorbid male and female latent classes were the ‘Moderate RD’ (4.2 % and 3.1%), and the ‘Severe RD’ (3.9% and 3.0). There were no comorbid cases found in both ‘Unique Severe RD’ and ‘Combined/RD’ latent cases in the ‘no RD’ category for both genders. The comorbidity of the ‘Moderate RD’ latent class in both males and females with ‘no RD’ was 2.2% and 1.5%, which could be used as an indicator for the presence of affected cases that the ‘no RD’ category could not detect.

Table 5.14
Sex prevalence among the nine ADHD-RD latent classes and RD diagnosis

Sex Variable	9- ADHD/RD Latent Classes	Reading Disability		Total
		no RD	yes RD	
Male	Few Symptoms	2014(98.8%)	24 (1.2%)	2038 (100%)
	Predominantly Hyp-Imp	274 (97.5%)	7 (2.5%)	281 (100%)
	Moderate RD	75 (34.9%)	140 (65.1%)	215 (100%)
	Predominantly Inattentive	240 (98.8%)	3 (1.2%)	243 (100%)
	Severe RD	4 (3.0%)	130 (97%)	134 (100%)
	Predominantly Inattentive/RD	12 (9.5%)	114 (90.5%)	126 (100%)
	Combined	117 (91.4%)	11 (8.6%)	128 (100%)
	Unique Severe RD	0 (0.0%)	97 (100%)	97 (100%)
	Combined & RD	0 (0.0%)	87 (100%)	87 (100%)
Total		2736(81.7%)	613 (18.3%)	3349 (100%)
Female	Few Symptoms	2371 (99.5%)	13 (0.5%)	2384 (100%)
	Predominantly Hyp-Imp	216 (97.7%)	5 (2.3%)	221 (100%)
	Moderate RD	49 (33.3%)	98 (66.7%)	147 (100%)
	Predominantly Inattentive	136 (93.2%)	10 (6.8%)	146 (100%)
	Severe RD	2 (2%)	97 (98%)	99 (100%)
	Predominantly Inattentive/RD	1 (1.8%)	55 (98.2%)	56 (100%)
	Combined	49 (94.2%)	3 (5.8%)	52 (100%)
	Unique Severe RD	0 (0.0%)	50 (100%)	50 (100%)
	Combined & RD	0 (0.0%)	31 (100%)	31 (100%)
Total		2824(88.6%)	362 (11.4%)	3186 (100%)

5.3.5 Characterisation of the nine ADHD-RD latent classes with Zygoty

Table 5.15 shows the distribution of zygoty among the nine latent classes. There were more DZ twins (1319; 56%) than MZ twins (1031; 44%) in the study. Some latent classes can account for concordant MZ, though they are very small in number or equal to DZ. For example, the number of ‘Moderate RD’ assigned to Twin One of MZ twins compared to ‘Severe RD’, ‘Predominantly Inattentive with RD’, and ‘Unique Severe RD’ assigned to Twin Two is relatively higher than DZ twins with the same assigned latent classes (4:3, 3:2, and 3:2 respectively). Furthermore, the number of ‘Unique RD’ assigned to Twin One of MZ twins compared to Twin Two assignments of ‘Moderate RD’, ‘Predominantly Inattentive with RD’, and ‘Combined with RD’ assigned to Twin One MZ compared to ‘Unique Severe RD’ assignments to Twin Two is also higher than for DZ twins which does not contain any individuals with the same assigned latent classes (3:0, 1:0, and 3:0 respectively). DZ prevalence can be seen in the total number for each latent class of Twin 1 ‘MZ’ and ‘DZ’ groups in each latent

class compared to the same Twin 2 'MZ' and 'DZ' groups: 713:879, 82:101, 60:73, 54:79, 37:51, 27:42, 18:32, 18:39, and 22:23 respectively (Table 5.15).

The traditional method to determine heritability is by comparing concordance rates of MZ twins versus DZ twins (Tishler & Carey, 2007). Heath et al. (Heath et al., 2003) stated that LCA can be considered an alternative statistical method for determining zygosity based on zygosity questionnaire items. LCA is also considered an effective tool to determine zygosity when genotyping information is not present (Heath et al., 2003). Todd et al. (2001) stated that by comparing the degree of MZ and DZ concordance between twins in the same and different latent classes, researchers can determine if these latent classes are heritable or not and also can recognise a genetic influence up on that particular latent class. According to this, when the Chi-Square test was calculated for the concordant twins, it showed a significant difference between MZ to DZ ($\chi^2 = 50.104$; $p < 0.01$), which indicates that those latent classes are distinctive heritable groups. One exception was the number of concordant MZ twins for the 'Predominantly Inattentive-RD' latent class compared to DZ twins, where there were more DZ children than MZ children (11 and 10 respectively) (Table 5.15).

Similarly, Table 5.16 shows how the proportion of concordant versus discordant pairs differs between MZ and DZ twins. The Chi-Square test showed significant differences between concordant and discordant, MZ and DZ twins for all nine classes except the 'Predominantly Inattentive-RD' class. These significant results indicated that these latent classes are heritable.

Table 5.15

The cross-tabulation of LC-9 Twin 1 with LC-9 Twin 2 by zygosity

		LC-9 for Twin 2										
LC-9 Groups	Zyg	Few Symptoms	Pre Hyp-Imp	Mod RD	Pre Inatt	Sev RD	Pre Inatt/RD	Combined	Uniq Sev RD	ComRD	Total	
LC-9 For Twin 1	Few Symptoms	MZ	665 (64.5%)	12 (1.2%)	10 (1.0%)	13 (1.3%)	8 (0.8%)	2 (0.2%)	2 (0.2%)	1 (0.1%)	0 (0.0%)	713 (69.2%)
		DZ	679 (51.5%)	48 (3.6%)	43 (3.3%)	39 (3.0%)	20 (1.5%)	13 (1.0%)	15 (1.1%)	12 (0.9%)	10 (0.8%)	879 (66.6%)
	Predominantly Hyp-Imp	MZ	22 (2.1%)	49 (4.8%)	1 (0.1%)	5 (5.0%)	0 (0.0%)	0 (0.0%)	4 (0.4%)	0 (0.0%)	1 (0.1%)	82 (8.0%)
		DZ	45 (3.4%)	24 (1.8%)	7 (0.5%)	9 (0.7%)	4 (0.3%)	1 (0.1%)	5 (0.4%)	6 (0.5%)	0 (0.0%)	101 (7.7%)
	Moderate RD	MZ	5 (0.5%)	0 (0.0%)	42 (4.1%)	3 (0.3%)	4 (0.4%)	3 (0.3%)	0 (0.0%)	3 (0.3%)	0 (0.0%)	60 (5.8%)
		DZ	42 (3.2%)	4 (0.3%)	11 (0.8%)	4 (0.3%)	3 (0.2%)	2 (0.2%)	4 (0.3%)	2 (0.2%)	1 (0.1%)	73 (5.5%)
	Predominantly Inattentive	MZ	16 (1.6%)	4 (0.4%)	1 (0.1%)	29 (2.8%)	0 (0.0%)	3 (0.3%)	1 (0.1%)	0 (0.0%)	0 (0.0%)	54 (5.2%)
		DZ	35 (2.7%)	14 (1.1%)	4 (0.3%)	11 (0.8%)	3 (0.2%)	3 (0.2%)	6 (0.5%)	1 (0.1%)	2 (0.2%)	79 (6.0%)
	Severe RD	MZ	4 (0.4%)	0 (0.0%)	5 (0.5%)	0 (0.0%)	23 (2.2%)	2 (0.2%)	0 (0.0%)	3 (0.3%)	0 (0.0%)	37 (3.6%)
		DZ	32 (2.4%)	2 (0.2%)	3 (0.2%)	1 (0.1%)	7 (0.5%)	4 (0.3%)	1 (0.1%)	1 (0.1%)	0 (0.0%)	51 (3.9%)
	Predominantly Inattentive & RD	MZ	2 (0.2%)	2 (0.2%)	3 (0.3%)	4 (0.4%)	2 (0.2%)	10 (1.0%)	1 (0.1%)	0 (0.0%)	3 (0.3%)	27 (2.6%)
		DZ	12 (0.9%)	8 (0.6%)	0 (0.0%)	5 (0.4%)	1 (0.1%)	11 (0.8%)	3 (0.2%)	1 (0.1%)	1 (0.1%)	42 (3.2%)
	Combined	MZ	1 (0.1%)	1 (0.1%)	1 (0.1%)	1 (0.1%)	0 (0.0%)	0 (0.0%)	13 (1.3%)	0 (0.0%)	1 (0.1%)	18 (1.7%)
		DZ	7 (0.5%)	9 (0.7%)	3 (0.2%)	7 (0.5%)	1 (0.1%)	1 (0.1%)	2 (0.2%)	0 (0.0%)	2 (0.2%)	32 (2.4%)
	Unique Severe RD	MZ	1 (0.1%)	0 (0.0%)	3 (0.3%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)	13 (1.3%)	0 (0.0%)	18 (1.7%)
		DZ	21 (1.6%)	4 (0.3%)	0 (0.0%)	4 (0.3%)	1 (0.1%)	0 (0.0%)	1 (0.0%)	8 (0.6%)	0 (0.0%)	39 (3.0%)
	Combined & RD	MZ	1 (0.1%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	2 (0.2%)	1 (0.1%)	0 (0.0%)	3 (0.3%)	14 (1.4%)	22 (2.1%)
		DZ	6 (0.5%)	3 (0.2%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	3 (0.2%)	3 (0.2%)	0 (0.0%)	6 (0.5%)	23 (1.7%)
Total	MZ	717 (51.5%)	68 (6.6%)	66 (6.4%)	56 (5.6%)	39 (3.8%)	22 (2.1%)	21 (2.0%)	23 (2.2%)	19 (1.8%)	1031 (100%)	
	DZ	879 (66.6%)	116 (8.8%)	71 (5.4%)	80 (6.1%)	42 (3.2%)	38 (2.9%)	40 (3.0%)	31 (2.4%)	22 (1.7%)	1319 (100%)	

Note: LC-9=Latent Class-9; Zyg.=zygosity; Pre. Hyp.-Imp.=Predominantly Hyperactive-Impulsive; Mod. RD=moderate Reading Disability; Pre. Inatt.=Predominantly Inattentive; Sev.RD=severe RD; Pre. Inatt./RD=Predominantly Inattentive and Reading Disability; Uniq. Sev. RD= Unique Severe RD; Com.RD=Ccombined and Reading Disability.

Table 5.16

Chi-square test of the proportion of concordant versus discordant MZ and DZ twin pairs for each latent class

T1*T2 Latent Class	Zygotity	Concordant twins	Discordant twins	Chi-Square
1. T1*T2 Few Symptoms	MZ	665	48	$\chi^2=76.83, p <0.01$
	DZ	679	200	
2. T1*T2 Predominantly Hyp.-Imp.	MZ	49	33	$\chi^2=24.45, p <0.01$
	DZ	24	77	
3. T1*T2 Moderate RD	MZ	42	18	$\chi^2=41.46, p <0.01$
	DZ	11	62	
4. T1*T2 Predominantly Inattentive	MZ	29	25	$\chi^2=24.13, p <0.01$
	DZ	11	68	
5. T1*T2 Severe RD	MZ	23	14	$\chi^2=22.39, p <0.01$
	DZ	7	44	
6. T1*T2 Predominantly Inattentive & RD	MZ	10	17	$\chi^2=0.913, p >0.05$
	DZ	11	31	
7. T1*T2 Combined	MZ	13	5	$\chi^2=23.88, p <0.01$
	DZ	2	30	
8. T1*T2 Unique Severe RD	MZ	13	5	$\chi^2=14.15, p <0.01$
	DZ	8	31	
9. T1*T2 Combined & RD	MZ	14	8	$\chi^2=6.42, p <0.05$
	DZ	6	17	

Note: T1=Twin 1; T2= Twin 2

5.4 Discussion

This study aimed to apply Latent Class Analysis (LCA) to ADHD and RD subtypes in order to obtain informative genetic phenotypes that would be effective in performing a genotyping analysis. The prevalence of ADHD/RD latent subtypes showed that the most prevalent subtype is ‘Predominantly Hyperactive-Impulsive’ (7.7%); however, by adding the prevalence of all Inattentive latent classes (including the ‘Predominantly Inattentive’ (6.0%) and the ‘Predominantly Inattentive and Reading Disability’ subtypes (2.8%), the total prevalence of the Predominantly Inattentive subtypes (8.2%) would be the highest among the latent classes. Nearest to this type of class, the total prevalence for both ‘Combined’ and ‘Combined and Reading Disability’ will give a total prevalence of 4.6%.

Rasmussen et al. (2002; 2004) previously applied the LCA approach to an ADHD Australian sample, in order to replicate their findings with adolescent female twins from Missouri on a male and female Australian sample. Both studies found the eight latent class model was the best fit. The classes in this model included one “few symptom” class, three “mild to moderate ADHD” classes, three ‘severe ADHD’ classes, and one “unique ADHD” class. However, the study considered only six latent classes. The 18 *DSM-IV* ADHD item ratings for two latent classes were different as they were associated with broad confidence intervals. Comparing the latent classes from Rasmussen et al’s study (2002) with the current study, both studies share the same number of ADHD latent classes, after excluding the two latent classes described above from Rasmussen et al’s study (2002). There are still slight phenotypic differences in relation to ADHD severity between the two studies.

The total gender differences among the ADHD-RD latent classes, excluding unaffected individuals, showed that they were more prevalent among boys than girls: 20.1% to 12.3% (Table 5.14). However, it was not obvious if there was a relationship between the structure of LC-9 and gender differences in the classes. Differences in gender prevalence occurred in each latent class. The ‘Predominantly Inattentive’ latent class showed slightly greater prevalence for girls (0.3%) than boys (0.1%) (Table 5.14). This could be due to the presence of higher genetic contributions between the domains in females than in males.

5.4.1 Examining the 18 DSM-IV ADHD and seven RD item endorsements

The aim of examining the parents’ ratings for the 18 *DSM-IV* ADHD questionnaire items and the seven RD items for their children was to measure the differences between the *DSM-IV* ADHD subtypes, the RD category, and the nine ADHD/RD latent classes. This helps to determine the validity of the ADHD and/or RD phenotypes based on their correspondence with the *DSM-IV* items and the seven RD items.

Rasmussen et al.(2002) stated that when using latent class analysis, individuals with comparable ratings across all symptoms, based on statistical probabilities, form natural groups. This conclusion does not take into account the total number of symptoms or the presence of impairment. Based on these findings, this idea can be further developed to

compare the accuracy of both ADHD and RD continuous and categorical data. The ratings for the 18 items were found to be higher for the *DSM-IV* subtypes; however, LCA proved to be more effective in identifying the presence of ADHD in individuals overall. This conclusion is borne out by referring to the results above, which show that LCA identified a greater number of cases of ADHD than the DSM-IV approach.

The endorsements of the seven RD items (Willcutt, Boada et al., 2003) were examined systematically with the ‘yes RD’ and ‘no RD’ categories (Table 5.9), the nine ADHD-RD latent classes (Table 5.10 and Table 5.11), and the *DSM-IV* ADHD subtypes (Table 5.12). The seven RD item endorsements showed relatively consistent correspondence with the severity of the RD phenotype. The more highly the RD items are endorsed, the more severe is the RD phenotype. However, the RD item endorsements with the three RD latent classes showed a minor discrepancy. Rather than having strong RD item endorsement for “Slow reading more than other children of the same age”, and “Reading below expectancy level” with the ‘Unique Severe RD’, these items were endorsed more highly with the ‘Severe RD’ latent class. The reason for this could be that the LCA clustering for these two items tended to have greater correspondence with the ‘Severe RD’ latent class instead of the ‘Unique Severe RD’ latent class.

The weakest RD item endorsements among the seven items in Table 5.9 to Table 5.12 were with the “Difficulty learning the days or months” and the “Difficulty learning letter names” items. This might be due to the weak validity and reliability that the “Difficulty learning letter names” item exhibited compared to the other items when reassessed by Willcutt et al. (2003). This could also be applicable to the “Difficulty learning letter names” item, as it also showed a minor cross-loading (0.34) on the factor analysis. Although these two items were endorsed strongly (0.90 and 0.87 respectively) with the ‘Unique Severe RD’ latent class, their endorsement was extremely low with the ‘Moderate RD’ (0.25 and 0.07) and ‘Severe RD’ (0.17 and 0.10) latent classes (Table 5.10). This indicates that the LCA might effectively cluster these two symptoms and assigned them to the ‘Unique Severe RD’ latent class. This is because that LCA has the ability to robustly dissect RD symptoms into distinctive and genetically informative phenotypic groups.

Moreover, the ADHD latent classes (Table 5.11) and the *DSM-IV* ADHD subtypes (Table 5.12) that were endorsed highly with the seven RD items were the ‘Combined/RD’ latent class, and the *DSM-IV* Combined subtype, then the ‘Predominantly Inattentive-RD’ latent class and the *DSM-IV* Inattentive subtype. In contrast, the least endorsed latent class and least endorsed *DSM-IV* ADHD subtype with the seven RD items were the ‘Predominately Hyperactive-Impulsive’ latent class and the *DSM-IV* Hyperactive-Impulsive subtype. It was expected that the Hyperactive-Impulsive subtype would be the least endorsed with the RD items, as Willcutt et al. (2000; 2003) had found the genetic contribution between the *DSM-IV* Hyperactive-Impulsive subtype was insignificant with RD, whereas the *DSM-IV* Inattention subtype exhibited significant genetic contribution.

Table 5.17
The correspondence between the Willcutt’s (2003) seven RD items and the RD components

RD Items	RD Components
• Item 1	• Helps to assess Spelling
• Item 2 & Item 6	• Help to assess Verbal Learning and memory
• Item 3	• Helps to assess Phonological awareness
• Item 4	• Helps to assess rapid memory
• Item 5 & Item 7	• Help to assess overall Reading ability

Examining RD item endorsements with LCA enabled this study to specify the RD components among the three RD latent classes and the two ADHD-RD comorbid latent classes. Table 5.17 shows the correspondence between the seven RD items and the RD components: spelling, verbal learning and memory, phonological awareness, rapid memory, and overall reading ability.

Table 5.18

Matching up the Predominant Phenotypic RD item(s) with each RD Latent Class

RD Latent Class	Predominant Phenotypic RD item(s)
1. Moderate RD Latent Class	Predominantly spelling item
2. Severe RD	Predominantly rapid memory item Predominantly overall reading ability items Predominantly spelling item
3. Predominantly Inattentive RD	Predominantly spelling item Predominantly rapid memory item Predominantly overall reading ability items
4. Unique Severe RD	All items are predominant
5. Combined RD	All items are predominant

Each of the three RD latent classes and the two ADHD-RD comorbid latent classes were matched up with the related RD components (Table 5.18). Upon examination of the predominant RD items in each RD and ADHD-RD latent class, it was found that RD item 1 (spelling), was predominant in the ‘Moderate RD’ latent class. The most predominant of the seven RD items with the ‘Severe RD’ latent class were rapid memory, the overall reading ability, and spelling. However, all the seven RD items were found predominant with the ‘Unique Severe RD’ latent class. With the ‘Inattentive-RD’ latent class, the predominant RD items were spelling, rapid memory item, and overall reading ability, whereas, with the ‘Combined RD’ latent class, all RD items were predominant. For supporting these findings Wolf & Bowers (1999) hypothesised a theory related to RD called double-deficit theory of reading disability. It proposes that a deficit in both phonological awareness and rapid naming gives rise to the lowest level of reading performances. It constitutes the most severe form RD, rather than individuals with deficits in only one of these reading composite skills.

The aim of assigning the predominant RD components to the ADHD-RD latent classes was to specify the phenotypic criteria for each latent class in order to robust genetic association between the latent class and a particular item. To do this would be a substantial advance in relating the ADHD-RD phenotype to a particular genetic marker.

The study demonstrates that LCA has the capacity to cluster individuals into phenotypically homogenous groups in order to detect genetic association.

5.4.2 Zygoty

The shaded diagonal in Table 5.15 between T1 and T2 showed higher concordance rates among MZ twins than DZ twins, except for the ‘Few Symptoms’ and the ‘Predominantly Inattentive’ latent classes. The concordance among MZ and DZ pairs was explored to determine the presence of genetic effects that contribute to the ADHD-RD latent classes. The diagonals between the same ADHD-RD latent classes were significantly higher among MZ twins than DZ twins, indicating a presence of common genetic effects for MZ twins. However, two latent classes where DZ twins rated slightly higher than MZ twins were the ‘Few Symptoms’ and the ‘Predominantly Inattentive’ classes.

Willcutt et al.’s (2003) findings of the presence of genetic entities due to attributed genetic influence in the comorbidity between ADHD/RD for the ‘Predominantly Inattentive/RD’ and the ‘Combined/RD’ latent classes did not show the same pattern of zygoty in this study. The former latent class was nearly the same in the number of the concordant MZ and DZ twin pairs, indicating lower genetic contribution to this than to the latter latent class, in which the concordant MZ twin pairs were double the number of the concordant DZ twins, indicating a high genetic contribution.

5.4.3 Conclusion

In conclusion, LCA showed its efficiency in refining the phenotypes of ADHD alone, RD alone, and ADHD-RD comorbidity, and its ability to classify them in homogenous groups based on clusters of symptoms, suggesting that most of the latent classes may be robust enough to use in molecular genetic studies. The only class that has not showed heritability is the ‘Predominantly Inattentive/RD’ latent class based on comparing the comparison rates of MZ twins versus DZ twins (Table 5.16). It’s also suggested that the comorbid ADHD-RD latent classes may be genetically distinctive from ADHD alone and RD alone. The LCA’s production of the nine ADHD-RD latent classes comes from its ability to dissect phenotypes into several robust dimensions.

Table 5.19

The overlapping and distinct cases between the nine ADHD-RD latent classes and the DSM-IV ADHD subtypes by 'no RD' and 'yes RD' Categories

LC-9 Groups	Overlapping Cases	distinct Cases	Total Cases
Few Symptoms	4375 (98.94%)	47 (1.06%)	4422 (100%)
Predominantly Hyp.-Imp.	13 (2.59%)	489 (97.41%)	502 (100%)
Moderate RD	238 (65.7%)	124 (34.3%)	362 (100%)
Predominantly Inattentive	28 (7.2%)	361 (92.8%)	389 (100%)
Severe RD	227 (97.4%)	6 (2.6%)	233 (100%)
Predominantly Inattentive & RD	169 (92.86%)	13 (7.14%)	182 (100%)
Combined	26 (14.44%)	154 (85.56%)	180 (100%)
Unique Severe RD	147 (100%)	0 (0.0%)	147 (100%)
Combined & RD	118 (100%)	0 (0.0%)	118 (100%)
Total	5341 (81.73%)	1194(18.27%)	6535 (100%)

To confirm this efficiency more over the *DSM-IV* diagnostic criteria, Table 5.19 shows the overlap of the nine ADHD-RD latent classes with the *DSM-IV* ADHD subtypes by 'no RD' and 'yes RD' categories which confirms the ability of LCA in identifying the cases that the *DSM-IV* categories could not pick up. For instance, out of 502 cases of the 'Predominantly Hyperactive-Impulsive' latent class, the *DSM-IV* Hyperactive Impulsive subtype only picked up 13 (2.59%) cases, whereas the 'Predominantly Hyperactive-Impulsive' latent class identified a further 489 (97.4%) cases. In total, there were 1194 (18.27%) cases that the *DSM-IV* ADHD diagnostic criteria could not pick up. The ability of LCA to pick up more cases than DSM-IV does not mean that all chosen individuals by LCA are clinical cases. Previously, Hudziak et al., 1998, Neuman et al., 1999, Rasmussen et al. 2002 used LCA to refine ADHD classification in population-based samples of children through combining probability of symptom endorsement and overall symptom profile to create a set of eight distinct classes of ADHD symptoms. These classes are Few Symptoms, Mild combined, Mild Inattentive, Severe Combined, Severe Inattentive, Moderate Inattentive, Sever Hyperactive-Impulsive, and Moderate Combined. Although five latent classes are not clinically relevant, three of these classes are so, which are Severe Combined, Severe Inattentive, and Severe Hyperactive-Impulsive. Todd et al. (2002)

explained that the severe inattentive latent class was associated with academic problems, family problems, and referral to health care providers.

Willcutt (2008) explained that “because LCA approach often includes a substantially higher number of individuals for each class, this may increase power to detect differences between classes in comparisons to analyses of the DSM-IV subtypes” (Willcutt, Personal communication).

The performance of LCA in identifying cases was also significantly better in most of the other latent classes than was the *DSM-IV* defined categories. The phenotypes of LCA cases may be genetically informative for use in genotyping analysis. Although previous literatures (e.g., Todd et al., 2005) showed LCA may be a better approach in genotyping analysis, and may be more genetically informative than DSM-IV; this is still considered as a hypothesis and needs further investigations and confirmation.

CHAPTER 6: GENETIC MODEL FITTING

6.1 Introduction

In regard to whether ADHD-RD comorbidity is a genetically distinctive group from ADHD without Reading Disability (RD), Latent Class Analysis (LCA) in chapter five found distinctive classes of ADHD subtypes with and without comorbid RD, suggesting that the comorbid ADHD-RD classes may be genetically distinct from ADHD alone and RD alone. However, the nature of the genetic effects of each comorbid ADHD subtype still needs more investigation. In addition, the study also aimed to investigate the genetic and environmental effects of each ADHD subtype and RD, and to investigate whether ADHD subtypes and RD have the same genetic effects or whether each subtype has different genetic effects.

Previous findings indicated that the considerable ADHD-RD comorbidity is in part attributable to some of the same candidate genes for both disorders (Friedman et al., 2003) such as the region of chromosome 6p21.3 that is a susceptibility locus for RD (Cardon et al., 1994) and also for ADHD (Willcutt et al., 2002). Furthermore, the DRD4 gene might be a susceptible candidate gene for ADHD-RD comorbidity based on evidence by Levitt et al. (1997) that showed the important role of this neurotransmitter in the development of the brain (Hsiung, Kaplan, Petryshen, Lu, & Field, 2004). Willcutt et al. (2007) found that common genetic influences significantly contributed to RD-ADHD comorbidity, and this genetic attribution was stronger between Inattention and RD than between Hyperactivity-Impulsivity and RD.

The quantitative genetic approach by using a genetic model fitting from a structural equation modelling program called Mx Statistical Modelling (Neale, Boker, Xie, & Maes, 2006; Neale & Cardon, 1992), was used to investigate the above problems. As Mx is available as a free download from <http://www.vcu.edu/mx/> and is a useful model-fitting program, it is commonly used in the field of behavioural genetics. It utilises a Graphical User Interface (GUI) that enables the drawing of path diagrams to explain the model and its fit to the data. Mx modelling is based on a totally different set of assumptions about the relationship of ADHD to RD than LCA.

6.2 Methodology

6.2.1 Participants

The Mx statistical genetic modelling was applied to 2610 twin Australian families including MZ and DZ twins but not to their siblings. The participants were recruited from ATAP and consisted of 1130 (43.8%) monozygotic twin pairs and 1455 (56.2%) dizygotic twin pairs. The age range of the sample was from 4 years to 18 years old, with an average age of 12.93 years old +/- 3.3 years. A full explanation of participants' recruitment, the measures used for zygosity, ADHD and RD was described in chapter 3. The descriptive statistics for the ADHD and RD groups were described in chapter 4.

6.2.2 Analyses

6.2.2.1 Data transformation, standardisation and assumption testing

Generally, statistical transformation is used on data as a remedy for outliers, failures of normality, linearity, and homoscedasticity. It was found that most of the data variables were not normally distributed. These variables include age, Inattention score, Hyperactive-Impulsive score, combined score, and Reading Disability score. In addition, all of these variables were kurtotic and positively skewed. Logarithmic, square root, and inverse transformations were performed to approximate a normal distribution. All transformed variables had skewed and kurtosis values of less than 1. ADHD subtypes were transformed to root-square transformations to approximate normality, whereas RD scores remained untransformed as it was found that this best approximated normality.

6.2.2.1.1 Assumption testing for zygosity

A non-parametric test (distribution-free) was used to examine if the means of MZ and DZ twins were the same. It was tested by a Mann Whitney U-test, which was used since the assumption of normality or equality of variance were violated. This, like many non-parametric tests, uses the ranks of the data rather than their raw values to calculate the statistic. Assumption testing was performed to confirm if means of MZ and DZ twins were equal. The Mann Whitney U-test was applied on zygosity for ADHD-subtypes' scores and RD scores. The test determines whether both MZ and DZ twins came from identical populations or come from different populations, which can

help provide a basis for comparison if any of ADHD and RD score variables were independent from zygosity.

6.2.2.1.2 Assumption testing for age

To examine the effect of age on the scores for the ADHD subtypes and RD, data was split into two groups based on the median of age. This was performed in order to test if both groups came from similar or different populations. Then the correlations of the Inattentive, Hyperactive-Impulsive, Combined, and RD scores were calculated, in order to fit a univariate and bivariate models for all phenotypes.

6.2.2.1.3 Assumption testing for sex

The Mann Whitney U-test was used to test the hypothesis that gender differences exist when variations ADHD-subtype or RD scores with sex occur in either identical or different populations. The Mann Whitney U-test found a gender difference, based on the differences between the sexes already found in populations with ADHD. The disorder is more prevalent in boys than girls, ranging from 3:1 to 8:1 (Rhee, Waldman, Hay, & Levy, 2001).

Univariate model fitting (Univariate twin analyses)

Broadly speaking, the use of Structural Equation Modelling (SEM) can indicate the degree to which a quantitative phenotypic variance has its roots in genetic and/or environmental factors (Mather & Jinks, 1977). The statistical package Mx (Neal et al, 2006) and ML estimation procedures are the tools used in structural equation modelling. In the SEM paradigm, the genetic and environmental components of a trait are each further separated into two subtypes: Additive (A) and Non-Additive (D) genetic influences, and common/shared (C) and unique (E) environmental effects respectively. “A” stands for the total effects of alleles at all loci that influence the trait. By contrast, “D” stands for the relations between alleles at the same locus (dominant D) or in different loci (epistasis). In the context of family members, environmental factors – shared (C) and unique (E) – contribute to phenotypic resemblance and phenotypic difference respectively (Ulrich, Gervil, Kyvik, Olesen, & Russell, 1999). Taking components A, D, C and E above into account, total phenotypic variance (P) of a trait can be expressed as $P = A + D + C + E$ (Rijsdijk & Sham, 2002).

Prior to fitting the univariate genetic and environmental structural equation models to the twin data, the twin-pair correlations were first assessed for each ADHD subtype and RD. The relationship between the MZ correlation value (rMZ) and the DZ correlation value (rDZ) provided an indication as to which Mx model would provide the best fit. If the correlation for MZ was less than twice the correlation for DZ, then the best model fitting would be the common environment model with or without additive genes (ACE, AE or CE). If the correlation for MZ was greater than twice the correlation for DZ twins, then the best model fitting would be the non-additive genetic influences (D) with or without additive genes (ADE or DE) model, and/or contrast effects. C and D can not occur together as the choice between C or D is definitional. If the MZ correlation and DZ correlation are equal, the common environment model (CE) would provide the most informative results, whereas if the MZ correlation is less than the DZ correlation, the genetic influences are not suggested (Hudziak, Derks, Althoff, Rettew, & Boomsma, 2005). These relationships between rMZ, rDZ and the corresponding univariate genetic model fitting assumptions are summarized in Table 6.1 below (Neale & Cardon, 1992).

Table 6.1
The four genetic model fitting assumptions

Univariate genetic model fitting	Genetic and Environmental effects	Family source of variance	Twin Correlation
ACE Model	Then: Additive (A) > zero Common (C) > zero Unique (E) > zero Dominance (D) = zero	Additive genetic & Common environmental effects	MZ ≈ 2DZ
AE Model	OR Additive (A) > zero Unique (E) > zero Common (C) = zero Dominance (D) = zero	OR Additive genetic effects	
ADE Model	Additive (A) > zero Dominance (D) > zero Unique (E) > zero Common (C) = zero	Additive genetic & non-Additive genetic effects	MZ > 2DZ
CE Model	Unique (E) > zero Common (C) > zero Additive (A) = zero Dominance (D) = zero	Common environmental effects	MZ = DZ

**This table was developed from Neale & Cardon (1992), p. 170.*

The differing correlation values between MZ and DZ twins for components A and D is key in enabling the estimation of different variance components. MZ twins correlate 1.0 for both A and D as they have identical genetic material, whereas DZ twins correlate only 0.5 for A and 0.25 for D because dizygotic twins share half of their segregating genes on average. Shared environmental factors (C) are independent of zygosity, and thus correlate 1.0 for both DZ and MZ twins. Unique environmental factors (E) must remain uncorrelated, as this value represents an environmental influence that is not experienced by both twins, whether they are MZ or DZ (Hudziak et al., 2005). The effect of E can be estimated by examining phenotypic differences between MZ twins, as this is the only factor that can account for such differences (Rijsdijk & Sham, 2002).

In order to achieve better accuracy in the estimates of the relative influence of additive genetic, non-additive genetic, shared environmental, and non-shared environmental factors on the particular ADHD subtype, and on the RD dimension, a univariate genetic and environmental model is fitted to the twin-pair correlations. The matrices of twin pair correlation can utilise a maximum-likelihood model fitted to the Mx models of correlation matrices, maximising the fit between the model and the data, and, consequently, determines those parameter estimates, providing the best possibility of model fitting and the fewest data discrepancies. A full ACE model was fitted to the data after the matrices were input into Mx structural equation modelling program as represented by the genetic ACE model (Figure 6.1). The univariate genetic modelling by Mx Statistical Modelling (Neale et al., 2006) was used as the best for the purpose. It was conducted to examine genetic and environmental contributions to ADHD subtypes and RD among the entire sample.

In addition, it was necessary to also determine if genetic and environmental factors can cause comorbidity and correlations between ADHD subtypes and RD. Modelling was utilised to carry out the twin study, comprising MZ and DZ twin pairs. SPSS software was first used for the exploratory analysis of the data and subsequently, Mx Statistical Modelling for univariate analyses of each ADHD subtype and RD score. A series of univariate genetic and environmental models were fitted to the data, obtained after the approximation of the relative influence of A, D, C, and E factors on each of the ADHD

subtypes and RD, separately and in combination. The model fit was assessed by combining the various parameters A, C, D, and E. The parameters a, c, and e in Figure 6.1 represent the observed phenotype on the latent factors A, D, and E and show the level of relationship between the observed phenotype and the latent factors. It is possible to explain the variance proportion of the genetic and environmental effects by squaring a, d (or c), and e (Hudziak et al., 2005).

How well a model fits the data can be measured by examining its chi-square (χ^2) statistics. Should the χ^2 value be so large as to be statistically significant, this indicates that the model is a relatively poor fit to the data. Conversely, if χ^2 proves statistically insignificant, this indicates that the model is a good fit. In the case of a poorly fitting model, adding or removing some of the four parameters may improve the fit, and this can be determined by comparing the χ^2 value obtained initially with the χ^2 value obtained after the addition or removal of certain parameters (Legrand, McGue, & Lacono, 1999). The number of observed statistics minus the number of parameters being estimated in the model gives the degrees of freedom (df) value for the χ^2 test explained in the foregoing (Neale & Cardon, 1992; Rijdsdijk & Sham, 2002).

The Akaike's Information Criterion (AIC) and Root Square Error of Approximation (RMSEA) are utilised to assess the general fit of models. Both are used for purposes of comparison when the parameters of one model do not overlap with the parameters of the other. The AIC combines the χ^2 value of the model's fit with degrees of freedom, helping to determine the criteria for the best fitting model. The lowest negative AIC indicates the best fitting model (Ulrich et al., 1999). The RMSEA offers a measure of difference for each degree of freedom and is a measure of proximity of fit. An RMSEA value of 0.05 is considered as an indicator of good fit, and values up to 0.08 stand for reasonable errors of estimate among the sample (Rebollo & Boomsma, 2006). RMSEA values between 0.00 and 0.05 indicate excellent fit, and the values between 0.05 and 0.08 good fit.

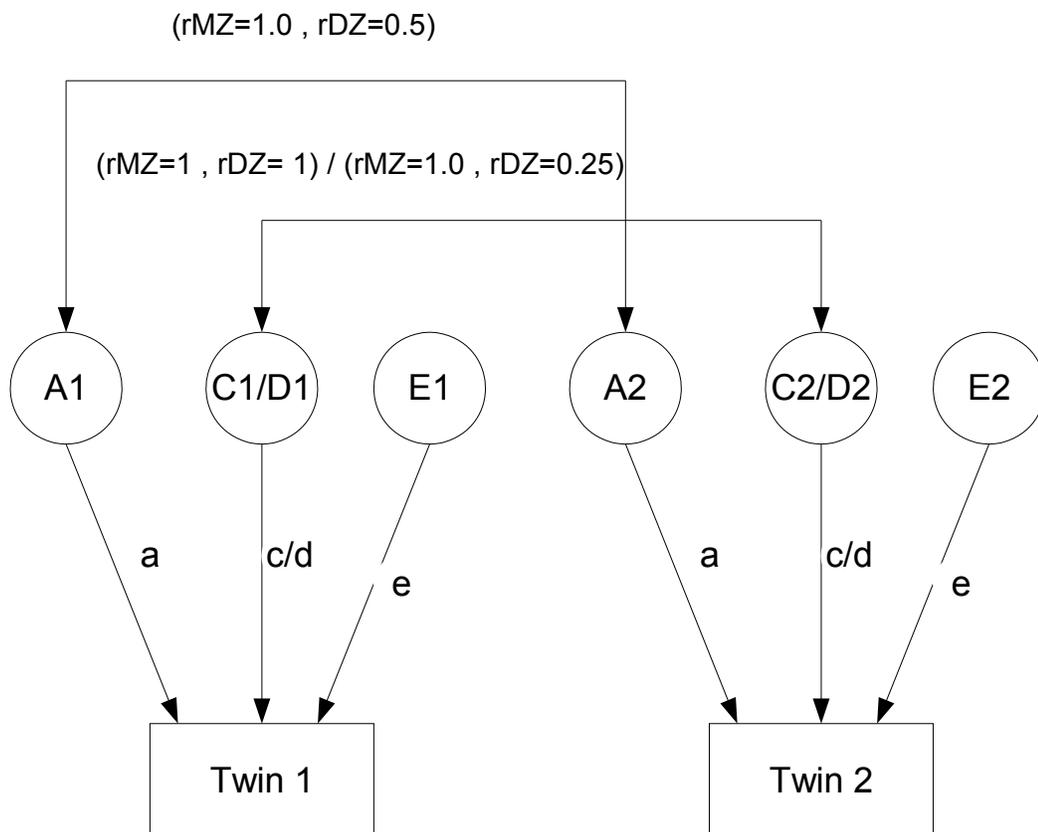


Figure 6.1 Univariate model for Mx modelling

rMZ= Monozygotic twins correlation; *rDZ*= Dizygotic twins correlations; *A* = Additive genetic factors; *C*= Shared environmental effects; *D*= Dominance genetic factors; *E*= Nonshared environmental effects, *a,c,d,e*= Loadings of observed phenotype on latent factors *A, C, D, E*.

6.2.2.3 *Bivariate model fitting*

Phenotypic relations among ADHD subtypes and Reading Disability were investigated by calculating of bivariate correlations. When a high correlation exists between each ADHD subtype and RD, the shared and the separate genetic and environmental influences can be studied with bivariate genetic and environmental Mx modelling. The bivariate model fitting included the breakdown of the correlation among each ADHD subtype and RD measures into additive or non-additive genetic, shared environmental influences, and non-shared environmental influences, to estimate the phenotypic correlations of ADHD subtypes with RD. Common-pathway bivariate environmental and genetic models were fitted to the twin-pair correlations in order to achieve better accuracy in the estimates of the relative influence of additive genetic (A), shared environmental (C), and non-shared environmental (E) factors on correlation of each ADHD subtype with RD. Study of the extent of the overlap of ADHD subtypes and RD can be detected with the use of full ACE model. This model enables the estimation of common genetic and environmental influences, which can be distinguished by both measures as well as the other specific effects taken into account by one or other measure. The genetic and environmental variables (A, C, and E) determine this latent variable, with the possibility of specific genetic and environmental factors giving rise to the disorders. The comparison-nested models and the full model enabled the investigation of the presence of particular genetic and environmental effects. Figure 6.2 is a diagrammatic representation of a common-pathway bivariate model. Parameters can be eliminated in the same way as for univariate models but the process includes the factors specific to each measure. For each measure, the factors A, C, or D were excluded to examine if the common factors contributed to the variation relative to the specific factor.

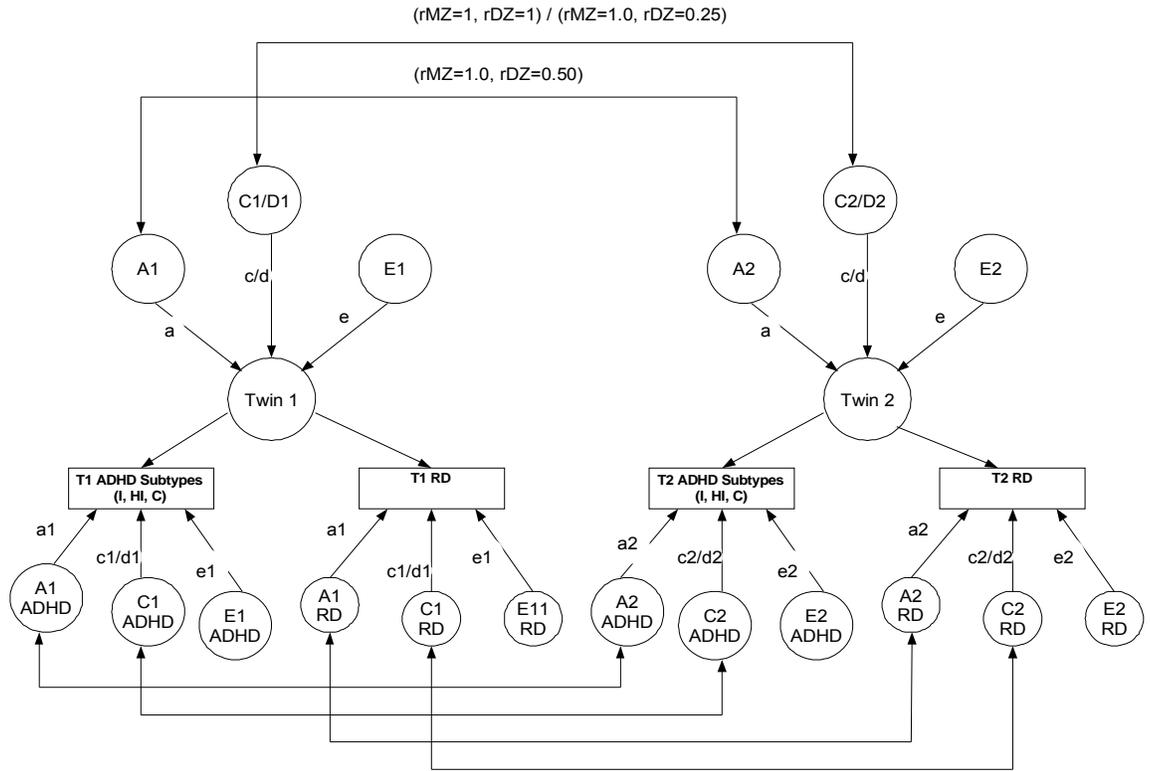


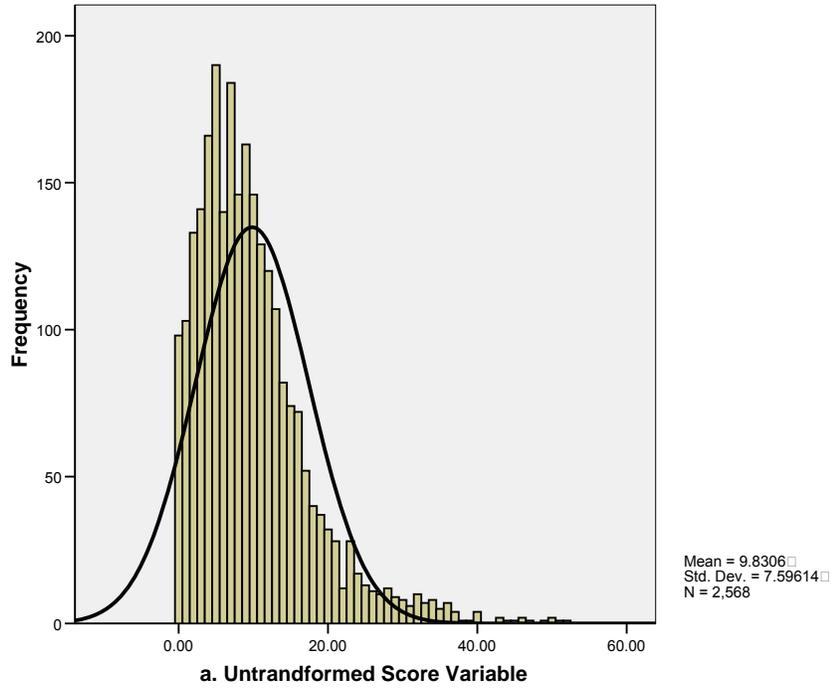
Figure 6.2 Bivariate model for Mx modeling

rMZ= Monozygotic twins correlation
rDZ= Dizygotic twins correlations
A =Additive genetic factors
C =Shared environmental effects
D =Dominance genetic factors
E= Nonshared environmental effects
a,c,d,e= Loadings of observed phenotype on latent factors *A, C, D, E*.
I = Inattention
HI= Hyperactive – Impulsive
C= Combined
RD= Reading Disability

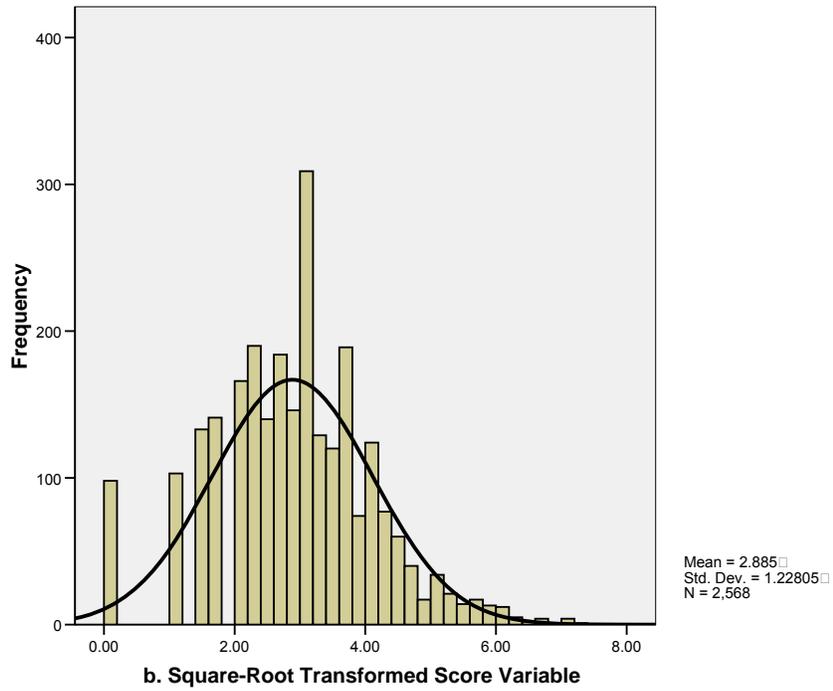
6.3 Results

6.3.1 Data transformation and standardisation

After checking the normality for ADHD subtype and RD scores, it was found that the RD score variables were best without transformation. Score variables for the Inattentive, Hyperactive-Impulsive, and Combined subtypes were transformed to square-root values (Figure 6.3) after a value of '1' was added to each ADHD score in order to obtain approximate normality.



a. Untransformed Score Variable (e.g. DSM-IV Combined symptoms for Twin 2)



b. Square-root transformed score variable (e.g. DSM-IV Combined symptoms for Twin 2)

Figure 6.3 An example of distribution of untransformed and square-root transformed score variable.

6.3.2 Assumption testing for age limitation

As showed earlier the minimum age of this sample was 4 years old and the maximum was 18 years old with a range of 14.5 years, and a median of 13 years old. To examine whether age has affect on the scores for ADHD subtypes or RD, and whether the total sample comes from a similar or different population, the whole sample was divided into two groups based on the median age; the first group of individuals aged 13 years old and younger, and the other group of individuals aged over 13 years old. Consequently, the phenotypic univariate and bivariate correlations for Inattentive, Hyperactive-Impulsive, and Combined subtypes, and for RD, were obtained for both age groups (See Appendices A2.1-A2.8). Then a univariate and bivariate genetic model was fitted to the MZ and DZ twin-pair correlations. The full ACE and ADE models, and reduced models of AE, CE and DE models were also fitted by providing variance-covariance matrices to Mx for both age groups of each phenotype. The results showed that the age effect had no influence on Mx univariate and bivariate models and gave consistent results among the two age groups for each phenotype.

6.3.3 Assumption testing for sex limitation

As expected, the Mann Whitney U-test found gender differences among ADHD and RD phenotypes(as also shown in Table 4.20 and Table 4.27). This result led to examine whether gender has influence on Mx genetic modelling. Therefore, the phenotypic correlations for the five sex sets of each phenotype (Inattentive, Hyperactive-Impulsive, Combined and RD category) ‘Dizygotic female twins’ (DZ f), ‘Dizygotic male twins’ (DZ m), ‘Monozygotic female twins’ (MZ f), ‘Monozygotic male twins’ (MZ m), and ‘Dizygotic opposite-sex twins’ (DZ OS), were fitted to Mx models (Tables 2). The genetic and environmental model fittings were performed on full models: ACE, ADE and the reduced models AE, CE, and DE. The results of Mx model fitting of ADHD subtypes (Inattention, Hyperactivity-Impulsivity, and Combined) and RD, for the five sets based on the variable of sex, showed that ADHD-subtype scores came from a homogenous population, as ACE model showed to be the model with best fitting (Tables 6.3-6.6), whereas RD scores came from a heterogeneous population, because its opposite-sex correlation was low due to sex limitation.

For sex limitation a bivariate correlation was performed between twin one and twin two for each ADHD-subtype score and RD score, in which the subjects were selected by sex. To test the sex limitation, a univariate genetic analysis was performed by fitting the univariate model simultaneously to all five sets for each score. All of the genetic and environmental model fittings (the full model (ACE and ADE) and reduced models (AE, CE, and DE) were tested. Comparing and obtaining consistent results among the five models for each score would mean that there were no sex limitations, and indicate that the population is homogenous.

Table 6.2
Monozygotic and dizygotic univariate sex correlations for ADHD subtypes and Reading Disability

Phenotype	Inattention	Hyp-Imp	Combined	RD
Monozygotic male	0.861 (0.84-0.89)	0.889 (0.89-0.91)	0.905 (0.89-0.92)	0.904 (0.89-0.92)
Monozygotic female	0.868 (0.85-0.89)	0.895 (0.88-0.92)	0.893 (0.88-0.91)	0.896 (0.88-0.92)
Dizygotic male	0.505 (0.43-0.58)	0.500 (0.43-0.57)	0.550 (0.48-0.62)	0.418 (0.34-0.5)
Dizygotic female	0.476 (0.4-0.56)	0.598 (0.53-0.67)	0.559 (0.49-0.63)	0.271 (0.18-0.37)
Opposite Sex	0.375 (0.31-0.44)	0.524 (0.46-0.58)	0.483 (0.43-0.55)	0.192 (0.12-0.27)

Correlation is significant at the 0.01 level (2-tailed)

Confidence Interval-95% values are shown between brackets.

Table 6.3

Male and Female model of the Inattentive subtype

<i>ACE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2
$\chi^2 = 9.416$	0.92	0.13	-	0.37	0.85	0.02	-	0.13
P = 0.667								
d.f = 12								
AIC = -14.584								
RMSEA = 0.014								
<i>*AE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2
$\chi^2 = 9.590$	0.93	-	-	0.33	0.87	-	-	0.11
P = 0.727								
d.f = 13								
AIC = -16.410								
RMSEA = 0.013								
<i>CE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2
$\chi^2 = 581.054$	-	0.79	-	0.61	-	0.62	-	0.38
P = 0.000								
d.f = 13								
AIC = 555.054								
RMSEA = 0.259								
<i>ADE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2
$\chi^2 = 9.590$	0.93	-	0.00	0.37	0.87	-	0.00	0.13
P = 0.652								
d.f = 12								
AIC = -14.410								
RMSEA = 0.014								

* *Best fit model.*

Table 6.4
Male and Female model of the Hyperactive-Impulsive Subtype

<i>*ACE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a²</i>	<i>c²</i>	<i>d²</i>	<i>e²</i>
$\chi^2 = 6.057$ P = 0.913 d.f = 12 AIC = -17.943 RMSEA = 0.008	0.84	0.43	-	0.33	0.71	0.18	-	0.11
<i>AE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a²</i>	<i>c²</i>	<i>d²</i>	<i>e²</i>
$\chi^2 = 28.183$ P = 0.009 d.f = 13 AIC = 2.183 RMSEA = 0.035	0.93	-	-	0.32	0.90	-	-	0.10
<i>CE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a²</i>	<i>c²</i>	<i>d²</i>	<i>e²</i>
$\chi^2 = 598.436$ P = 0.000 d.f = 13 AIC = 572.436 RMSEA = 0.267	-	0.83	-	0.56	-	0.69	-	0.31
<i>ADE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a²</i>	<i>c²</i>	<i>d²</i>	<i>e²</i>
$\chi^2 = 28.183$ P = 0.005 d.f = 12 AIC = 4.183 RMSEA = 0.037	0.93	-	0.00	0.32	0.90	-	0.00	0.10

** Best fit model.*

Table 6.5
Male and Female model of the Combined subtype

<i>*ACE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a²</i>	<i>c²</i>	<i>d²</i>	<i>e²</i>
$\chi^2 = 6.276$ P = 0.902 d.f = 12 AIC = -17.724 RMSEA = 0.000	0.87	0.38	-	0.32	0.76	0.14	-	0.10
<i>AE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a²</i>	<i>c²</i>	<i>d²</i>	<i>e²</i>
$\chi^2 = 20.803$ P = 0.077 d.f = 13 AIC = -5.197 RMSEA = 0.030	0.94	-	-	-0.31	0.90	-	-	0.10
<i>CE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a²</i>	<i>c²</i>	<i>d²</i>	<i>e²</i>
$\chi\chi^2 = 669.617$ P = 0.000 d.f = 13 AIC = 643.617 RMSEA = 0.281	-	0.83	-	0.56		0.69	-	0.31
<i>ADE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a²</i>	<i>c²</i>	<i>d²</i>	<i>e²</i>
$\chi^2 = 20.803$ P = 0.053 d.f = 12 AIC = -3.197 RMSEA = 0.032	0.94	-	0.00	0.31	0.90	-	0.00	0.10

** Best fit model.*

Table 6.6
Male and Female model of the Reading Disability

<i>ACE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 76.834$ P = 0.000 d.f = 0 AIC = 52.834 RMSEA = 0.065	0.95	0.00	-	0.32	0.90	0.00	-	0.10
<i>AE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 76.834$ P = 0.000 d.f = 13 AIC = 50.834 RMSEA = 0.060	0.95	-	-	0.32	0.90	-	-	0.10
<i>CE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>E</i> ²
$\chi^2 = 1040.588$ P = 0.000 d.f = 13 AIC = 1014.588 RMSEA = 0.346	-	0.74	-	0.67		0.55	-	0.45
<i>*ADE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 17.479$ P = 0.132 d.f = 12 AIC = -6.521 RMSEA = 0.028	0.47	-	0.83	0.32	0.22	-	0.68	0.10
<i>DE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 22.235$ P = 0.052 d.f = 13 AIC = -3.765 RMSEA = 0.025	-	-	0.95	-0.32	-	-	0.90	0.10

** Best fit model.*

This table (6.6) indicates a presence of sex limitation with Reading Disability. A further confirmation for this was shown when the AE model appeared to be the best fit model when applied to MZ and DZ males for RD (Table 6.7), whereas the ADE model was the best fit model when applied to MZ and DZ females for RD (Table 6.8). This was expected, as girls are affected by the same RD symptoms as boys, but a different degree of RD symptoms is seen in girls than in boys. This leads to the conclusion of the effect of difference in sex on RD. On the contrary, this is not the case with ADHD subtypes as ADHD symptoms are more prevalent in boys than girls, and the sample did not exhibit sex limitation, which means that ADHD subtypes are not affected by sex differences. In fact the non-additive effects that appeared were false effects,

because the opposite-sex correlation was so low. In addition, the Confidence Intervals (CI) for the correlation of MZ female compared to the correlation of DZ female, as well as the CIs for the correlation DZ female compared to the correlation of opposite sex are different (Table 6.2).

Table 6.7
MZ and DZ males model on Reading Disability

<i>ACE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 0.797$ P = 0.850 d.f = 3 AIC = -5.203 RMSEA = 0.000	0.95	0.00	-	0.31	0.90	0.00	-	0.10
<i>AE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 0.797$ P = 0.933 d.f = 4 AIC = -7.203 RMSEA = 0.000	0.95	-	-	-0.31	0.90	-	-	0.10
<i>CE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>E</i> ²
$\chi^2 = 373.124$ P = 0.000 d.f = 4 AIC = 365.124 RMSEA = 0.428	-	0.83	-	0.55		0.70		0.30
<i>*ADE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 0.000$ P = 1.000 d.f = 3 AIC = -6.000 RMSEA = 0.000	0.88	-	0.37	-0.31	0.77		0.13	0.10
<i>DE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 19.188$ P = 0.001 d.f = 4 AIC = 11.188 RMSEA = 0.072	-	-	0.95	-0.31	-	-	0.90	0.10

* Best fit model.

Table 6.8
MZ and DZ females model on Reading Disability

<i>AE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2=17.396$ P = 0.002 d.f = 4 AIC = 9.396 RMSEA = 0.070	0.95	-	-	-0.33	0.90	-	-	0.10
<i>CE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>E</i> ²
$\chi^2=426.373$ P = 0.000 d.f = 4 AIC = 418.373 RMSEA = 0.467	-	0.80	-	0.60		0.64	-	0.36
<i>*ADE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2=0.000$ P = 1.000 d.f = 3 AIC = -6.000 RMSEA = 0.000	0.43	-	0.84	-0.32	0.19		0.71	0.10
<i>DE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2=0.963$ P = 0.915 d.f = 4 AIC = -7.037 RMSEA = 0.000	-	-	0.94	-0.32	-	-	0.90	0.10

* *Best fit model.*

6.3.3 Univariate model fitting

Correlation values (*r*) obtained for the three *DSM-IV* ADHD subtypes (Inattentive, Hyperactive-Impulsive, Combined) and Reading Disability for both MZ and DZ twins are shown in Table 6.9.

Table 6.9
Monozygotic and dizygotic univariate correlations for ADHD subtypes and Reading Disability

Phenotype	Monozygotic Twin	Dizygotic Twin
1. Inattentive Subtype	0.869**	0.449**
2. Hyperactive-Impulsive Subtype	0.893**	0.541**
3. Combined Subtype	0.901**	0.531**
4. Reading Disability	0.901**	0.288**

** *Correlation is significant at the 0.01 level (2-tailed).*

Correlations can help to determine the best model fitting to use. As can be seen from the correlation table above (Table 6.9), all MZ and DZ correlations for the Inattentive

($r_{MZ} = 0.869$, $r_{DZ} = 0.449$), Hyperactive- Impulsive ($r_{MZ} = 0.893$, $r_{DZ} = 0.451$), and Combined subtypes ($r_{MZ} = 0.901$, $r_{DZ} = 0.531$), indicated that the best fit model to detect the genetic and environment contributions is the ACE model. The rMZ is relatively equal to the double of rDZ, thus the best fitting model would be the “additive genes” (ACE). The MZ and DZ correlation for Reading Disability (RD, $r_{MZ} = 0.901$, $r_{DZ} = 0.288$) indicated that the best-fitting model was the “non-additive genetic” model (ADE), as the correlation of MZ was greater than double the correlation of DZ.

Table 6.10 presents the results of the Mx univariate modelling for the three *DSM-IV* ADHD subtypes and for Reading Disability. For the Inattentive subtype, the AE model was the best-fitting model, and is considered a sub-model of ADE (Table 6.10); while for the Hyperactive-Impulsive, and Combined subtypes, the best fitting model was the ACE model. For RD, the best model was ADE. Furthermore, it was observed that the effect of the additive genetic factor in the AE model with the Inattentive subtype ($A=0.92$) was higher than the effect of the additive genetic factor in the ACE model for both the Hyperactive-Impulsive ($A=0.84$) and Combined subtypes ($A=0.86$). Therefore, the heritability estimate (a^2) for the Inattentive subtype was higher ($a^2 = 0.86$) than either for the Hyperactive-Impulsive ($a^2 = 0.71$) or the Combined subtypes ($a^2 = 0.74$). For Reading Disability (RD), ADE was the poorest-fit model (Table 6.10), and showed a high estimate of the dominant genetic effect ($d^2=0.66$), and a relatively low estimate for the additive genetic effect ($a^2 = 0.25$). The AE and ADE models were not the best fitting models for Hyperactive-Impulsive or Combined subtypes (Table 6.10). The estimate of the shared- environment factor (C) made a relatively small contribution compared to the additive genetic factor (A) in the ACE model for the Inattentive ($c^2 = 0.03$), Hyperactive-Impulsive ($c^2 = 0.18$) and Combined ($c^2 = 0.16$) subtypes.

Table 6.10
Univariate model-fitting on ADHD subtypes and Reading Disability

1. Univariate Model-fitting on Inattentive Subtype									
<i>ACE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	
$\chi^2 = 0.000$	0.92	0.17	-	0.36	0.85	0.03	-	0.13	
P = 1.000									
d.f = 3									
AIC = -6.000									
RMSEA = 0.000									
<i>*AE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	
$\chi^2 = 0.504$	0.93	-	-	0.36	0.86	-	-	0.13	
P = 0.973									
d.f = 4									
AIC = -7.496									
RMSEA = 0.000									
					<div style="border-top: 1px solid black; border-bottom: 1px solid black; display: inline-block; padding: 2px;"> CI (0.91, 0.95) </div>				
<i>ADE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	
$\chi^2 = 0.504$	0.93	-	0.000	0.36	0.86	-	0.000	0.13	
P = 0.918									
d.f = 3									
AIC = -5.496									
RMSEA = 0.000									
2. Univariate Model-fitting on Hyperactive-Impulsive Subtype									
<i>*ACE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	
$\chi^2 = 0.000$	0.84	0.43	-	0.33	0.71	0.18	-	0.11	
P = 1.000									
d.f = 3									
AIC = -6.000									
RMSEA = 0.000									
					<div style="border-top: 1px solid black; border-bottom: 1px solid black; display: inline-block; padding: 2px;"> CI (0.80, 0.88) </div>				
3. Univariate Model-fitting on Combined Subtype									
<i>*ACE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	
$\chi^2 = 0.000$	0.86	0.40	-	0.31	0.74	0.16	-	0.10	
P = 1.000									
d.f = 3									
AIC = -6.000									
RMSEA = 0.000									
					<div style="border-top: 1px solid black; border-bottom: 1px solid black; display: inline-block; padding: 2px;"> CI (0.82, 0.90) </div>				
4. Univariate Model-fitting on Reading Disability									
<i>*ADE Model</i>	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	
$\chi^2 = 0.000$	0.50	-	0.81	0.31	0.25	-	0.66	0.10	
P = 1.000									
d.f = 3									
AIC = -6.000									
RMSEA = 0.000									
					<div style="border-top: 1px solid black; border-bottom: 1px solid black; display: inline-block; padding: 2px;"> CI (0.24, 0.66) </div>				

CI= Confidence Interval

*Best fit model.

6.3.4 Bivariate Mx modelling

Bivariate genetic model fitting was performed between each subtype of ADHD and RD to detect the contribution of the genetic factors and the environmental factors for

ADHD subtypes and RD, and also to detect the interaction between these two disorders. Figure 6.2 and tables 6.14 – 6.16 show the results of the model fitting. According to the bivariate correlations (Tables 6.11- 6.13) all of the twin1 twin2 MZ and twin1 twin2 DZ correlations for Inattention, Hyperactive-Impulsive, Combined subtypes with Reading Disability correlations were less than half the correlations of each ADHD subtype with itself. These correlations help in determining which model fitting would be best to adopt in order to detect the genetic and environment relationships. These correlations indicate that the non-additive genetic model would be the best to adopt.

Table 6.11
The MZ and DZ bivariate correlations between the Inattentive subtype and Reading Disability

MZ	Inatt T1	Inatt T2	RD T1	RD T2
Inatt T1	1	.869(**)	.379(**)	.342(**)
Inatt T2	.869(**)	1	.324(**)	.353(**)
RD T1	.379(**)	.324(**)	1	.901(**)
RD T2	.342(**)	.353(**)	.901(**)	1
DZ	Inatt T1	Inatt T2	RD T1	RD T2
Inatt T1	1	.449(**)	.389(**)	.174(**)
Inatt T2	.449(**)	1	.134(**)	.388(**)
RD T1	.389(**)	.134(**)	1	.288(**)
RD T2	.174(**)	.388(**)	.288(**)	1

** Correlation is significant at the 0.01 level (2-tailed)
T1= Twin 1; T2= Twin 2; MZ=Monozygotic; DZ=Dizygotic; Inatt=Inattention; RD= Reading Disability

Table 6.12
The MZ and DZ bivariate correlations between the Hyperactive-Impulsive subtype and Reading Disability

MZ	Hyp-Imp T1	Hyp-Imp T2	RD T1	RD T2
Hyp-Imp T1	1	.893(**)	.182(**)	.174(**)
Hyp-Imp T2	.893(**)	1	.177(**)	.192(**)
RD T1	.182(**)	.177(**)	1	.901(**)
RD T2	.174(**)	.192(**)	.901(**)	1
DZ	Hyp-Imp T1	Hyp-Imp T2	RD T1	RD T2
Hyp-Imp T1	1	.541(**)	.225(**)	.155(**)
Hyp-Imp T2	.541(**)	1	.143(**)	.247(**)
RD T1	.225(**)	.143(**)	1	.288(**)
RD T2	.155(**)	.247(**)	.288(**)	1

** Correlation is significant at the 0.01 level (2-tailed).
T1=Twin 1; T2=Twin 2; MZ=Monozygotic; DZ=Dizygotic; Hyp-Imp=Hyperactive-Impulsive; RD=Reading Disability

Table 6.13
The MZ and DZ bivariate correlations between the Combined subtype and Reading Disability

MZ	Comb T1	Comb T2	RD T1	RD T2
Comb T1	1	.901(**)	.318(**)	.294(**)
Comb T2	.901(**)	1	.284(**)	.310(**)
RD T1	.318(**)	.284(**)	1	.901(**)
RD T2	.294(**)	.310(**)	.901(**)	1
DZ	Comb T1	Comb T2	RD T1	RD T2
Comb T1	1	.531(**)	.350(**)	.184(**)
Comb T2	.531(**)	1	.154(**)	.361(**)
RD T1	.350(**)	.154(**)	1	.288(**)
RD T2	.184(**)	.361(**)	.288(**)	1

** Correlation is significant at the 0.01 level (2-tailed).

T1=Twin 1; T2=Twin 2; MZ=Monozygotic; DZ=Dizygotic; Comb=; RD= Reading Disability

Since the three ADHD subtypes were found to be correlated with RD (Tables 6.11-6.13), the extent to which their covariation was attributable to genes was estimated using bivariate Mx modelling. Genetic correlations were estimated for each ADHD subtype with RD (Figure 6.2). Overall, the genetic correlations were greater than double the correlation for RD, suggesting that there are substantial non-additive genetic effects (dominance effect) between the three ADHD subtypes with RD. This finding is important, in order to discover the genetic causes of ADHD-RD comorbidity.

According to the results of the bivariate genetic model fitting of each ADHD subtype with RD (Tables 6.14-6.16) that there are substantial non-additive genetic effects between the two disorders. All other models, including ACE, CE and AE, did not best represent the twin data.

Table 6.14 showed that the ADE model for the Inattentive subtype with RD was the best fitting model ($\chi^2=9.531$, $P=0.573$), with the higher shared additive genetic factor ($A=0.54$), lower shared non-additive genetic factor ($D=0.29$), and shared unique environmental factor ($E=0.18$). Furthermore, the additive genetic factors for the Inattentive subtype only ($A_{Inatt}=0.72$) and for RD only ($A_{RD}=0.74$) were higher than the shared one ($A_{Shared}=0.54$). The unique environmental factor for the Inattentive only

($E_{Inatt}=0.31$) and the RD only ($E_{RD}=0.26$) was higher than the shared unique environmental factor ($E_{Shared}=0.18$). There were non-additive genetic effects for the shared one ($D_{Shared}=0.23$) and for Reading Disability only ($D_{Inatt}=0.74$), which was the highest non-additive genetic effect. In addition, the ‘D’ for Inattentive subtype only was dropped from the model to examine if the model improved. The result showed that the probability of the additive (AE) genetic model ($P= 0.657$) was better than the probability of the ADE model ($P= 0.573$).

From table 6.15, the bivariate genetic model fitting for the Hyperactive-Impulsive subtype with RD was found to be significantly influenced by the non-additive genetic model ADE ($\chi^2 =28.265$, $P= 0.003$), with a moderately shared genetic factor for Hyp-Imp only ($A=0.45$) a nil shared non-additive genetic factor ($D= 0.00$) and a lower shared unique environmental factor ($E=0.11$). The additive genetic effect for the Hyperactive-Impulsive only subtype was mostly similar ($A_{Hyp-Imp}=0.82$) to the non-additive genetic effect for RD only ($A_{RD}=0.82$), however, the additive genetic effect for RD only was too low ($A_{RD}=0.05$). The unique environmental factors for Hyperactive-Impulsive only ($E_{Hyp-Imp}= 0.31$) and RD only ($E_{RD}= 0.30$) were higher than the shared unique environmental factor ($E_{Shared}= 0.11$). The highest non-additive genetic effect was for RD only ($D =0.083$). In addition, the ‘D’ for shared Hyp-Imp/RD and Hyp-Imp subtype only was dropped from the model to examine if the model improved. When this occurred, the model becomes an AE model and considered as a sub-model of ADE model. The result showed that the probability of the additive (AE) genetic model ($P= 0.008$) was better than the probability of the ADE model ($P= 0.003$).

Table 6.16 shows that the best genetic model fitting for the Combined subtype with RD was the non-additive model (ADE). Both measures indicated that there was shared additive genetic effect ($A_{shared}=0.56$), whereas the additive genetic factor for Combined only ($A_{Combined} =0.75$), was higher than the former. There were no dominant genetic effect for shared Combined/RD and Combined only ($D= 0$), whereas both disorders also have their own unique environmental factor. The unique environmental factors for the Combined subtype measured 0.26, and for RD measured 0.27. The best-fitting model was the ADE model ($\chi^2 =19.736$, $P= 0.049$) with a substantial non-additive

genetic effect for RD only ($D_{RD}=0.77$); however, this dominant genetic effect was zero with shared Combined/RD and Combined only ($D=0.000$). On the other hand, there was no additive genetic effect for RD only ($A_{RD}=0.00$). Because of this, both the A and D were dropped from this model to see if the model improved. The result showed that the model improved with a probability of 0.102.

Table 6.14

Bivariate model-fitting on the Inattentive subtype and Reading Disability of entire sample.

<i>ACE Model</i>	<i>Shared Inatt/RD</i>								<i>Inattentive Only</i>							<i>Reading Disability Only</i>								
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 67.144$ P = 0.000 d.f = 11 AIC = 45.144 RMSEA = 0.044	0.60	0.00	-	0.18	0.36	0.00	-	0.03	0.70	0.15	-	0.31	0.50	0.02	-	0.10	0.77	0.00	-	0.26	0.60	0.00	-	0.07
<i>AE Model</i>	<i>Shared Inatt/RD</i>								<i>Inattentive Only</i>							<i>Reading Disability Only</i>								
$\chi^2 = 67.557$ P = 0.000 d.f = 14 AIC = 39.557 RMSEA = 0.037	0.60	-	-	0.18	0.36	-	-	0.03	0.71	-	-	0.31	0.50	-	-	0.10	0.77	-	-	0.26	0.60	-	-	0.07
<i>CE Model</i>	<i>Shared Inatt/RD</i>								<i>Inattentive Only</i>							<i>Reading Disability Only</i>								
$\chi^2 = 1592.429$ P = 0.000 d.f = 14 AIC = 1564.429 RMSEA = 0.292	-	0.48	-	0.38	-	0.23	-	0.14	-	0.63	-	0.47	-	0.40	-	0.22	-	0.57	-	0.55	-	0.32	-	0.30
<i>ADE Model</i>	<i>Shared Inatt/RD</i>								<i>Inattentive Only</i>							<i>Reading Disability Only</i>								
$\chi^2 = 9.531$ P = 0.573 d.f = 11 AIC = -12.469 RMSEA = 0.003	0.54	-	0.23	0.18	0.29	-	0.05	0.03	0.72	-	0.00	0.31	0.52	-	0.00	0.10	0.08	-	0.74	0.26	0.01	-	0.55	0.07
<i>*ADE (Dropped d)</i>	<i>Shared Inatt/RD</i>								<i>Inattentive Only (dropped d)</i>							<i>Reading Disability Only</i>								
$\chi^2 = 9.531$ P = 0.657 d.f = 12 AIC = -14.469 RMSEA = 0.000	0.54	-	0.23	0.18	0.29	-	0.05	0.03	0.72	-	-	0.31	0.52	-	-	0.10	0.08	-	0.74	0.26	0.01	-	0.55	0.07
<i>ADE (Substitute D by C)</i>	<i>Shared Inatt/RD</i>								<i>Inattentive Only (Substitute D by C)</i>							<i>Reading Disability Only</i>								
$\chi^2 = 9.535$ P = 0.657 d.f = 12 AIC = -12.469 RMSEA = 0.000	0.54	-	0.23	0.18	0.29	-	0.05	0.03	0.41	0.59	-	0.31	0.17	0.35	-	0.10	0.08	-	0.74	0.26	0.01	-	0.55	0.07

* Most successful model fitting

Table 6.15

Bivariate model-fitting on Hyperactive-Impulsive subtype and Reading Disability of entire sample.

<i>ACE Model</i>	<i>Shared Hyp-Imp/RD</i>								<i>Hyperactive-Impulsive Only</i>								<i>Reading Disability Only</i>							
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 79.456$ P = 0.000 d.f = 11 AIC = 57.456 RMSEA = 0.047	0.44	0.00	-	0.10	0.19	0.00	-	0.01	0.73	0.39	-	0.31	0.53	0.15	-	0.10	0.87	0.00	-	0.30	0.76	0.00	-	0.10
<i>AE Model</i>	<i>Shared Hyp-Imp/RD</i>								<i>Hyperactive-Impulsive Only</i>								<i>Reading Disability Only</i>							
$\chi^2 = 96.544$ P = 0.000 d.f = 14 AIC = 68.544 RMSEA = 0.046	0.45	-	-	0.10	0.20	-	-	0.01	0.82	-	-	0.31	0.67	-	-	0.10	0.87	-	-	0.30	0.76	-	-	0.10
<i>CE Model</i>	<i>Shared Hyp-Imp/RD</i>								<i>Hyperactive-Impulsive Only</i>								<i>Reading Disability Only</i>							
$\chi^2 = 1637.996$ P = 0.000 d.f = 14 AIC = 1609.996 RMSEA = 0.296	-	0.40	-	0.23	-	0.16	-	0.05	-	0.73	-	0.50	-	0.53	-	0.25	-	0.63	-	0.63	-	0.40	-	0.40

Continued to Table

<i>ADE Model</i>	<i>Shared Hyp-Imp/RD</i>								<i>Hyperactive-Impulsive Only</i>								<i>Reading Disability Only</i>							
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2
$\chi^2 = 28.265$ P = 0.003 d.f = 11 AIC = 6.2652 RMSEA = 0.025	0.45	-	0.00	0.11	0.20	-	0.00	0.1	0.82	-	0.00	0.31	0.67	-	0.00	0.10	0.05	-	0.83	0.30	.003	-	0.69	0.10
<i>*ADE (Dropped-d)</i>	<i>Shared Hyp-Imp/RD (dropped-d)</i>								<i>Hyperactive-Impulsive Only (dropped-d)</i>								<i>Reading Disability Only</i>							
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2
$\chi^2 = 28.265$ P = 0.008 d.f = 13 AIC = 2.265 RMSEA = 0.023	0.45	-	-	0.11	0.20	-	-	0.1	0.82	-	-	0.31	0.67	-	-	0.10	0.05	-	0.84	0.30	.003	-	0.71	0.10
<i>DE Model</i>	<i>Shared Hyp-Imp/RD</i>								<i>Hyperactive-Impulsive Only</i>								<i>Reading Disability Only</i>							
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2
$\chi^2 = 211.667$ P = 0.000 d.f = 14 AIC = 183.667 RMSEA = 0.071	-	-	0.44	0.11	-	-	0.19	0.01	-	-	0.81	0.30	-	-	0.66	0.10	-	-	0.83	0.30	-	-	0.69	0.10
<i>ADE (Substitute D by C)</i>	<i>Shared Hyp-Imp/RD (dropped D)</i>								<i>Hyperactive-Impulsive Only (Substitute D by C)</i>								<i>Reading Disability Only</i>							
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	a^2	c^2	d^2	e^2
$\chi^2 = 28.265$ P = 0.005 d.f = 12 AIC = 4.265 RMSEA = 0.023	0.45	-	-	0.11	0.20	-	-	0.01	0.47	0.67	-	0.31	0.22	0.45	-	0.10	0.05	-	0.84	0.30	.003	-	0.70	0.10

** Most successful model fitting*

Table 6.16

Bivariate model-fitting on combined subtype and Reading Disability of entire sample.

<i>ACE Model</i>	<i>Shared Combined/RD</i>								<i>Combined Only</i>					<i>Reading Disability Only</i>										
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 57.827$ P = 0.000 d.f = 11 AIC = 35.827 RMSEA = 0.045	0.56	0.00	-	0.16	0.31	0.00	-	0.02	0.67	0.36	-	0.27	0.45	0.13	-	0.07	0.80	0.00	-	0.28	0.64	0.00	-	0.08
<i>AE Model</i>	<i>Shared Combined/RD</i>								<i>Combined Only</i>					<i>Reading Disability Only</i>										
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 68.892$ P = 0.000 d.f = 14 AIC = 40.892 RMSEA = 0.043	0.56	-	-	0.16	0.31	-	-	0.02	0.75	-	-	0.27	0.56	-	-	0.07	0.80	-	-	0.28	0.64	-	-	0.08
<i>CE Model</i>	<i>Shared Combined/RD</i>								<i>Combined Only</i>					<i>Reading Disability Only</i>										
	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²	<i>A</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>a</i> ²	<i>c</i> ²	<i>d</i> ²	<i>e</i> ²
$\chi^2 = 1585.190$ P = 0.000 d.f = 14 AIC = 1557.190 RMSEA = 0.308	-	0.48	-	0.33	-	0.23	-	0.11	-	0.70	-	0.42	-	0.50	-	0.18	-	0.60	-	0.55	-	0.36	-	0.30

Continued to Table

	Shared Combined/RD								Combined Only								Reading Disability Only								
	A	C	D	E	a ²	c ²	d ²	e ²	A	C	D	E	a ²	c ²	d ²	e ²	A	C	D	E	a ²	c ²	d ²	e ²	
<i>ADE Model</i>																									
$\chi^2 = 19.736$	0.56	-	0.00	0.16	0.31	-	0.00	0.03	0.75	-	0.00	0.26	0.56	-	0.00	0.07	0.00	-	0.77	0.27	0.00	-	0.60	0.07	
P = 0.049																									
d.f = 11																									
AIC = -2.264																									
RMSEA = 0.022																									
<i>*ADE (Dropped-d)</i>																									
	Shared Combined /RD (dropped-d)								Combined Only (dropped-d)								Reading Disability Only								
	A	C	D	E	a ²	c ²	d ²	e ²	A	C	D	E	a ²	c ²	d ²	e ²	A	C	D	E	a ²	c ²	d ²	e ²	
$\chi^2 = 19.736$	0.56	-	-	0.16	0.31	-	-	0.03	0.75	-	-	0.27	0.56	-	-	0.07	0.00	-	0.77	0.27	0.00	-	0.60	0.07	
P = 0.102																									
d.f = 13																									
AIC = -6.264																									
RMSEA = 0.019																									
<i>DE Model</i>																									
	Shared Combined/RD								Combined Only								Reading Disability Only								
	A	C	D	E	a ²	c ²	d ²	e ²	A	C	D	E	a ²	c ²	d ²	e ²	A	C	D	E	a ²	c ²	d ²	e ²	
$\chi^2 = 153.684$	-	-	0.55	0.16	-	-	0.30	0.03	-	-	0.75	0.27	-	-	0.56	0.07	-	-	0.77	0.27	-	-	0.60	0.07	
P = 0.000																									
d.f = 14																									
AIC = 125.684																									
RMSEA = 0.068																									
<i>ADE (Substitute D by C)</i>																									
	Shared Combined /RD (dropped-d)								Combined Only (Substitute D by C)								Reading Disability Only								
	A	C	D	E	a ²	c ²	d ²	e ²	A	C	D	E	a ²	c ²	d ²	e ²	A	C	D	E	a ²	c ²	d ²	e ²	
$\chi^2 = 19.736$	0.56	-	-	0.16	0.31	-	-	0.03	0.43	0.62	-	0.27	0.18	0.38	-	0.07	0.00	-	0.77	0.27	-	-	0.60	0.07	
P = 0.072																									
d.f = 12																									
AIC = -4.264																									
RMSEA = 0.021																									

* Most successful model fitting

6.4 Discussion

The results of the univariate genetic modelling have shown evidence of heritability for all of the *DSM-IV* ADHD subtypes, in support of previous findings that have also shown high heritability of ADHD Hudziak et al. (2005). Note the high heritability percentages for the *DSM-IV* ADHD subtypes: the heritability of the Inattentive subtype was 86%, of the Hyperactive-Impulsive subtype was 71%, and of the Combined subtype was 74%.

The best-fitting univariate model for the Inattentive subtype was AE. In addition, the AIC of the ADE model showed a value of -5.496, indicating that this model can be accepted. Since the non-additive genetic effect (D) was zero, this model was the same as the AE model. A genetic modelling study by Hudziak, Derks, Althoff, Rettew and Boomsma (2005) on ADHD using the Conners' Rating Scales found that both additive genetic factors (30%) and non-additive genetic factors (48%) played a role in the aetiology of the disorder. In the current study, the ACE model also showed an acceptable model ($\chi^2=0.000$, $P=1.000$, $AIC=-6.000$), with a high additive genetic effect ($a^2=0.85$), extremely low shared environmental effect ($c^2=0.03$), and low unique environmental effect ($e^2=0.13$).

The best fitting model for both the Hyperactive-Impulsive and Combined subtypes was the ACE model. Although all successful models showed ADHD subtypes were heritable, the results indicated that there is a variation in the heritability rates among ADHD subtypes as the additive genes for the Inattentive subtype was the highest ($a^2_{\text{Inattentive}}=0.86$), compared to the Hyperactive-Impulsive ($a^2_{\text{Hyp-Imp}}=0.71$), and Combined subtype ($a^2_{\text{Combined}}=0.74$). This might suggest that the rate of additive genes among ADHD subtypes is relatively different. In order to confirm this, further investigation is needed.

Levy, McStephen & Hay (2000) confirmed that the additive genetic factor is important in both Inattentive subtypes and Hyperactive-Impulsive subtypes; however, they found that a common environmental factor (C) is needed for Hyperactivity-Impulsivity but not for Inattentiveness. This conclusion has been supported in this study; the common environmental factor (C) for the Inattentive subtype was extremely low ($c^2_{\text{Inattentive}}=0.03$), whereas for Hyperactivity-Impulsivity, the common environmental factor (C) was moderately high ($c^2_{\text{Hyp-Imp}}=0.18$). The analysis also showed a moderate presence of unique

environmental effects in the three *DSM-IV* ADHD subtypes ($e^2_{\text{Inattentive}}=0.13$, $e^2_{\text{Hyp-Imp}}=0.11$, and $e^2_{\text{Combined}}=0.10$).

The univariate correlations for the Reading Disability measure showed that monozygotic twins had more than double the correlations of dizygotic twins, indicating the influence of non-additive genetic factors. The univariate analysis for Reading Disability showed that the best-fitting model fitting was the non-additive genetic factor (ADE) indicating that non-additive genes ($d^2_{\text{RD}}=0.66$) play a substantial role in the disorder, whereas the additive genetic effect was much lower ($a^2_{\text{RD}}=0.25$). In fact the non-additive effects that appeared were false effects, because the opposite-sex correlation was so low and limitation of sex was not dominant.

These results confirmed that there are gene(s) that contribute to both ADHD subtypes and to RD, leading to the question, do these genes cause both disorders, or does each disorder have its own genetic effects? Does every ADHD subtype also have a particular gene(s) or are these shared in the same genes? In addition, does ADHD-RD comorbidity result from different genes to those cause the individual disorder or subtype? To some extent bivariate analyses answers these questions.

Generally, the results of the bivariate analyses for each ADHD subtype with RD showed that both additive and non-additive genetic factors, in the ADE model, contributed in causing both disorders. This means that some genes on the same chromosome or alleles (polymorphism) at the same locus might be interacting together.

In the Combined and RD bivariate modeling, the ADE model showed the highest shared additive genetic effect ($a^2_{\text{Shared Combined/RD}}=0.31$) compared to the ADE model of Inattentive/RD ($a^2_{\text{Shared Inatt/RD}}=0.29$), and Hyperactive-Impulsive/RD ($a^2_{\text{Shared Hyp-Imp/RD}}=0.20$). These results indicate the degree of contribution of the shared genes in comorbidity of each ADHD subtype with RD. Willcutt et al. (2000) and Willcutt, DeFries et al. (2003) found a significant shared bivariate heritability effect in the RD/Inattention subtype ($h^2_{\text{Shared RD/Inatten}}=0.39$), and a weak shared bivariate heritability genetic effect in the RD/Hyperactive-Impulsive subtype ($h^2_{\text{Shared RD/Hyp-Imp}}=0.05$), with strong attributed common gene influences of the phenotypic overlap (95%) for the Inattentive/RD

comorbidity and low attributed common gene influences for Hyperactive-Impulsive/RD (21%). Furthermore, the estimates from the current study confirmed that the effects of the heritable shared additive genes for Hyperactive-Impulsive/RD were 20%, for Inattentive/RD were 29%, and for Combined/RD were 31%. These results lead to two conclusions; firstly the shared additive genes attributed to the Inattentive subtype with RD are more than shared additive genes attributed to the Hyperactive-Impulsive subtype with RD. This is supported by previous findings, that comorbidity of RD with the Inattentive subtype contributes more than the comorbidity of RD with Hyperactive-Impulsive and the Combined subtypes Stevenson (2001). Secondly, the contributed percentage of the same genes that exist among each ADHD subtype comorbid with RD is approximately 70%, however, about 30% of the shared genes of Inattentive ADHD-RD and Combined/RD are different from the shared genes of Hyperactive-Impulsive/RD. In addition, 93% of the shared additive genes of both Inattentive ADHD-RD and of Combined/RD are the same and only 7% of shared genes of Inattentive ADHD-RD are different from the shared genes of Combined/RD. So, it may be concluded that ADHD and RD are influenced by 70% of the same genes, and there are more shared genes between Inattentive ADHD-RD and Combined/RD comorbidity (93%) than between the Hyperactive-Impulsive/RD genes. In addition, it can be concluded that shared genes attributed to the comorbidity of each ADHD subtype with RD might be different to those acting on RD and ADHD as individual disorders.

Stevenson (2000a); Willcutt and Pennington (2002) suggested that a region on chromosome 6 (6p21.1) might be responsible for RD comorbidity with ADHD. Willcutt et al. (2002) hypothesised that the contribution of particular loci in chromosome 6p to ADHD/RD comorbidity can be due to a phenomenon called pleiotropy, where a particular gene can affect more than one phenotype. This hypothesis is supported by the findings from the current bivariate analyses: there were shared additive genetic effects indicating a presence of shared genes between each ADHD subtype and RD. This leads to the conclusion that similar shared additive genes are found in each ADHD subtype comorbid with RD. The expression of these particular shared genes can contribute to the aetiology of both ADHD and RD phenotypes to produce the comorbidity through the pleiotropy phenomenon.

Although the ADE model was the best bivariate model, the shared non-additive genetic effects were low in the Inattentive/RD model ($d^2_{\text{Shared RD/Inatt}}=0.05$), and were absent in the Hyperactive-Impulsive/RD and in the Combined/RD bivariate models. The additive genetic effects for RD only in the ADE models were low compared to in each separate ADHD subtype; i.e., the additive genetic effect for the Inattentive subtype only ($a^2_{\text{Inattentive only}}=0.52$: $a^2_{\text{RD only}}=0.01$), the Hyperactive-Impulsive only ($a^2_{\text{Hyp-Imp only}}=0.67$: $a^2_{\text{RD only}}=0.003$), and Combined only ($a^2_{\text{Combined only}}=0.56$, $a^2_{\text{RD only}}=0.00$). On the contrary, the non-additive genetic effects were high for Reading Disability only ($d^2_{\text{RD only}}=0.55, 0.71, 0.60$) in each ADE model. These results suggest that the aetiology of each ADHD subtype is influenced by additive genes, whereas RD is influenced by non-additive genetic effects, suggesting that RD aetiology can be caused by the interacting of particular alleles or genes on the same chromosome.

Although the bivariate analyses showed that ADE model was the best fit to the data, results showed that the dominant genetic effect was low for shared Inattentive/RD ($d^2_{\text{Shared RD/Inatt}}=0.05$), and was equal to zero with the shared Hyperactive-Impulsive/RD, shared Combined/RD, Inattentive ADHD only, Hyperactive-Impulsive only, and Combined only indicating that the interaction of the non-additive genes for ADHD subtypes was not significant. In the ADHD-RD bivariate modelling, the ADE model showed no dominant genetic effect in any ADHD subtype, leading to substitution of the non-additive genetic effect (D) with a common environmental effect (C), which resulted in the presence of this factor in the three ADHD subtypes ($c^2_{\text{Inatt only}}=0.35$, $c^2_{\text{Hyp-Imp only}}=0.45$, $c^2_{\text{Combined only}}=0.38$).

In conclusion, the best fitting model to represent the data in the bivariate analyses was the ADE model which also showed the effect of shared additive genes contributing to the comorbidity between each *DSM-IV* ADHD subtype and Reading Disability, suggesting that these shared loci or genes might be responsible for comorbidity through the pleiotropic phenomenon. The analyses also showed that the genetic effect found in RD were dominant, indicating involvement of interacting alleles or genes on the same chromosome. However, the dominant genetic effect between Inattentive ADHD and RD was minimal and was zero between RD and the Hyperactive-Impulsive and Combined subtypes. The bivariate analyses suggested an effect resulting from of the same shared

genes found among each ADHD subtype comorbid with RD; the degree of sharing is more similar between the comorbidities of the Inattentive and Combined subtypes comorbid with RD, and than between the Hyperactive-Impulsive subtype with RD. The study also found that this comorbidity contributed more to the presence of the Inattentive and the Combined subtypes and less to the Hyperactive-Impulsive subtype. Consequently, the relationship of these shared additive genes was higher between the Inattentive and Combined subtypes comorbid with RD, and less between Hyperactive-Impulsive ADHD and RD, suggesting particular genes play a role in comorbidity. In addition, the study found that ADHD-RD comorbidity is commonly influenced by 70% of same shared additive genes and 30% of shared additive genes of Hyperactive-Impulsive/RD are different from the shared genes of Inattentive ADHD-RD and Combined/RD. In addition, about 93% of the same shared additive genes were found between comorbidity of Inattentive ADHD-RD and of Combined/RD, and 7% of the shared additive genes were different between them.

In summary, for the question, “Does each disorder have its own genetic effects?”, the study findings suggest that the genetic components for RD only is different from the genetic components for ADHD only, given that the aetiology of ADHD subtypes was attributed to additive genetic effects, while that of RD was attributed to non-additive genetic effects. The additive genetic effects within ADHD subtypes were varied, suggesting relative additive-gene differences between ADHD subtypes.

Moreover, a tentative answer to the question, “Are ADHD and RD commonly influenced by the same or different genes, or does ADHD-RD comorbidity have distinct genes differing from those acting on RD and ADHD separately?”, the study found that ADHD and RD are commonly influenced by same genes, and there are more shared genes between Inattentive ADHD-RD and Combined/RD comorbidity than between shared genes of comorbid Hyperactive-Impulsive/RD. Also the shared genes attributed to the comorbidity of each ADHD subtype with RD might be different to those acting on RD and ADHD separately.

CHAPTER 7: ASSOCIATION AND HAPLOTYPE BLOCK ANALYSES

7.1 Introduction

There are two effective approaches to determine if genetics plays a role in the aetiology of any complex disorder. These two approaches are quantitative and molecular studies. One of the tools that quantitative genetics studies can use is statistical modelling programs such as Mx (Neale & Cardon, 1992). Mx enables genetic research using MZ and DZ twins to investigate common and dominant genetic effects, as well as the shared environmental and non-shared environmental effects for particular phenotype(s). In the molecular approach, linkage-and-association studies are used to identify gene(s) or Single Nucleotide Polymorphisms (SNPs) that contribute to a disorder.

The previous chapter showed that the aetiology of *DSM-IV* ADHD subtypes was due to additive genetic effects and ADHD's comorbidity with Reading Disability was due to shared genetic effects, indicating a role for particular genes from each disorder to cause this comorbidity. Identifiable genes may play a role in the comorbidity for each ADHD subtype with RD. Therefore the genotyping analysis aimed to investigate if ADHD candidate genes contributed to RD and if RD candidate genes contributed to ADHD subtypes, and which of these two gene groups contributed to ADHD-RD comorbidity. For this purpose, the study selected some Single Nucleotide Polymorphisms (SNPs) of ADHD candidate genes such as DRD4, DAT1 and others, and also some SNPs of RD candidate genes from chromosome 6p22.2.

There is a debate about the use of ADHD data identified by *DSM-IV* in molecular genetic studies, because of its inability to break down the phenotypes into homogenous groups. Therefore, as LCA can produce homogenous phenotypes, it was adopted in order to obtain genetically informative phenotypes for ADHD alone, RD alone, and for comorbid ADHD-RD. Accordingly, this study aimed to compare the *DSM-IV* criteria, and LCA categories, in order to evaluate which one would be more effective for performing genotyping analysis; *DSM-IV* or LCA? Would ADHD-RD individuals identified by *DSM-IV* and by LCA give similar or different genotyping results? Would each category have its

own genotyping results? Additionally, it was intended to determine if there are particular genes responsible for the comorbidity of each ADHD subtype with Reading Disability, especially with the Inattentive subtype.

In order to investigate the above problems, a genotype analysis, including a family-based association study and haplotype block analysis was carried out. In addition, this study sought to replicate previous genetic-association findings of ADHD and RD candidate genes on an Australian twin sample, as to the best of my knowledge none of the candidate genes used in this study has been replicated on an Australian twin sample. The family-based association approach was used to detect a genetic contribution between the nominated genes and these two disorders, while the haplotype block analysis was applied to detect the risk alleles of ADHD alone, RD alone, and ADHD-RD comorbidity from the selected genes.

An important point should be highlighted regarding the two categories used in the genotyping analysis. Despite identifying the *DSM-IV* ADHD subtypes based on the cut-off of six items, the actual data used in the family-based association study were the scores of the Unaffected, Inattentive, Hyperactive-Impulsive and Combined subtypes. This means that the *DSM-IV* ADHD data are continuous. On the other hand, the data used for the nine ADHD-RD latent classes were categorical (unaffected, affected, or do not know). Therefore, because *DSM-IV* ADHD data used was continuous and LCA data was categorical, two computer software programs were used for the family-based association study: The QTDT (Abecasis et al., 2000) for continuous data represented by *DSM-IV* score, and Haploview (Barrett et al., 2005), for discrete data represented by LCA (Barrett, 2007). By detecting the most statistically significant SNPs identified in the association analyses, we were able to perform a Linkage Disequilibrium (LD) mapping and haplotype-block analysis of the SNPs that contributed to ADHD and RD. These analyses help to detect variations responsible for causing the disorders and identifying genetic variations that increase susceptibility to ADHD, RD and ADHD-RD comorbidity, and to target risk alleles of ADHD and RD. The significance of these analyses might bring about a fuller understanding of the structure of the genetic variation at each ADHD-RD candidate gene. It might also help to identify a comprehensive and informative set of

ADHD-RD markers for association testing. The HapMap project (2003; 2005) is a resource for investigating complex disorders such as ADHD and RD by performing LD mapping, association, and haplotype-block analyses.

Faraone et al. (2005) reviewed the replicated genes that most significantly contribute to ADHD. Those genes are the Dopamine Transporter gene (DAT1), Dopamine Receptor D4 (DRD4), Synaptosomal-associated Protein of 25 kDa (SNAP-25), Sodium-dependent serotonin transporter (5HTT), and Catechol-O-Methyltransferase (COMT). Subsequently, Sklar (2005) constructed a model which illustrated the status of linkage disequilibrium relationships for SNPs across seven ADHD candidate genes (HTR1B, SLC6A4, DRD4, DRD5, SLC6A3, SNAP25, and DBH). The model is an extension of a meta-analysis conducted by Faraone et al. (2005) that found elevated odds ratios for ADHD in the seven genes. Sklar (2005) reported that molecular genetic studies for ADHD, plus the LD and haplotype map modelling, could occur because of the proliferation in LD and haplotype-block mapping studies detecting relevant genes contributing to the disorder.

This present study was designed to investigate ADHD-RD candidate genes through Single Nucleotide Polymorphisms (SNPs) of those candidate genes and some SNPs of the 6p21 region. The family-based association approach is a tool to detect whether any of these candidate SNPs are susceptible to ADHD and RD. Because participants in this study were identified using two different approaches - *DSM-IV* and Latent Class Analysis (LCA) (Hudziak et al., 1998; Neuman et al., 1999) - both the family-based association approach and haplotype analysis were performed on the *DSM-IV* ADHD subtypes, Reading Disability, and the nine ADHD-RD latent classes. Comparing these two approaches will ascertain if the results, in terms of the associated SNPs, contribute to each phenotype in the same way or to the same degree.

This chapter will present both the family-based association analyses and the haplotype-blocks mapping for ADHD and RD.

7.2 Methodology

7.2.1. Participants

The sample for genotyping analyses was derived from the LCA, *DSM-IV* ADHD and RD categories. For the family-based association study, thirty-seven twin families including the twins, their siblings and their parents were recruited, with a total of 190 individuals. There were 32 (16%) monozygotic twins, 42 (22%) dizygotic twins, and 42 siblings. The age range of the sample was from 4 years to 18 years old, with a mean age of 13.04 years old +/- 3.6 years. Table 7.1 shows frequency by sex for MZ twins, DZ twins, their siblings and their parents.

Table 7.1
Zygosity and sex frequencies of the genotyping sample

Category	Male	Female	Total
MZ	20	12	32
DZ	27	15	42
Siblings	21	21	42
Parents	37	37	74
Total	105	85	190

This study used three criteria for defining the affected and unaffected individuals with ADHD and Reading Disability: the *DSM-IV*; seven items for defining RD (Willcutt, Boada et al., 2003) , and Latent Class Analysis (LCA) (Neuman et al., 1999) for both ADHD and RD items.

7.2.2. Measures

7.2.2.1 Zygosity

Zygosity for the selected subjects was determined based on the zygosity measures described in chapter 3.

Researchers of behavioural genetics use zygosity in genotyping studies, such as family-based association studies, by selecting genetically informative twin families. Such studies can help to distinguish between two types of effect: genotypic association effects

between family components and within family components (Gosso et al., 2006). MZ twins can be genetically informative to the within-family component when paired with non-twin siblings, while DZ twins, whether paired or not with non-twin siblings, are considered to be genetically informative to both the within-family and the between-family components.

7.2.2.2. Ascertainment of DSM-IV ADHD subtypes

The ADHD subtypes phenotypic assessments by recruited twin families for the association study were based on a parent-report questionnaire, the Twin and Sibling Questionnaire (Hay & Levy, 2004). All of the 18 items assessing *DSM-IV* criteria for ADHD symptoms included in this questionnaire were based on the Australian Twin Behaviour Rating Scale (ATBRS) (Levy et al., 1996; Levy et al., 1997). These symptoms in children had been observed over the previous 12 months.

Table 7.2 shows the number of children recruited with each ADHD subtype. As the sample involved twin families, the approach used was a family-based association analysis. The association study was performed on all recruited families including parents, twins, and their siblings. The numbers of children who were identified as Inattentive and as Hyperactive-Impulsive were incidental in selection of participants, as most of the participants were diagnosed with Combined ADHD, with and without Reading Disability. Usually with molecular genetic studies, it is preferable to recruit a large sample, as this increases the validity of the results. Unfortunately, this study was limited only to 37 twin families because of limitation of funds.

7.2.2.3 Reading Disability measures

A seven-item questionnaire developed by Erik Willcutt and others (2003) was used in the Twin and Sibling Questionnaire to assess RD symptoms. Details of this measure were described in Chapter Three. Table 7.2 shows the numbers of recruited children identified as having one of the ADHD subtypes, RD and Inattentive or Combined ADHD comorbid with RD.

Table 7.2
The frequencies of RD and DSM-IV ADHD subtypes recruited for the genotyping analysis

<i>DSM-IV ADHD and RD</i>	<i>Frequency</i>
No ADHD	37
Inattentive	14
Hyperactive-Impulsive	4
Combined	35
RD	26
Parents	74
Total	190
Comorbid Inattentive/RD	6
Comorbid Combined/RD	18

7.2.2.4 ADHD-RD latent class measures

LCA is often considered as a factor analysis categorical variant, resulting in the identification of distinct diagnostic subtypes. LCA can also be used as a classification tool. Chapter 5 reported the result of the LCA which identified nine latent classes conducted with the sample (n=6535) of 2610 Australian twin families. Latent class models were fitted to the parents' responses about their offspring for the 18 *DSM-IV* ADHD symptoms and the seven RD items. The latent class analysis was performed by a Latent Class Analysis Program (LCAP) Version 2.34 (Neuman et al., 1999). Table 7.3 shows numbers of individuals recruited for the association study in each latent class. These individuals are the same ones shown in Table 7.2, but identified by different criteria.

As mentioned earlier, the association analysis was mainly designed to be performed on the 'Combined', 'Combined/RD', and any of the Reading Disability latent classes; however, families included in this genetic analysis also contained other children identified with other ADHD-RD latent classes. If any of those selected individuals had their other twin or sibling diagnosed with different LCA criteria, then all the children of these families were included in the analysis.

Table 7.3
The frequencies of the nine latent classes

Latent Class – 9	Frequency
1. Few Symptoms	37
2. Predominantly Hyperactive-Impulsive	4
3. Moderate Reading Disability	8
4. Predominantly Inattentive	6
5. Severe Reading Disability	8
6. Predominantly Inattentive & Reading Disability	3
7. Combined	17
8. Unique Severe Reading Disability	11
9. Combined & Reading Disability	22
Parents	74
Total	190

Table 7.4 shows the overlap between the selected latent classes with *DSM-IV* ADHD subtypes and with RD. 116 children appear in this table: the remaining 74 individuals were parents who were not included in the *DSM-IV* and LCA identification criteria, but were included in the family-based association study and haplotype block analysis. There was a discrepancy between the phenotype assigned for 13 children based on the two diagnostic approaches.

Table 7.4

The assignment of the selected latent ADHD-RD classes with DSM-IV ADHD subtypes by Reading Disability

<i>RD</i>	<i>ADHD RD Latent Classes</i>	<i>Total DSM-IV ADHD</i>				<i>Total</i>
		No ADHD	Inatt	Hyp-Imp	Combined	
RD absent	Few Symptoms	36	0	0	0	36
	Predominantly Hyp-Imp	0	<i>1*</i>	3	0	4
	Predominantly Inattentive	0	5	0	<i>1*</i>	6
	Severe RD	<i>1*</i>	0	0	0	1
	Combined	<i>1*</i>	<i>1*</i>	<i>1*</i>	14	17
	Combined & RD	<i>1*</i>	0	0	1	2
<i>Total</i>		38	7	4	17	66
RD present	Few Symptoms	1	0	0	0	1
	Moderate RD	8	0	0	0	8
	Severe RD	7	0	0	0	7
	Predominantly Inattentive & RD	0	2	0	<i>1*</i>	3
	Unique Severe RD	9	<i>2*</i>	0	0	11
	Combined & RD	<i>1*</i>	<i>2*</i>	0	17	20
<i>Total</i>		26	6	0	18	50

RD= Reading Disability, *Inatt*= Inattention, *Hyp-Imp*= Hyperactivity-Impulsivity

*RD**: Cases where LCA did not match with DSM-IV ADHD by RD

Table 7.5 shows the family structure of the recruited thirty-seven twin families (190 individuals). The minimum number of children in each family was two children either MZ or DZ twins, plus their parents, whereas the maximum number of children per each twin family was four children: two children as MZ or DZ twins and two children as non-twin siblings and their parents. Some families were composed of three children, two of them as MZ or DZ twins, with a non-twin sibling, and their parents.

Table 7.5

Family structure of the recruited families in the genotyping analysis

Family Structure	Phenotype	children
<u>I. Parents + Twins:</u> 3 families (12 individuals including parents)	<u>3 Families have:</u> 1 individual: Severe RD 5 individuals: Combined and RD	6
<u>II. Parents + Twins+ 1 Sibling:</u> 26 families (130 individuals Including parents)	<u>9 families have:</u> 27 Unaffected <u>17 Families have:</u> 6 Individuals: unaffected 2 individuals: Pred. Hyp.-Imp. 6 individuals: Moderate RD 3 individuals: Pred. Inattentive 7 individuals: Severe RD 2 individuals: Pred. Inattentive and RD 11 individuals: Combined 7 individuals: Unique Severe RD 7 individuals: Combined and RD	78
<u>III. Parents + Twins+ 2 Siblings:</u> 8 families (48 Individuals Including parents)	<u>8 families have:</u> 4 Individuals: unaffected 2 individuals: Pred. Hyp.-Imp. 2 individuals: Moderate RD 3 individuals: Pred. Inattentive 1 individuals: Pred. Inattentive and RD 6 individuals: Combined 4 individuals: Unique Severe RD 10 individuals: Combined and RD	32
Total: 190		116

7.2.3. Participants' recruitment for Genotyping Analysis

A recent method for DNA collection was used. The ORAgene™ DNA Self-Collection Kit is a non-invasive system for collecting DNA from saliva. It is an easy to use, reliable method of self-administered DNA collection. As demonstrated by Keddache and Lem (2004) DNA collected by ORAgene™ can provide the same DNA as that isolated from blood. Chartier and Birnboim (2004) reported that ORAgene™ is a suitable method for obtaining a high amount of DNA with considerably less bacterial contamination than buccal swabs. Each nominated family, including parents, twins and siblings was sent the saliva kit package.

7.2.3.1 Genotyping analysis

The SNP genotyping protocol was performed at the Queensland Institute of Medical Research (QIMR) laboratory by using a Sequenom MassARRAY Matrix-Assisted Laser Desorption/Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS). It can determine SNP allele frequency and is currently being used for analysis of SNPs. The candidate SNPs can be evaluated by chip-based MALDI-TOF MS called DNA MassARRAY™. DNA MassARRAY™ uses samples in chip-based, high-density arrays. This system accurately finds SNPs in individual DNA samples, or alternatively determines SNP allele frequencies. Buetow et al. (2001) applied this method and reported that mass spectrometry (MS) can yield a rapid SNP genotyping. Werner et al (2002) also used MALDI-TOF MS of primer extension assays in order to perform quantitative analysis of allele frequencies of SNPs, and stated that the MALDI-TOF MS method offers distinctive advantages such as the automation and simultaneous analysis of a large number of subjects, low costs for SNP assay establishment, and savings in the amount of DNA required.

The DNA collection and the genotyping analysis were performed in the genetic epidemiology laboratory at Queensland Institute of Medical Research (QIMR) in Brisbane-Queensland. As QIMR performed the protocol extraction of DNA from the saliva kits, they provided the procedure (see Appendix A3.1). Assays were designed to type 25 SNPs; however, four SNPs failed during the design and testing stage and were excluded. The remaining twenty-one SNPs came from nine ADHD-RD candidate genes (DAT1 ‘SLC6A3’, DRD4, HTR1B, COMT, SNAP25, KIAA0319, MRS2L, and THEM2) (Table 6.7). These four SNPs that failed were rs10535985, rs2038137, rs6039806, and rs6911855. The genotyping protocol plus the Polymerase Chain Reactions (PCRs) for the 21 SNPs are found in Appendices A3.2 and A3.3

QIMR lab performed the study of the candidate gene SNPs, based on previous linkage and association genetic studies which found genes that were candidates for ADHD and RD comorbidity because of their contributions to both disorders (Cope et al., 2005; Feng, Crosbie et al., 2005; Feng, Wigg et al., 2005; Francks et al., 2004; Shifman et al., 2002). Table 7.6 shows the candidate gene SNPs used in this association study. These SNPs were

selected from candidate genes and chromosomal regions contributing to ADHD and RD. These genes are DRD4, DAT1, SNAP25, COMT, and HTR1B, as well as genes from the 6p22.2 region. These genes are: MRS2L, KIAA0319, TTRAP, and THEM2.

Table 7.6
ADHD-RD candidate genes selected in SNPs assays

<u>SNP ID</u>	<u>*rs ID</u>	<u>Gene</u>	<u>Chromosome</u>	<u>Location</u>
SNP 1	rs3758653	DRD4	11p15.5	626399
SNP 2	rs27072			1447522
SNP 3	rs6347	SLC6A3 (DAT1)	5p15.33	1464412
SNP 4	rs362549			10217890
SNP 5	rs362987			10225452
SNP 6	rs362998			10225621
SNP 7	rs1051312	SNAP25	20p12.2	10235088
SNP 8	rs737865			18310121
SNP 9	rs4680			18331271
SNP 10	rs165599	COMT	22q11.21	18336781
SNP 11	rs2793422	MRS2L		24526327
SNP 12	rs4504469			24696863
SNP 13	rs2179515	KIAA0319		24736182
SNP 14	rs6935076			24752301
SNP 15	rs2143340	TTRAP		24767050
SNP 16	rs3777664	THEME2	6p22.2	24801825
SNP 17	rs2000292			78223664
SNP 18	rs6297			78228660
SNP 19	rs6296			78228979
SNP 20	rs6298			78229711
SNP 21	rs130058	HTR1B	6q14.1	78230000

* 'rs ID' is a reference to the SNP cluster created by NCBI dbSNP. Usually, SNPs are indexed by two different accession numbers in NCBI dbSNP: the **HANDLE | ID / NCBI | ssASSAY ID** forms which refer to an individual submission record, and the **NCBI | rsSNP ID** form which refers to the abstracted SNP and all associated records.

7.2.4 Statistical Analysis

The family-based genetic associations were calculated by using two kinds of software; Quantitative Transmission Disequilibrium Test (QTDT) (Version 2.5.1) (Abecasis et al., 2000) , and Haploview (Version 4.0 beta 15) (Barrett et al., 2005). QTDT was downloaded from <http://www.sph.umich.edu/csg/abecasis/QTDT/download/>, and Haploview was downloaded from

<http://www.broad.mit.edu/mpg/haploview/download.php>. The reason for using two software programs for performing the family-based genetic association tests was because QTDT can give more accurate results for association tests with continuous data, as the *DSM-IV* ADHD scores and by the the seven RD scores. Haploview software on the other hand is used with categorical data, as with the nine latent classes produced by LCA.

McGrath, Smith, and Pennington (2006) stated that differences in results between studies of complex disorders might be due to several factors, including different population selection and analysis techniques, differences in the test SNPs and genetic heterogeneity. These variations can result in inability to replicate an association, or an association with a different allele of an SNP, or different peaks of association within the same region. QTDT allows the analysis of quantitative or distinct traits in nuclear families, with or without parental genotypes. In addition, it incorporates variance components in the analysis of family data and includes exact estimation of p-values for analysis of small samples and non-normal data.

7.2.4.1 QTDT

Analysis of quantitative traits within nuclear families of varying sizes was possible using the Quantitative Transmission Disequilibrium (Abecasis et al., 2000). These authors introduced a general linkage-disequilibrium test by an orthogonal model for analysing the quantitative traits of nuclear families with or without the parental genotype information, which greatly increases the power of the data, and for the analysis of larger sibling relationships, in which identification of segregating alleles is more efficient. Untransmitted alleles act as an internal control for transmitted alleles, leading to a very robust experimental design. The method was based on Fulker, Cherny, Sham, and Hewitt's (1999) work, in that association effects are divided into between-family and within-family components. The latter has no confounding population-substructure effects, regardless of the composition of nuclear families. Abecasis et al. (2000) refuted the idea that the orthogonal method in other models - by Spielman et al. (1993), Allison (1997), and Rabinowitz (1997) - that provide disequilibrium in minimal family configurations, cannot detect linkage in nuclear families of any configuration, in the absence of disequilibrium. Under the flexible variance component framework, tests were

carried out between each of the SNPs and ADHD-RD phenotypes. These were tests of population stratification, total association, and within-family association.

In conducting these family-based genetic association tests, the QTDT analyses employed the orthogonal association model which divides the genotypic association effect into two discrete categories: orthogonal between-family components (β_b); and orthogonal within-family components (β_w) (Abecasis et al., 2000). It is important to note that population stratification or admixture has an effect on the between-family association component. The within-family component, on the other hand, is significant only where LD exists as a result of close linkage. Total association tests which amalgamate the within-family (β_w) and between-family (β_b) components may yield false positive or negative results due to effect of population stratification on the between-family (β_b) component. In order to allow for the influence of admixture on the association test results, population stratification was tested based on whether $\beta_b = \beta_w$ as proposed by Fulker, Cherny, Sham, & Hewitt (1999). Should $\beta_b = \beta_w$, the total association test mentioned previously can be applied to the entire population. In the context of this research, the relatively modest sample size meant that the total association test was more powerful than both the within-family and population stratification tests. Significant results obtained using the total association test are not nullified by the presence of a large degree of population stratification, due to the fact that any false association created does not affect the validity of the within-family component, which thus acts as a traditional test of association (Gosso et al., 2006).

Population stratification is considered as one of the obstacles in association studies, as it can give false-positive associations. This originates because the population contains mixed ethnicity, and some time a particular trait in one ethnic group is more frequent than in the other groups, and this shows positive associations with any allele that also happens to be more common in that group (Gosso et al., 2006). This is a greater problem for case-control study designs, and is less so for family-based study designs, as the within-family design in the QTDT minimises for this effect (Abecasis et al., 2000). In addition, Brookes et al. (2006) stated that the Transmission Disequilibrium Test (TDT)

provides excellent protection from population stratification effects. Accordingly, the QTDT model used in this association test was ‘The Total Evidence for Association’ (AT), which evaluates the total evidence for association for both within and between pairs. This model also correlates the sum of the phenotypic score with the sum of the number of risk alleles for each pair. The model for the Population Stratification (AP) was not applied because the former model contains the within-family design.

7.2.4.2 *Haploview*

Haploview software (Barrett et al., 2005) was also used to perform a family-based genetic association test, Linkage Disequilibrium (LD) and haplotype-block association analysis. Each phenotype was required to have two input files; the linkage pedigree format and marker information file. The linkage pedigree format file contained columns of family, individual, father, mother, gender, affected status, and marker genotypes. The affection status column of the each trait was also coded as 0 for unknown, 1 for unaffected and 2 for affected. The marker information file contained two columns, marker name and marker position for 21 SNPs. Each genetic marker was presented by two columns (one for each allele) and coded from 1 to 4, where: 1=A, 2=C, 3=G, and 4=T.

7.2.4.2.1 *Determining pairwise statistics LD by Haploview*

Haploview was used in this study to construct and compute an LD map for the 21 SNPs within a certain distance of each other, on which the LD between all possible pairs of inter-SNPs was measured by the coefficient D' . Haploview software maximised the information available from a pedigree for both LD analysis and the association test. For TDT association testing and for LD analyses, all available transmissions from parent-offspring in the pedigree file were utilised. Haploview was also used to define haplotype blocks, and to prepare plots of inter-SNP linkage disequilibrium (Gabriel et al., 2002). Haploview software usually allows measuring LD by coefficient D' or squared correlation coefficient r^2 values including viewing pairwise r^2 values to meet Gabriel’s definition (Barrett et al., 2005) of a haplotype block. The coefficient D' was calculated for each pairwise combination of SNPs using genotype data for the 21 SNPs from the 37 families.

Haploview software can also represent a graphic model for the studied SNPs which are in LD. Because LD between individual SNPs varies in each gene, the software is designed to demonstrate a graphic model exhibiting the degree of LD strength for each SNP by D' . Abecasis et al., (2000) suggested that D' values of greater than 0.33 are considered the minimum useful amount of LD.

The squared correlation coefficient r^2 is another helpful tool to measure LD. The squared correlation coefficient r^2 is considered to be the preferred calculation of population geneticists, with its values varying between zero and one; the former indicating the two markers are in complete equilibrium while the latter means that the same information exists in the two markers (Pritchard & Przeworski, 2001; Wall & Pritchard, 2003). The value of r^2 is determined by the division of D^2 by the product of the four allele frequencies at the two SNPs. Perfect LD occurs when r^2 is equal to 1. In this case, r^2 indicates the presence of recombination between a pair of haplotypes. This also indicates that the separation of SNPs has not occurred, and the allele frequency remains the same. Useful information can be obtained with the intermediate values of r^2 as information about one SNP can be used to calculate the effect of the second SNP (Pritchard & Przeworski, 2001; Wall & Pritchard, 2003).

7.3 Results

7.3.1 Estimation of heterozygosity rates for the 21 SNPs

The underlying heterozygosity rates for the 21 SNPs among 190 subjects were estimated using the Haploview software. This estimation included the marker's observed heterozygosity (ObsHET), the marker's predicted heterozygosity (PredHET), the Hardy-Weinberg equilibrium p value (HWpval), and the Minor Allele Frequency for each marker (MAF) (Table 7.7)

Hardy-Weinberg equilibrium showed probabilities ranged from 0.1032 to 1.0, indicating that the population was in Hardy-Weinberg equilibrium at the 21 SNPs. The Higher the ObsHET and PredHET, the more Minor Allele Frequencies rates can be obtained for an SNP. In order to avoid the rare heterozygous genotypes, MAF had to be > 0.05 . In this study, the percentage of SNPs with $MAF < 0.05$ was 4.60%, while the percentage of SNPs

with MAFs >0.05 was 95% (Table 7.7). According to this, rs4680 and rs165599 SNPs for the COMT gene, and rs2143340 for TTRAP gene showed the highest observed and predicted heterozygosity rates, with the highest MAFs respectively (0.478, 0.493, and 0.493) (Table 7.7). In contrast, the lowest MAF was rs2793422 SNP for the MRS2L gene; therefore, this SNP was not counted for genotyping analysis. Tagging single nucleotide polymorphisms (tag-SNPs) selection criteria was defined as SNPs with an MAF above 0.05. Furthermore, the percentages of non-missing genotypes (%Geno) and the number of fully genotyped family markers were estimated, and the observed genotype frequencies of all SNPs were within the distribution expected according to HWE. Because MZ twins have identical genotypes, they were considered as one genotype, when estimating allele frequencies. An Australian association study (Treloar et al., 2005) between five SNPs of the progesterone receptor gene and endometriosis on a sample of 1055 triads of affected women plus two parents, showed that all five SNPs were in Hardy-Weinberg equilibrium (probabilities 0.37–0.84) and the minor allele frequencies ranged from 0.061 to 0.241. Haploview detected 1.14% of mendelain errors, which were all eliminated from the analysis.

Table 7.7

List of selected SNPs with their estimated heterozygosity rates

#	Name	Position	ObsHET	PredHET	HWpval	%Genotype	FamTrios	MendErr	MAF	M.A
1	DRD4(rs3758653)	626399	0.399	0.361	0.8048	99.5	35	0	0.236	T:C
2	DAT1(rs27072)	1447522	0.511	0.453	0.2796	99.5	35	0	0.347	A:G
3	DAT1(rs6347)	1464412	0.413	0.446	0.8301	100.0	36	0	0.336	A:T
4	SNAP25(rs362549)	10217890	0.33	0.412	0.288	97.9	32	0	0.29	C:T
5	SNAP25(rs362987)	10225452	0.217	0.256	1.0	100.0	36	0	0.151	T:C
6	SNAP25(rs362998)	10225621	0.444	0.398	0.2629	100.0	36	0	0.274	C:T
7	SNAP25(rs1051312)	10235088	0.217	0.236	1.0	100.0	36	0	0.137	G:A
8	COMT(rs737865)	18310121	0.431	0.444	0.7379	99.5	35	0	0.333	G:A
9	COMT(rs4680)	18331271	0.541	0.499	0.9396	97.9	32	0	0.478	A:G
10	COMT(rs165599)	18336781	0.586	0.5	0.8563	98.4	33	0	0.493	A:C
11	MRS2L(rs2793422)	24526327	0.096	0.094	1.0	98.9	34	0	0.049	C:T
12	KIAA0319(rs4504469)	24696863	0.307	0.265	0.2549	100.0	36	0	0.158	T:C
13	KIAA0319(rs2179515)	24736182	0.332	0.333	1.0	98.9	34	0	0.211	A:G
14	TTRAP(rs6935076)	24752301	0.497	0.441	0.2218	100.0	36	0	0.329	C:T
15	TTRAP(rs2143340)	24767050	0.545	0.5	0.3175	100.0	36	0	0.493	G:A
16	THEME2(rs3777664)	24801825	0.365	0.41	0.3674	100.0	36	0	0.288	G:C
17	HTR1B(rs2000292)	78223664	0.196	0.185	1.0	94.7	30	0	0.103	A:G
18	HTR1B(rs6297)	78228660	0.365	0.41	0.3674	100.0	36	0	0.288	C:T
19	HTR1B(rs6296)	78228979	0.423	0.41	1.0	100.0	36	0	0.288	A:G
20	HTR1B(rs6298)	78229711	0.519	0.492	0.1032	100.0	36	0	0.438	G:A
21	HTR1B(rs130058)	78230000	0.42	0.413	0.7717	99.5	35	0	0.292	A:G

- # is the marker number.

- Name is the marker ID specified (only if an info file is loaded).

- Position is the marker position specified (only if an info file is loaded).

- ObsHET is the marker's observed heterozygosity.

- PredHET is the marker's predicted heterozygosity (i.e. $2*MAF*(1-MAF)$).

- HWpval is the Hardy-Weinberg equilibrium p value, which is the probability that its deviation from H-W equilibrium could be explained by chance.

- %Geno is the percentage of non-missing genotypes for this marker. The genotyping analysis was performed by QIMR laboratory.

- FamTrio is the number of fully genotyped family trios for this marker (0 for datasets with unrelated individuals).

- MendErr is the number of observed Mendelian inheritance errors (0 for datasets with unrelated individuals).

- MAF is the minor allele frequency (using founders only) for this marker.

- M.A. is the minor allele for this marker.

7.3.2. Single Locus Association Analyses

7.3.2.1. QTDT

7.3.2.1.1 DSM-IV ADHD and RD subtypes

The family-based association results for *DSM-IV* ADHD subtypes and Reading Disability (Table 7.8) provided evidence of significant association with two candidate SNPs. The rs3777664 SNP of the *THEM2* gene on the 6p22.2 region was associated with the Inattentive subtype (AT: $P=0.0323$) in 116 probands, whilst the rs3758653 SNP of *DRD4*

gene was associated with RD (AT: $P=0.0231$) in 115 probands. The remaining SNPs did not show significant evidence for association; however, two SNPs were closely associated. These were rs3777664 SNP of the MRS2L gene on chromosome 6p22.2 for the Combined subtype (AT: $p=0.0857$), and rs3758653 SNP of the TTRAP gene on chromosome 6p22.2 for Reading Disability (AT: $p=0.0788$).

Table 7.8

Total evidence for association for DSM-IV ADHD subtypes and Reading Disability

<u>DSM4</u>	<u>SNP</u>	<u>Gene</u>	<u>Chr</u>	<u>Chi Seq</u>	<u>P-value</u>	<u>d.f (x)</u>	<u>Probands</u>
1. Inattention	rs3777664 (SNP 16)	THEM2	6p22.2	4.58	0.0323	112	116
2. Hyp-Impulsive	rs2793422 (SNP 11)	MRS2L	6p22.2	2.127	0.1448	112	116
3. Combined	rs3777664 (SNP 16)	THEM2	6p22.2	2.95	0.0857	112	116
4. RD	rs2143340 (SNP 15)	TTRAP	6p22.2	3.09	0.0788	111	115
	rs3758653 (SNP 1)	DRD4	11p15.5	5.16	0.0231	111	115

7.3.2.2. Haploview

7.3.2.2.1 The nine latent ADHD-RD classes

The results of single locus family-based association analysis for the nine latent ADHD RD latent classes demonstrated ten out of twenty-one SNPs showed genetic evidence for association (Table 7.9). Out of the nine ADHD-RD latent classes, these significant associations occurred with six ADHD-RD latent classes: ‘Moderate RD’, ‘Predominantly Inattentive’, ‘Severe RD’, ‘Predominantly Inattentive and RD’, ‘Combined’, and ‘Unique Severe RD’ latent classes.

Both the rs4680 and rs165599 SNPs on the COMT gene showed association with the ‘Predominantly Inattentive and RD’ latent class ($\chi^2=5.0$, $P=0.0253$) with over-transmission of allele A (T:U ratio = 5:0); however the ‘Moderate RD’ latent class exhibited association with only the latter SNP ($\chi^2=4.455$, $P=0.0348$) with over-transmission of allele A (T:U ratio =5:0), whereas the former SNP did not show association with the ‘Combined RD’ latent classes ($\chi^2=2.882$, $P=0.0896$). Likewise, both rs6296 and rs2000292 SNPs of the HTR1B gene were associated with the ‘Severe RD’

and 'Combined' latent classes ($\chi^2=4.5$, $P=0.0339$; $\chi^2=6.0$, $P=0.0143$ respectively) with over-transmission of allele A (T:U ratio =7 :1) for the former, and with over-transmission of allele A (T:U ratio =6: 0) for the latter, this could be one of the reasons that this SNP did not exhibit significant association with the 'Combined RD' latent class ($\chi^2=3.0$, $P=0.0833$). The p value for rs6298 SNP of the same gene was not significant ($\chi^2=3.571$, $p=0.0588$) with the 'Predominantly Inattentive RD' latent class, however, it was close to $p<0.05$.

In addition, there were two Reading Disability candidate markers: the first was the rs2179515 SNP on the KIAA0319 gene, which revealed association with both the 'Predominantly Inattentive' and the 'Unique Severe RD' latent classes ($\chi^2=5.0$, $P=0.0253$) with over-transmission of allele A (T:U ratio =5: 0) in both latent classes. Furthermore, two SNP, rs6347 and rs27072 for the DAT1 (SLC6A3) gene, displayed association with both 'Combined' ($\chi^2=7.0$, $P=0.0082$) and 'Severe RD' ($\chi^2=4.5$, $p=0.0339$) latent classes, with over-transmission of allele G (T:U ratio =7: 0) for the former, and with over-transmission of allele A (T:U ratio =7: 1) for the latter. The second RD candidate marker was the rs2143340 on the TTRAP gene which demonstrated association with the 'Predominantly Inattentive RD' latent class ($\chi^2 =4.0$, $P=0.0455$) with over-transmission of allele A (T:U ratio =4: 0). Another two ADHD candidate markers; the rs27072 SNP on DAT1 gene, and the rs362987 on SNAP-25 gene displayed significant association with the 'Combined' ($\chi^2=8.067$, $p=0.00045$) and 'Unique Severe RD' ($\chi^2=4.0$, $p=0.0455$) respectively. These two SNPs also revealed over-transmission of the G allele and T allele with a transmitted: untransmitted (T:U) ratio of 13:2 for the former SNP and 4:0 for the latter.

Table 7.9

Association- single markers for the nine latent ADHD-RD classes

1. Association- Single Marker table for Pred Hyp-Imp Class					
SNP ID	Name	Over-transmitted	T:U	Chi Square	P value
SNP 6	SNAP25(rs362998)	T	3:0	3.0	0.0833
2. Association- Single Marker table for Moderate RD latent Class					
SNP 9	COMT(rs4680)	G	8:2	3.6	0.0578
SNP 10	COMT(rs165599)	C	9:2	4.455	0.0348
3. Association- Single Marker table for Predominantly Inattentive latent Class					
SNP 13	KIAA0319(rs2179515)	A	5:0	5.0	0.0253
SNP 20	HTR1B(rs6298)	G	6:1	3.571	0.0588
4. Association- Single Marker table for Severe RD latent Class LC5					
SNP 12	KIAA0319(rs4504469)	T	6:1	3.571	0.0588
SNP 19	HTR1B(rs6296)	A	7:1	4.5	0.0339
SNP 20	HTR1B(rs6298)	G	7:2	2.778	0.0956
5. Association- Single Marker table for Predominantly Inattentive RD latent Class LC6					
SNP 9	COMT(rs4680)	A	5:0	5.0	0.0253
SNP 10	COMT(rs165599)	A	5:0	5.0	0.0253
SNP 14	TTRAP(rs6935076)	T	3:0	3.0	0.0833
SNP 15	TTRAP(rs2143340)	A	4:0	4.0	0.0455
6. Association- Single Marker table for Combined latent Class LC7					
SNP 2	DAT1(rs27072)	G	13:2	8.067	0.0045
SNP 4	SNAP25(rs362549)	T	9:3	3.0	0.0833
SNP 7	SNAP25(rs1051312)	G	7:0	7.0	0.0082
SNP 17	HTR1B(rs2000292)	A	6:0	6.0	0.0143
7. Association- Single Marker table for Unique Severe RD Class LC8					
SNP 5	SNAP25(rs362987)	T	4:0	4.0	0.0455
SNP 6	SNAP25(rs362998)	C	9:3	3.0	0.0833
SNP 10	COMT(rs165599)	C	8:2	3.6	0.0578
SNP 13	KIAA0319(rs2179515)	A	5:0	5.0	0.0253
8. Association- Single Marker table for Combined RD Class LC9					
SNP 7	SNAP25(rs1051312)	A	6:1	3.571	0.0588
SNP 8	COMT(rs737865)	G	13:5	3.556	0.0593
SNP 9	COMT(rs4680)	G	12:5	2.882	0.0896
SNP 10	COMT(rs165599)	C	11:4	3.267	0.0707
SNP 12	KIAA0319(rs4504469)	C	11:4	3.267	0.0707
SNP 17	HTR1B(rs2000292)	A	3:0	3.0	0.0833

This family-based association analysis was designed to mainly target the Combined latent classes with and without RD, and the RD latent classes. While the study recruited numbers of these latent classes (Table 7.4), it accidentally recruited some numbers for the other latent classes. Although individual numbers of these latent classes were lower than the Combined RD latent class (22 individuals), the former latent classes exhibited some genetic association, whereas the latter did not. One of the limitations was the low sample size in this study. As commonly known, the bigger the sample, the higher the chances to obtain genetic association. Despite this the association test for the ‘Combined RD’ latent class showed results close to $p < 0.05$.

This indicates promising results if the sample size were increased. However, I attempted to re-examine this latent class again by looking to the family structure of those families within this latent class. It was found that there were two MZ twin families without siblings, who were considered as non-informative as they have identical genetic structure. Another family whose parent’s genotype information was incomplete, meant that Haploview could not use their genetic information. These three individuals were eliminated from the sample. Then the association test was applied on this revised latent class. The SNPs which were close to < 0.05 showed significant associations (Table 7.10).

Table 7.10
Association- single markers for the ‘Combined RD’ latent class

Association- Single Marker table for Combined RD Class LC9					
SNP ID	Name	Over-transmitted	T:U	Chi Square	P value
SNP 8	COMT(rs737865)	G	13:4	4.765	0.029
SNP 9	COMT(rs4680)	G	12:3	5.4	0.0201
SNP 10	COMT(rs165599)	C	11:3	4.571	0.0325
SNP 12	KIAA0319(rs4504469)	C	11:3	4.571	0.0325

All three COMT SNPs showed associations: the rs737865 ($\chi^2=4.765, p =0.029$), rs4680 ($\chi^2 =5.4, p =0.0201$), rs165599 ($\chi^2=4.571, p =0.0325$). The transmitted : untransmitted (T:U) ratios of 13:4, 12:3, and 11:3, also showed over-transmission of the alleles G, G, and C

respectively. The rs165599 in the KIAA0319 gene also showed an association ($\chi^2=3.486$, $p=0.0325$), with over-transmission of allele C (T:U ratio = 11: 3).

7.3.3 Haplotype Mapping Analyses

7.3.3.1. Pair-wise linkage disequilibrium analysis and Construction of SNP Blocks

Both D' and r^2 were used as pair-wise measures of Linkage Disequilibrium (LD) among the 21 markers for 190 subjects used in the haplotype mapping analysis by Haploview. The haplotype-blocks mapping analysis showed the presence of one significant haplotype block among the twenty-one SNPs according to inter-marker LD coefficient plot with the *DSM-IV* ADHD subtypes, RD category, and the nine ADHD-RD latent classes (Figure 7.11). This haplotype block spans 5 kb and covers the SNP 9 (rs4680) and SNP 10 (rs165599) regions of the COMT gene, based on a 95% confidential interval on D' values (Ardlie et al., 2002). The pair-wise marker-to-marker LD calculated by D' and r^2 statistics (Ardlie et al., 2002; Pritchard & Przeworski, 2001; Wall & Pritchard, 2003). All SNPs were tagged by the htSNPs used in this study with the criteria of $D' > 0.80$ and $r^2 > 0.80$. The test showed a single LD block consisting of SNP 9 and SNP 10, which selected htSNPs with $D'=1$, its CI=0.94-1.0, and its $r^2=0.97$. The captured allele test showed that 20 SNPs in 20 tests captured 21 out of 21 (100%) alleles at $r^2 \geq 0.80$, with maximum mean r^2 of 0.999.

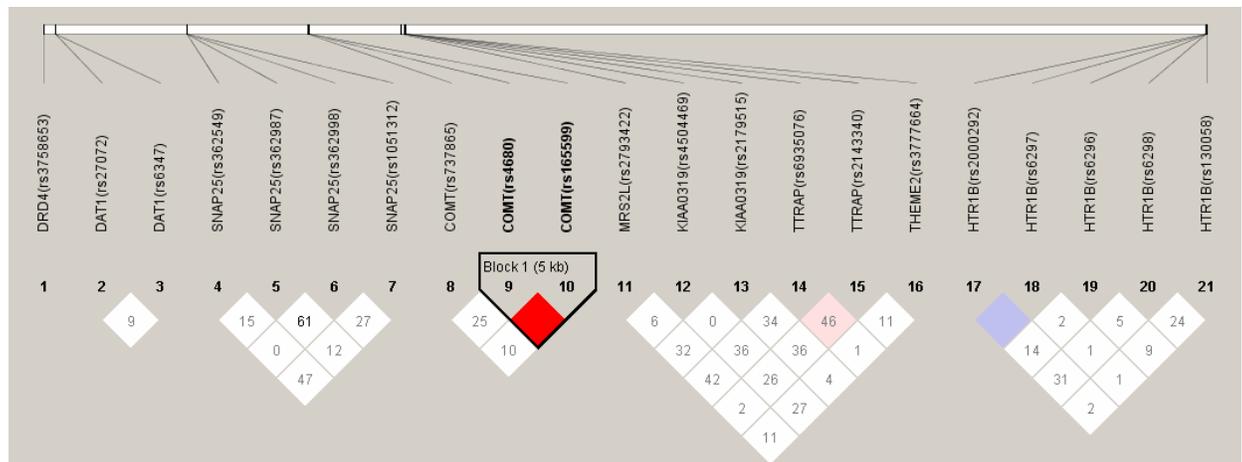


Figure 7.1 Diagram depicting the genomic organisation of the 21 SNP markers and showing Linkage Disequilibrium plot of all SNPs revealing one significant haplotype block containing two htSNPs; rs4680 and rs165599, on the COMT gene.

Although LD is used when SNPs are on the same chromosome, it can be also calculated for SNPs on different chromosomes in order to test for map error. The pairwise LD is used when SNPs are on the same chromosome. Consequently, the 21 SNPs used in this study are markers from six chromosomes, i.e., each group of SNPs are located on the same chromosome; the two DAT1 SNPs located on chromosome 5, the four SNAP25 SNPs located on chromosome 20, the three COMT SNPs located on chromosome 22, the six SNPs of MRS2L, KIAA0319, TTRAP, and THEME2 genes, as well as the five HTR1B SNPs are all located on chromosome 6. Based on this, Haploview was applied to determine LD map for the particular SNPs that located on the same chromosome. The finding showed a significant LD block containing two SNPs of COMT gene located on the same chromosome (Figure 7.1).

7.3.3.2 Haplotype-based association analysis

SNP 9 (rs4680) revealed a single-locus association with the ‘Predominantly Inattentive RD’ latent class (Table 7.9). This significant haplotype block was analysed for the presence of haplotype-block association, including haplotype block frequencies for each *DSM-IV* ADHD subtype, RD category and the nine ADHD RD Latent classes. The analysis revealed a presence of three risk haplotypes (“alleles”) (‘AA’, ‘GC’, and ‘AC’) for the haplotype block located within a block of SNP 9 (rs4680) and SNP 10 (rs165599) on the COMT gene. The risk haplotype population frequencies for the detected haplotype block were 0.507 for ‘AA’, 0.478 for ‘GC’, and 0.015 for ‘AC’ haplotypes. The haplotype analysis showed no evidence of significant haplotype association with the three *DSM-IV* ADHD subtypes and the RD category (Table 7.11), and three ADHD-RD latent classes (‘Predominantly Hyperactive-Impulsive’, ‘Predominantly Inattentive’, and ‘Severe RD’ latent classes) (Table 7.12).

However, it demonstrated significant haplotype association with the other five ADHD RD latent classes (Table 7.13). The ‘AA’ and ‘GC’ risk haplotypes were significant with these ‘Moderate RD’ (AA: T:U=1.0 : 8.0, $\chi^2 = 5.444$, $p=0.0196$; ‘GC’: T:U=8.0 : 1.0, $\chi^2 = 5.511$, $p=0.0189$), ‘Predominantly Inattentive RD’ (‘AA’: T:U=5.0 : 0.0, $\chi^2 = 5.0$, $P=$

0.0253; ‘GC’: T:U=0.0 : 5.0, $\chi^2 = 5.0$, $P= 0.0253$), ‘Unique Severe RD’ (‘AA’: T:U=1.0 : 7.0, $\chi^2 = 4.5$, $P= 0.0339$; ‘GC’: T:U=7.0 : 1.0, $\chi^2 = 5.565$, $P= 0.0326$), and ‘Combined RD’ (‘AA’: T:U=3.0 : 12.0, $\chi^2 = 5.376$, $P= 0.0204$; ‘GC’: T:U=12.0 : 3.0, $\chi^2 = 5.426$, $P= 0.0198$). However, the ‘AC’ risk haplotype was significant with the fifth latent class: ‘Combined’ (T:U=3.9: 0.0, $\chi^2=3.939$, $p=0.0472$). In addition, the ‘AA’ risk haplotype for both DSM-IV Combined subtype and Reading Disability category exhibited P-values close to >0.05 : $\chi^2 = 3.563$, $P= 0.0591$; $\chi^2 = 3.741$, $P= 0.0531$ respectively (Table 7.12).

Table 7.11
Haplotype block analyses for DSM-IV ADHD subtypes

ADHD RD Phenotypes	Haplotype	Haplotype Association- Block: htSNPs (rs4680 and rs165599)			
		Freq	T:U	χ^2	P- value
1. DSM-IV Predominantly Hyperactive Impulsive Subtype	AA	0.507	2.0 : 2.0	0.0	1.0
	GC	0.485	2.0 : 2.0	0.0	0.9939
	AC	0.015	0.0 : 0.0	NaN	0.0
2. DSM-IV Predominantly Inattentive Subtype	AA	0.514	9.0 : 6.0	0.6	0.4386
	GC	0.471	4.0 : 9.0	1.923	0.1655
	AC	0.015	2.0 : 0.0	2.0	0.1573
3. DSM-IV Predominantly Combined Subtype	AA	0.514	9.0 : 19.0	3.563	0.0591
	GC	0.478	19.0 : 11.0	2.159	0.1417
	AC	0.015	0.0 : 0.0	NaN	0.0
4. Reading Disability (RD)	AA	0.507	16.0 : 29.0	3.741	0.0531
	GC	0.478	29.0 : 15.9	3.796	0.0514
	AC	0.015	0.0 : 0.1	0.089	0.7652

- T:U is the ratio of transmission to non transmission of the overtransmitted allele

Table 7.12
Haplotype block analysis for the nine latent classes

ADHD RD Phenotypes	Haplotype	Haplotype Association- Block: htSNPs (rs4680 and rs165599)			
		Freq	T:U	χ^2	P- value
1. Predominantly Hyperactive Impulsive Latent Class- LC2	AA	0.507	2.0 : 2.0	0.0	1.0
	GC	0.478	2.0 : 2.0	0.0	0.9878
	AC	0.015	0.0 : 0.0	0.031	0.8608
2. Moderate RD Latent Class- LC3	AA	0.507	1.0 : 8.0	5.444	0.0196
	GC	0.478	8.0 : 1.0	5.509	0.0189
	AC	0.015	0.0 : 0.0	0.03	0.8628
3. Predominantly Inattentive Latent Class- LC4	AA	0.507	4.0 : 2.0	0.667	0.4142
	GC	0.478	1.0 : 4.0	1.8	0.1797
	AC	0.015	1.0 : 0.0	1.0	0.3173
4. Severe RD Latent Class- LC5	AA	0.507	3.0 : 1.0	1.0	0.3173
	GC	0.478	1.0 : 3.0	1.0	0.3173
	AC	0.015	0.0 : 0.0	NaN	0.0
5. Predominantly Inattentive RD Latent Class- LC6	AA	0.507	5.0 : 0.0	5.0	0.0253
	GC	0.478	0.0 : 5.0	5.0	0.0253
	AC	0.015	0.0 : 0.0	NaN	0.0
6. Combined Latent Class- LC7	AA	0.507	4.0 : 9.0	1.923	0.1655
	GC	0.478	7.0 : 5.9	0.087	0.7679
	AC	0.015	3.9 : 0.0	3.939	0.0472
7. Unique Severe RD Latent Class- LC8	AA	0.514	1.0 : 7.0	4.5	0.0339
	GC	0.471	7.0 : 1.0	4.565	0.0326
	AC	0.015	0.0 : 0.0	0.031	0.8598
8. Combined RD Latent Class- LC9	AA	0.507	3.0 : 12.0	5.376	0.0204
	GC	0.478	12.0 : 3.0	5.426	0.0198
	AC	0.015	0.0 : 0.1	0.06	0.8069

- Overtransmitted is the allele overtransmitted to affected offspring.
- T:U is the ratio of transmission to non transmission of the overtransmitted allele

7.4 Discussion

7.4.1 Family-based association analysis on the DSM-IV ADHD subtypes and RD

continuous data

This study investigated a family-based genetic association of twenty-one candidate SNPs (Table 7.6) on a sample consisting of 190 individuals (37 twin families) diagnosed by three different but related measures: the *DSM-IV* ADHD criteria, Willcutt's Reading Disability seven items (Willcutt, Boada et al., 2003), and Latent Class Analysis (LCA) (Hudziak et al., 1998; Neuman et al., 1999; Rasmussen, Neuman et al., 2002). The selection of the thirty-seven twin families was based mainly on the LCA, which defined as 'Combined', 'Combined & RD', 'Moderate RD', 'Severe RD', or 'Unique Severe RD'.

However, other ADHD and RD phenotypes were also included. The purpose for choosing these latent classes was to strengthen the sample as its size was low. In addition, the purpose of selecting families who had at least one child diagnosed by one of the three RD latent classes was to investigate the genetic nature of RD alone and its comorbidity with ADHD.

The goal of the family-based association study was to investigate whether ADHD RD individuals identified based on DSM-IV ADHD diagnostic criteria or LCA would give better association results. This study investigated the genetic contribution of twenty-one SNP markers from nine ADHD and RD candidate genes (DRD4, DAT1, SNAP-25, COMT, MRS2L, KIAA0319, TTRAP, THEME2, and HTR1B) with several phenotypes for ADHD and RD (*DSM-IV* ADHD subtypes, Reading Disability category, and nine ADHD-RD latent classes).

The family-based genetic association study was performed by using the QTDT program (Abecasis et al., 2000) on the *DSM-IV* ADHD classifications, and Willcutt's seven RD items. The results showed only two evidences of association. The first was rs377664 SNP from the THEM2 gene which was associated with the Inattentive subtype. The SNP of this gene is located in 6p22.2 region, suggesting this region might contribute to the Inattentive subtype as previous studies suggested its contribution to RD. This is supported by Willcutt et al's (2002) study that suggested that the quantitative trait locus for RD on chromosome 6p is also susceptible to ADHD.

The other association was rs3758653 SNP from the DRD4 gene which was associated with Reading Disability. This result suggests that the 7-repeat allele of the 48-bp tandem repeat in exon 3 of the DRD4 might be implicate in the aetiology of RD. This is supported by two studies; Levitt, Harvey, Friedman, Simansky, and Murphy (1997) who showed evidence of the involvement of neurotransmitters in brain development. Based on this, Hsiung, Kaplan, Petryshen, Lu, and Field (2004) suggested that DRD4 is considered a candidate gene for RD, independent of the relationship between ADHD and RD. This relationship was based on Willcutt et al's. (2003) finding that both the Inattentive subtype of ADHD and Reading Disability can co-occur. They found evidence of bivariate

heritability ($h^2=0.39$). Accordingly, Hsiung et al. (2004) performed a linkage and association study on 14 markers at and around DRD4, and revealed evidence of significant linkage but not significant linkage disequilibrium between RD and DRD4.

As Faraone, Doyle, Mick, and Biederman (2001) reported a significant association of the 7-repeat allele of the 48-bp tandem repeat in exon 3 of the DRD4 with ADHD, these results imply that the 6p22.2 region may contribute to ADHD, especially to the Inattentive subtype, and that, in addition to DRD4 contributing to ADHD, DRD4 may also contribute to RD aetiology. Further investigations are required to confirm this relationship between Inattention and RD and DRD4 gene and the overlap of RD candidate genes with ADHD, and vice versa.

7.4.2 Family-based association analysis on the nine ADHD-RD latent classes

The association analysis was also performed on the discrete data of the ADHD-RD represented by LCA. The analysis explored a variety of significant associations of the 21 SNPs with ADHD and RD. These significant SNPs were from six ADHD and RD candidate genes: DAT1, SNAP-25, COMT, KIAA0319, TTRAP, and HTR1B.

7.4.2.1 DRD4 gene

Despite the association of the DRD4 7-repeat 48-bp VNTR gene having been confirmed with ADHD (Faraone et al., 2001; Faraone et al., 2005; Tahir et al., 2000), the rs3758653 SNP (i.e., is one of DRD4 polymorphisms -906 T>C was selected and examined in this study) did not show association with ADHD. Other SNPs for the DRD4 gene have been examined (Brookes et al., 2006), and showed association with ADHD (rs180955, rs747302, rs9195457). Since we did not include those SNPs, in this study, we were not able to confirm an association of DRD4 SNPs with ADHD, although it is possible that are associated.

7.4.2.2 DAT1 gene

DAT1 or SLC6A3 is considered the most common candidate gene in genetic studies of ADHD. One of the VNTR located in the 3' untranslated region (3'UTR), which is the 10-repeat allele (480 bp), was replicated in several ADHD genetic studies (Barr, Xu et al.,

2001; Cook et al., 1995; Curran, 2001; Waldman et al., 1998). The presence of a strong association of haplotype blocks, containing the 10-repeat allele with alleles of markers located in exon 9 (rs6347) and intron 9 (rs8179029), with ADHD (Barr, Xu et al., 2001) has been reported. In addition, Feng et al. (2005) studied VNTR polymorphisms in DAT1 including the *MspI* polymorphism (rs27072) located 480 bp upstream of the VNTR, the *DraI* DNA change (T/C) located 134 bp downstream of the VNTR, and the exon 9 (rs6347) and intron 9 (rs8179029) polymorphisms. Their findings indicate an association of DAT1 with ADHD; however, this association was not seen with alleles of VNTR. It was only observed with the *MspI* polymorphism (rs27072) (P value= 0.009), with a transmission of G allele, implicating the contribution of 3' region of DAT1 on ADHD.

Accordingly, this study has examined the rs6347 in exon 9, and the rs27072 of *MspI*. The former, with G allele over-transmission, showed strong evidence of association with the 'Combined' latent class (P value= 0.0045), while the former did not show the association. This result replicated a previous study by Feng et al. (2005) and supported the implication of DAT1 gene with ADHD. In this instance, as our study used two classifications, the *DSM-IV* and LCA, the single-locus association result specified this genetic contribution with the combined subtype, rather than ADHD in general. This has led to the conclusion that the rs27072 of *MspI* polymorphism on 3' region of DAT1 affects the Combined subtype. This association was obtained from Latent Class-defined categories, rather than *DSM-IV*-defined categories. This finding can be supported by Todd et al.'s (2001) argument that LCA is a more appropriate approach for determining genetic contributions than *DSM-IV* ADHD diagnostic criteria.

7.4.2.3 *SNAP-25 gene*

Two out of four SNAP-25 SNPs (rs362549, rs362987, rs36299, and rs1051312), showed association with two latent classes. These two SNPs were rs1051312, which showed a strong association with the 'Combined' latent class (P value= 0.0082), and rs362987, which showed association with the 'Unique Severe RD' latent class (p - value= 0.0455). The association found in the latter SNP is supported by Feng et al. (2005) findings, which found that rs362549, rs362987, and rs362998 of the SNAP-25 gene showed an association (P value= 0.012, P value= 0.039, and P value= 0.019 respectively) with the

three subtypes of *DSM-IV* ADHD in a Toronto sample (Feng, Crosbie et al., 2005), whereas no evidence for association was observed with the Irvine sample, which included only the *DSM-IV* Combined subtype. To my knowledge, this is the first study to show the association of the marker rs1051312 with the ‘Combined’ latent class; however, this association needs to be replicated and confirmed. As SNAP-25 is a candidate gene for ADHD, the association of rs362987 with the ‘Unique Severe RD’ latent class might indicate there could be some shared genes between ADHD and RD. In contrast, there were non-significant associations of the other SNPs such as the rs1051312 with the ‘Combined RD’ latent class (P value= 0.0588), and with the ‘Unique Severe RD’ (P value= 0.0833), and with the ‘Combined’ latent class (P value= 0.0833). A small sample size could be the reason for obtaining these non-significant results; increasing the sample size might convert these results into significant ones.

7.4.2.4 *COMT* gene

There were three SNPs from the Catechol-O-Methyltransferase (*COMT*) gene utilized in the study (rs4680, rs165599, and rs737865). These SNPs exhibited association six times with three latent classes. The rs4680 showed association two times, one with the ‘Predominantly Inattentive RD’ latent class (P value= 0.0253), the other one was with the ‘Combined RD’ latent class (P value= 0.0201). The rs165599 SNP showed association with the ‘Moderate RD’, ‘Predominantly Inattentive RD’ and the ‘Combined RD’ latent classes (P values= 0.0348, 0.0253, and 0.0325 respectively). The rs737865 SNP expressed association among the ‘Combined RD’ latent class (P value= 0.0201). There is a pattern among the mentioned associations: all associations occurred in the classes that involve RD, either as independent RD classes, as such ‘Moderate RD’ latent class, or in comorbid form as in the ‘Predominantly Inattentive RD’ and the ‘Combined RD’ latent classes. Several studies argued that the *COMT* gene might be an aetiological genetic factor for ADHD; however, other studies did not show this association. Generally, several replicated studies of the *COMT* gene polymorphism did not show clear evidence of association with ADHD. Recently, Cheuk and Wong (2006) performed an association meta-analysis on twelve studies and found no significant association between the *COMT* gene polymorphism and ADHD. Turic et al. (2005) tested the rs165599 and rs737865 SNPs in

children with ADHD and their parents and found no evidence for association with the disorder. In contrast, the rs4680 SNP of COMT gene (Val allele) demonstrated significant association in previous studies on ADHD (Eisenberg et al., 1999; Qian et al., 2003; Thapar et al., 2005). In addition, the SNP also expressed significant association with DSM-IV Hyperactive-Impulsive subtype (Eisenberg et al., 1999) . This SNP was found to be significant, based on the occurrence of over-transmission of the Met allele in boys, but not in girls (Qian et al., 2003), and also showed significant association (P value= 0.002) with birth weight and gene environment interaction (COMT x birth weight) (Thapar et al., 2005) .

According to the significant results obtained from this study, it is suggested that COMT gene might be a susceptible gene for ADHD-RD comorbidity. To my knowledge, no previous studies investigated if COMT was a candidate gene for RD or might be a candidate for the comorbid ADHD and RD. Based on this finding, the study concluded that there is a shared pattern found among the above mentioned associations that all associations occurred in classes that involve RD, either independent RD classes or in comorbid form. This suggests COMT should be considered as a candidate gene for RD or for the comorbid ADHD and RD. The study found two possible reasons to support this suggestion. Firstly, based on the genetic fitting models, the bivariate analysis showed a presence of shared additive gene effects between ADHD subtypes and RD, especially the Inattentive and Combined subtypes. This shared genetic relationship can be represented by finding the association of the COMT gene with the comorbid Inattentive and Combined latent classes with RD. The second possibility is also based on the genetic fitting model, as the genetic effect that RD exhibited was dominant, indicating a presence of non-additive gene(s) that might contribute to the aetiology of RD. It could be that the RD genetic component in this sample is present in the COMT gene as a result of interaction between its alleles. The evidence of this conclusion is the association of this gene found with the 'Moderate RD' latent class. However, the shared non-additive genetic effects were insignificant between all ADHD subtypes and RD. Therefore, the first possibility is more logical to accept. Because the sample size of this study was small, further association studies are needed to confirm if COMT gene can be considered as an aetiological factor for ADHD-RD comorbidity.

7.4.2.5 *KIAA0319 and TTRAP genes*

Both rs2179515 and rs4504469 SNPs of the KIAA0319 gene and rs2143340 SNP from the TTRAP gene were included in this study. These genes are located between D6S276 and D6S1554 regions (Deffenbacher et al., 2004; Francks et al., 2004) of the 6p chromosome (Cardon et al., 1994; Cardon et al., 1995; Grigorenko et al., 2000). The association of the KIAA0319 gene's SNPs appeared in three different latent classes. The rs2179515 SNP showed association with both the 'Predominantly Inattentive' (P value= 0.0253) and the 'Unique Severe RD' (P value= 0.0253) classes, whereas the rs4504469 SNP of the same gene was expressed in the 'Combined RD' latent class (P value= 0.0325). In addition, the association of rs2143340 SNP from the TTRAP gene was also found with the 'Predominantly Inattentive RD' (P value= 0.0253) latent class. The rs4504469 and rs2179515 SNPs, plus four extra SNPs of the KIAA0319 gene and rs2143340 of TTRAP gene, recorded strong evidence for association with Reading Disability. Accordingly, our results confirm Cope et al's study (2005) of the contribution of KIAA0319 and TTRAP genes to Reading Disability. The difference between our findings to Cope et al's. (2005) is the application of LCA with Reading Disability, which produced a wide range of RD phenotypes, including the 'Unique Severe RD' latent class, which exhibited association.

Furthermore, three SNPs (rs2179515, rs4504469, and rs2143340) also exhibited association with the Inattentive and Combined latent classes, suggesting they are candidate genes for ADHD as well. No previous studies investigated this association before; however, Willcutt et al (2002) suggested that QTL for RD on chromosome 6p boosts its susceptibility to ADHD. According to this, the study suggested that both KIAA0319 and TTRAP genes might be genes susceptible to ADHD. Although the SNPs of KIAA0319 and TTRAP genes exhibited associations, the pattern of these associations was inconsistent. The association appeared once with the Inattentive class only, once with Reading Disability class only, and twice with comorbid latent classes (Inattentive RD, and Combined RD). These inconsistent results could not specify the kind of the genetic effects: additive or non-additive. They also could not conclude if this genetic effect is shared effect between Inattentive and Combined ADHA and RD, or if it is an

independent effect from RD or from ADHD latent classes. Thus, more investigations are required to confirm these results and answer the above questions.

7.4.2.6 HTR1B gene

The serotonin 1B receptor gene (HTR1B) was also involved in this study, denoted by five candidate SNPs for ADHD which were examined for RD: rs2000292, rs6297, rs6296, rs6298, and rs130058. The rs6296 SNP exhibited association with the ‘Severe RD’ latent class (P value= 0.0339), whereas rs2000292 SNP showed association with the ‘Combined’ latent class (P value= 0.0143). Smoller et al. (2006) examined these SNPs plus others and reported that the rs6296, rs6298 and rs6297 SNPs are known as the common synonymous G861C, C129T, and 3’UTR SNP (A1180G) polymorphisms respectively. In addition, they also reported that the rs130058 SNP is identified as a promoter SNP, whereas rs2000292 SNP exists independently between block 3 and the flanking haplotype block of 142 kb covering the HTR1B gene. They found that rs2000292, rs6297, and rs6296 SNPs demonstrated association with the DSM-IV Inattentive subtype. Another recent study by Ickowicz et al. (2007) attempted to replicate Smoller et al.’s (2006) results on rs2000292, rs6297, rs6296, rs6298, and rs130058 markers; however, their findings did not show evidence for association between ADHD and the HTR1B gene, on either categorical or quantitative trait data.

Comparing the results of our study with those of Smoller et al. (2006), we found that rs2000292 SNP exhibited association with the ‘Combined’ latent class, but in Smoller et al.’s. (2006) study this SNP expressed association with the Inattentive subtype. Therefore, they suggested HTR1B may be susceptible gene for the Inattentive subtype. On the other hand, our findings concluded that that HTR1B gene may be susceptible gene for the Combined latent class. The results also showed an association of rs6296 SNP with the ‘Severe RD’ latent class. To my knowledge, no previous studies have tested any of HTR1B SNPs with Reading Disability. If I adopt both Willcutt et al.’s (2002, 2003) hypotheses of pleiotropy, and the belief that both ADHD and RD might be alternate forms of the same disorder, this genetic association supports these hypotheses. It is therefore recommended to consider HTR1B as a pleiotropic gene for ADHD and RD.

Further investigations are required to confirm this association and to investigate other potential candidate genes for ADHD-RD comorbidity.

Despite obtaining some significant results in all of the association tests there were few transmissions and non-transmissions of the alleles. The reason for this is the small sample size.

Despite the significant genetic associations obtained in this study, it is important to point out that these significant findings are nonetheless borderline because of the low transmissions and non-transmissions of the alleles. This might indicate a presence of false-positive associations. Mitchell, Cutler, and Chakravarti (2003) described several conditions that can cause false-positive associations, such as genotyping errors both detectable and undetectable. In the current study, Haploview detected 1.14% genotyping errors, which errors may have resulted from the difficulty of assigning genotypes to heterozygous individuals relative to homozygotes. The reason is that, using genotyping technology, SNPs are represented by two alleles: one of the alleles is marked while the other is tested but not marked. If an individual is heterozygous and one of the alleles fails to be identified this individual will appear as homozygous (Cutler et al., 2001). Based on Mitchell et al's (2003) model and predictions, I found two assumptions that might cause the undetectable errors. First, there could be some alleles of unequal frequency transmitted in an unbalanced way. Second, the bias of transmitted alleles (T) seems to increase susceptibility to the disorder, as well as the scarcity of the untransmitted alleles (U), which, to be untransmitted, would seem to be protective alleles. This suggests that many reported TDT-derived associations between disorders and marker alleles may be false positives.

The reliable way to validate these associations is to increase the sample size by genotyping more affected families.

7.4.3. Haplotype Block Analysis

The haplotype analysis showed a presence of two ht SNPs (rs4680 and rs165599) on the COMT gene from the single haplotype block produced from the analysis. These two

htSNPs exhibited significant associations with five latent classes: the ‘Moderate RD’ and ‘Unique Severe RD’, pure RD latent classes; the ‘Predominantly Inattentive RD’ and ‘Combined RD’ latent classes, comorbid latent classes; and the ‘Combined’ latent class, pure ADHD latent class (Table 7.13). The results also showed a presence of three risk haplotypes (alleles) for those two htSNPs (Figure 7.11), represented by AA, GC, and AC alleles. The AA and GC risk alleles exhibited significant association with ‘Moderate RD’, ‘Predominantly Inattentive RD’, ‘Unique Severe RD’ and ‘Combined RD’ latent classes, whereas the AC risk allele exhibited significant association only with the ‘Combined’ latent class. This outcome suggests that both AA and GC risk alleles of rs4680 and rs165599 (ht SNPs) on the COMT gene can be considered aetiological factors for RD, and components for ADHD-RD comorbidity. In addition, these alleles can be considered as an aetiological factor for phenotypic RD criteria reinforce the connection between the presences of genetic factors in the phenotypic RD criteria.

7.4.3. Conclusion

This study concluded that using LCA-defined ADHD-RD categories in genetic association studies and haplotype-block analysis is more efficient than the use of *DSM-IV*-defined ADHD categories. This is because the nine ADHD-RD latent classes were more symptomatically homogenous than those defined by *DSM-IV* to the extent that the identification of distinctive clusters of symptoms represents more aetiologically pure forms of disorders. This clustering is believed to be the most robust and successful factor in reducing the genetic heterogeneity among ADHD and RD phenotypes. This argument can be explained more by two studies as examples: first, the evidence of RD association with KIAA0319 gene found by Cope et al. (2005) which was replicated in this study as well; however, Cope et al’s study did not specify the phenotypic components for RD (e.g. spelling, verbal learning and memory, phonological awareness, rapid memory etcetera). Second, neither did Feng et al’s. (2005; 2005) studies specify which ADHD subtypes (Inattentive, Hyperactive-Impulsive, or Combined) showed associations. On the other hand, the genetic associations that found with the phenotypes of the Predominantly Inattentive, Predominantly Inattentive and RD, Combined, Combined and RD, Moderate RD, Severe RD, Unique Severe RD latent classes have been specified.

From the above results, it can be concluded that there is a genetic overlap between ADHD and RD, as some of the ADHD candidate genes exhibited association with some ADHD latent classes as well as with RD latent classes and ADHD-RD comorbid latent classes. Similarly, some of the RD candidate genes showed association with RD phenotypes, ADHD phenotypes and ADHD-RD comorbid phenotypes.

The advantage of using LCA is that it has the ability to cluster each group based on homogenous symptomology, which helps to reduce the genetic heterogeneity, and so produces homogenous genetic groups based on their similar phenotypes. This can increase the robustness of the association genetic analysis to detect the unique—if any—genetic contribution to each particular phenotype.

CHAPTER 8: GENERAL DISCUSSION

Latent Class Analysis (LCA), a twin genetic-fitting model, and a family-based association study including haplotype analyses, were carried out in order to understand the genetic components of ADHD, RD, and their comorbidities. This chapter discusses the model fitting findings with the genetic association and haplotype results. In addition, the chapter examines the implications of all three studies on the ADHD, RD, and ADHD-RD comorbidity phenotypes and discusses to what extent the study made a significant contribution to this area of inquiry. Finally, the chapter highlights the limitations of the study and provides recommendations and future directions.

8.1 Significant findings of the research

The major finding of this research is that the use of ADHD-RD latent classes as a categorical data source is suitable for performing genetic association studies and haplotype block analyses, and was more efficient than the use of *DSM-IV*-defined ADHD subtypes. The symptoms within each of the nine ADHD-RD latent classes display greater homogeneity, and the existence of such clearly-delineated symptom clusters allows for greater aetiological precision in identifying the influence of genetics on ADHD and its subtypes.

The genetic modelling analyses involved a sample of 2611 Australian twin families, examined for significant heritability among traits and detection of molecular genetic associations. The aim of the twin genetic fitting model was to detect evidence for the presence of genetic factors among *DSM-IV* ADHD subtypes as well as Reading Disability in the sample, and if this genetic effect is shared between ADHD subtypes and RD. Where there is a shared genetic effect, which ADHD subtype does this genetic effect contribute to more with RD?

The family-based association study was performed on two different classification criteria: *DSM-IV* and the nine ADHD/RD latent classes. When the association test for ADHD and RD data using the *DSM-IV* criteria was performed by QTDT, it showed only

two associations: one for the Inattentive subtype with rs3777664 SNP of the THEM2 gene (P- value =0.0323), and one for Reading Disability with rs3758653 SNP of DRD4 gene (p - value =0.0231). The association test for the nine ADHD/RD latent classes showed 15 significant associations. The single-locus association results showed an overlap of some RD candidate SNPs with ADHD Inattentive and Combined subtypes. A number of ADHD candidate SNPs were found to be associated with Reading Disability. These associations between COMT, SNAP-25, and KIAA0319 genes and the distinctive ADHD/RD latent classes indicate that the genes act differentially on RD alone, ADHD alone, and comorbid ADHD-RD latent classes.

8.1.1 Significant findings for ADHD alone

The results of the univariate analysis of the Inattentive subtype showed that the AE model was the best fit with the data. This model indicated a presence of both the additive genetic ($a^2=0.86$), and unique environmental ($e^2=0.13$) effects that twins do not share. This suggested that the Inattentive subtype can be affected by additive genes and unique environmental factors. Both the Hyperactive-Impulsive and Combined subtypes showed the ACE model was the best fit to their data as additive genes ($a^2=0.71$ and $a^2=0.74$ respectively), and common ($c^2=0.18$ and $c^2=0.16$ respectively), and unique environmental ($e^2=0.11$ and $e^2=0.10$ respectively), influences affected these ADHD subtypes.

One of the study's aims was to distinguish between the ADHD subtypes, as described by the *DSM-IV* ADHD phenotyping, by isolating particular genetic components' contribution to only one subtype and therefore, to be able to ascribe some responsibility for that subtype's phenotype. The study confirms that ADHD subtypes are highly heritable, with results replicating previous twin studies (e.g., Gjone, 1996; Levy et al., 1997; Martin et al., 2002). The univariate analysis showed that the highest heritability was for the Inattentive subtype ($a^2= 0.86$), and the Hyperactive-Impulsive subtype had the least heritability ($a^2= 0.71$). The heritability of the Combined subtype was intermediate ($a^2=0.74$).

The problem of heterogeneity among *DSM-IV* ADHD subtypes is an obstacle to examine the distinction between the subtypes. However, having homogenous ADHD subtype groups can solve this problem by refining the phenotypes. Szatmari et al. (2007)

argued that identifying psychiatric disorders (e.g., ADHD) based on *DSM-IV* criteria caused heterogeneity, because *DSM-IV* diagnostic criteria contained various symptoms, ranging from severe symptoms causing deep impairment to few symptoms without impairment. Accordingly, it could be possible that candidate ADHD genes cannot be found using *DSM-IV* criteria. The key to overcoming this problem may be to refine the ADHD phenotypes so that there would be an ‘informative phenotype’ for performing genetic analysis. Szatmari et al. (2007) argued that an informative phenotype would be more Mendelian-like and could be transmitted within the pedigree in less complex ways, not like the *DSM-IV* phenotype. The informative phenotype can be categorised into component, intermediate and covariate phenotypes (Szatmari et al., 2007). Because ADHD is a complex disorder influenced by multiple genes, and each ADHD subtype has a wide range of heterogeneous phenotypes controlled by different genetic mechanisms, this study hypothesised that component phenotypes can effectively describe the fundamental characteristics of each subtype, instead of selecting a *DSM-IV* subtype that contains a wide range of phenotypes. Latent Class Analysis (LCA) can identify component phenotypes, as this analysis has the capability of segregating ADHD phenotypes into appropriate symptom clusters and to re-classify the heterogeneous ADHD phenotypes into distinctive homogenous groups, producing valid genetically informative phenotypes. As explained earlier in the Latent Class Analysis chapter, the ADHD latent classes were valid and replicated the Rasmussen et al. studies (2002; 2004).

Nine latent classes produced from the LCA can be divided into three groups; a group containing three ADHD latent classes, which matched the *DSM-IV* ADHD subtypes; a second group containing three RD latent classes; and a third group containing two ADHD-RD comorbid latent classes. This section will focus on the first ADHD group, which includes the *DSM-IV* ‘Predominantly Hyperactive-Impulsive’, ‘Predominantly Inattentive’, and ‘Combined’ latent classes. There were significant differences between the concordant MZ twins and concordant DZ twins, with MZ twin concordance being double that of DZ twins, indicating that the latent classes are heritable and may contain genes that contribute to the three ADHD latent classes (Table 5.15 and Table 5.16).

One of the aims of this study was to identify the susceptible genes of ADHD and to refine the ADHD subtypes' phenotypes in order to obtain genetically informative phenotypes for ADHD. The tool utilised for obtaining the informative phenotypes was LCA. Out of the nine latent classes produced by LCA, three latent classes represented three common DSM-IV ADHD subtypes. These latent classes were the 'Predominantly Hyperactive-Impulsive', the 'Predominantly Inattentive', and the 'Combined' latent classes. The other aim was to find out whether candidate genes of ADHD are same ones or if each subtype has its own genes, as it might be that some particular gene(s) would contribute to only one subtype, and would be responsible for the subtype's phenotype. A family-based association test was applied on both the *DSM-IV* ADHD subtypes and the ADHD latent classes by using 21 SNPs from nine candidate genes. The results exhibited only one genetic association result of the rs3777664 SNP from THEME2 gene with the Inattentive subtype; contrary to this, two latent classes showed association with four SNPs from four candidate genes. The rs2179515 SNP from KIAA0319 gene was associated with the 'Predominantly Inattentive' latent class, whereas the other three SNPs (rs27072 from DAT1, rs1051312 from SNAP25, and rs2000292 from HTR1B) were associated with the 'Combined' latent class.

Why did the *DSM-IV* ADHD family-based association test find only one significant association but LCA had four genetic associations? When *DSM-IV* identifies cases based on a cut-off score, it does not efficiently differentiate among the 18 ADHD symptoms, which causes a heterogeneous arbitrary classification of the ADHD diagnosis. The *DSM-IV* criteria did not pick up cases that LCA did and the result was that the *DSM-IV* subtypes did not overlap with the nine ADHD/RD latent classes. Furthermore, although the endorsement probabilities for the 18 *DSM-IV* symptoms were found to be higher for the ADHD subtypes, LCA proved to be more effective in identifying the presence of ADHD in individuals overall, as LCA identified a greater number of cases of ADHD than the *DSM-IV* approach.

Based on the earlier argument by Szatmari et al. (2007), this study found ADHD latent classes to be clinically homogenous and composed of biologically similar and shared common genetic risk factor behaviors that cluster together, whereas the *DSM-IV* ADHD

diagnostic criteria is clinically heterogeneous and composed of multiple behaviours that hang together. With the *DSM-IV* diagnostic criteria, affected family members may be concordant for some aspects of the phenotype but not others, reflecting intra-family heterogeneity; while with latent classes, family members are concordant for one aspect of the phenotype but not others, reflecting intra-family homogeneity. Under such circumstances, and unless expressly addressed in the association analysis, the use of *DSM IV* diagnosis has less power to detect susceptible genes, whereas LCA has more power to detect susceptible genes, because of its ability to neatly index genetic liability (Rasmussen, Neuman et al., 2002).

The family-based association study did not show association of the 21 markers with the ‘Predominantly Hyperactive-Impulsive’ latent class, despite the zygoty of this latent class showing significant genetic effects. The genetic modeling of the *DSM-IV* Hyperactive-Impulsive subtype showed a high heritability of “0.71”. The reason for not having an association on the molecular level could be that the families selected for the genotyping analysis did not contain enough individuals identified as Hyperactive-Impulsive. Another reason might be that the 21 markers were not genetically associated with this latent class; therefore, it is recommended that a new SNP assay be designed containing more SNPs than the candidate genes used in this study, as it is more likely that a genetic association with this latent class will then be found.

The ‘Predominantly Inattentive’ latent class exhibited association with rs2179515 from the KIAA0319 gene. No previous study examined this gene’s association with the Inattentive subtype; therefore, this association has potential significance but is considered speculative until further replication studies have been performed to validate the finding. Cope et al. (2005) found an association of this gene with Reading Disability. Several studies have showed that KIAA0319 has a relatively specific expression in the brain, particularly in the developing cerebral neocortex when neurogenesis and neuronal migration are in progress; hence, this gene produces protein mainly in the nervous tissue, especially on the cell surface, regulating adhesion between adjacent neurons (Fisher & Francks, 2006; Francks et al., 2004; Paracchini et al., 2006). Accordingly, it is suggested that irregularities during neural migration of the neurological mechanisms might be

involved in the RD aetiology ((Paracchini et al., 2006). Because Inattentive ADHD and RD co-occur more frequently, than the Hyperactive-Impulsive subtype with RD (Stevenson, 2001; Willcutt, DeFries et al., 2003), this study also suggests that irregularities during neural migration of the neurological mechanisms might be involved in the aetiology of Inattention. Further investigations are needed to confirm this relationship.

The Combined latent class demonstrated three associations with three SNPs from three different genes: rs27072 from DAT1; rs1051312 from SNAP25; and rs200292 from HTR1B. Several previous association and meta-analysis studies confirmed the contribution of these genes to the aetiology of ADHD (e.g., Bobb, Castellanos, Addington, & Rapoport, 2004; Brookes et al., 2006; Faraone et al., 2005; Feng, Crosbie et al., 2005; Feng, Wigg et al., 2005; Smoller et al., 2006). This is one of the first studies to replicate genetic associations of these SNPs on ADHD using the Australian twin sample. These findings partially support the assertion that these genes contribute to the Combined subtype but not to the other ADHD subtypes, and that they are partly responsible for the subtype's phenotype. According to the quantitative genetic modeling, the ADHD subtypes might be influenced both by shared common genes and specific genes for each subtype, meaning that there would be particular genes contributing to each ADHD subtypes and shared genes contributing to the three ADHD subtypes. Feng, Wigg et al's. study (2005) found that the DAT1 gene was associated with the Combined subtype. In addition, Brookes et al. (2006) reported that SNPs of the DAT1 and SNAP25 genes exhibited an association with the Combined subtype, but the HTR1B gene did not. However, other studies showed association of this gene with ADHD in general (Hawi et al., 2002; Quist et al., 2003). Increasing the sample size and testing more SNPs for these genes may help to distinguish the genetic component for each ADHD subtype, as well as the shared genes for all ADHD subtypes. Brookes et al's (2006) study has a sound methodology to adopt for this purpose.

8.1.2 Significant findings for RD alone

The ADE model was the best fitting model for Reading Disability, with the additive gene effect ($a^2=0.25$), the non-additive gene effect ($d^2=0.68$), and the unique environmental effect ($e^2=0.10$). Furthermore, the non-additive effect was false: the opposite-sex

correlation was very low due to the sex limitation, not dominance. Therefore, the RD model gave an uncertain D effect because of low opposite-sex correlation.

When the family-based association test for RD data was performed by QTDT, it showed only one association for Reading Disability defined by the *DSM-IV* with the rs3758653 SNP of the DRD4 gene (p -value = 0.0231). The association test for the three RD latent classes, performed by Haploview, showed four significant associations with the latent classes. The single-locus association results showed overlap of some ADHD candidate SNPs with Reading Disability: rs165599 from COMT, rs6296 from HTR1B, and rs362987 from SNAP25 genes. None of these association results have been studied previously and therefore, these are considered findings of potential significance until further replication studies have been performed.

The association result obtained for the rs2179515 from the KIAA0319 gene with the 'Unique Severe RD' latent class replicates previous results in that it showed significant association with this gene (Cope et al., 2005; Luciano et al., 2007; Paracchini et al., 2006). The difference between this study and previous studies is that the current study used LCA, which produced the three RD latent classes. According to the association results, each RD latent class appears to have different genetic components; however, further investigations are needed to confirm this by designing new SNP assays containing a wide range of candidate genes represented by genetic variant markers (SNPs).

The purpose of using LCA on Reading Disability (RD) was to refine the phenotypic components of RD in order to obtain genetically informative RD phenotypes. LCA was successful in isolating three new distinctive RD latent classes; 'moderate RD', 'Severe RD' and 'Unique Severe RD' latent classes. These RD latent classes have the advantage of being genetically informative phenotypes, as each RD latent class is clinically homogenous and composed of biologically similar, shared-risk factor behaviours that cluster together. The RD cut-off category found in the *DSM-IV* was not able to broadly cover the seven RD items, which were clinically heterogeneous and composed of variant behaviours that weakened the power to detect genetic associations and susceptibility

genes. The LCA allowed the detection of susceptible genes because of its ability to neatly index genetic liability which facilitated more efficient genetic analyses (Rasmussen, Neuman et al., 2002). Homogeneity of the ‘moderate RD’ latent class came from clustering of the spelling item; and homogeneity of the ‘Severe RD’ latent class came from clustering of ‘spelling’, ‘rapid memory’, and ‘overall reading’ items. The symptom cluster for the ‘Unique Severe RD’ included all of the seven RD items. These results suggest the differentiation of RD phenotypes and their classification into distinctive groups, leading to increased understanding of whether these RD latent classes have the same genetic components or if each RD latent class has its own genetic component(s). These three RD latent classes can be used in further studies to further explore the relationship between the RD genetics and the RD phenotypic characteristics.

The study also performed a haplotype analysis in order to determine the haplotype blocks that could be responsible for the aetiology of RD. The haplotype analysis showed that both AA and GC risk alleles of rs4680 and rs165599 (ht SNPs) on the COMT gene were associated with the ‘Moderate RD’ and ‘Unique Severe RD’ latent classes, considering these risk alleles as an aetiological factor for phenotypic RD components. LCA, in this instance, grouped each RD latent class based on symptom similarities. The symptoms homogeneity among these two RD latent classes led to obtain this significant haplotype block.

Evidence provided by Byrne et al. (2006; 2007) found a strong genetic factor existed in some RD components, such as phonological awareness, rapid naming, and verbal memory. These findings also support the presence of genetic factors among RD phenotypic components. This study extended Byrne et al’s examination and attempted to correlate the RD phenotypic components with the ‘Moderate RD’ and ‘Unique Severe RD’ latent classes. In summary, the haplotype analysis showed association of both AA and GC risk alleles of rs4680 and rs165599 (ht SNPs) on the COMT gene with RD, suggesting them as aetiological factors for phenotypic RD components.

8.1.3 Significant findings of ADHD-RD comorbidity

The bivariate analysis exhibited a genetic overlap between ADHD subtypes and RD, and showed the presence of shared genetic effects between each ADHD subtype with RD; however, it was stronger with Inattentive and Combined ADHD than the Hyperactive-Impulsive subtype. The Inattentive ADHD-RD comorbid relationship was previously found to be stronger than that between Hyperactive-Impulsive ADHD and RD (Willcutt, Pennington, & DeFries, 2000). The bivariate analysis between each ADHD subtype with RD showed that the best fitting genetic model, in all three shared ADHD subtypes, with RD was the ADE model, indicating a presence of additive genetic effects between ADHD subtypes and RD. This additive effect was higher for the shared Combined/RD ($a^2=0.31$) than the shared Inattentive/RD ($a^2=0.29$), and shared Hyperactive-Impulsive/RD ($a^2=0.20$). In addition, the bivariate analysis also showed the AE models were best for each ADHD subtype alone, whereas for RD alone, the ADE were best models, despite additive genetic effects being significantly low, while the non-additive genetic effects (d^2) were moderately higher, ranging from 0.55 to 0.71. In fact, the non-additive effects that appeared were spurious, because of the lower opposite-sex correlation ($r=0.192$), and the differences for the correlations' Confidence Interval values for MZ female compared to DZ male and for DZ female compared to Opposite sex.. The low opposite-sex correlation obtained from the current findings raises the issue of whether the aetiology of RD is different for boys and girls. Past studies have indicated that this may be the case given that the prevalence of RD in boys is usually found to be higher than in girls. Although this could not be investigated further in the current study due to limited resources, it is an important issue for future studies employing quantitative and molecular genetic analyses.

Zumberge, Baker, and Manis (2007) reported that the genetic overlap of the three *DSM-IV* ADHD subtypes with Reading Disability showed that 95% of the phenotypic covariance was found between Inattentive ADHD and RD, indicating a strong phenotypic and genetic relationship between Inattentive ADHD and RD. Only 21% covariance was found between the Hyperactive-Impulsive subtype and Reading Disability (Willcutt, Pennington, & DeFries, 2000). Furthermore, a recent study by Willcutt, Pennington, Olson, and DeFries (2007) suggested that ADHD may be more highly heritable in a comorbid

ADHD-RD disorder than in ADHD alone. Findings from the current study suggest the presence of major shared genetic effects between each ADHD subtype with RD, and also minor and different genetic effects that differentiate each comorbid ADHD subtype with RD. This conclusion was based on the different heritability rates obtained for the bivariate analyses ($a^2_{\text{Shared Inattention/RD}}=0.29$, $a^2_{\text{Shared Hyp-Imp/RD}}=0.20$, $a^2_{\text{Shared Combined/RD}}=0.31$). This suggests that the heritability of the Combined/RD comorbidity is stronger than the heritability of other two ADHD subtypes comorbid with RD (Willcutt et al., 2002).

This is the first time LCA has been used to assess the comorbidity of ADHD and RD. Effectively, LCA confirmed the ADHD-RD comorbidity by creating two distinctive latent classes; the ‘Predominantly Inattentive/RD’ and ‘Combined/RD’ latent classes. The former latent class replicated previous findings for the presence of a shared genetic contribution between the Inattentive subtype and RD (Willcutt, DeFries et al., 2003; Willcutt & Pennington, 2000b); however, LCA did not produce a comorbid class for RD with the ‘Predominantly Hyperactive-Impulsive’ latent class that was also supported by a low shared genetic contribution between the Hyperactive-Impulsive subtype and RD (Willcutt, DeFries et al., 2003; Willcutt & Pennington, 2000b). Interestingly, LCA was also able to demonstrate that RD is strongly comorbid with the Combined subtype, by creating the ‘Combined/RD’ latent classes. The chi square test for zygoty of the ‘Combined/RD’ latent class showed significant differences among concordant and discordant MZ and DZ twins, indicating the presence of a higher genetic factor among this latent class. However, the chi square test for zygoty of the ‘Predominantly Inattentive RD’ latent class was not significant among concordant and discordant MZ and DZ twins. Regardless of this result, this latent class is still considered to be heritable and is influenced by genetic factors. The reason for this contrary result could be the number imbalance among the twins sample.

This study asserts that both the ‘Predominant Inattentive RD’ and the ‘Combined RD’ latent classes are significant and can help in understanding the comorbidity between ADHD and RD. Each latent class has a homogenous and genetically informative phenotype helping to understand the comorbidity between the two domains, and also

helping to reveal if the comorbidity of ADHD subtypes with RD have the same genetic components or if each comorbid ADHD subtype has its own genetic components. These two latent classes conform to the strong comorbid relationship of the *DSM IV* Inattentive and Combined subtypes with RD, and the weak comorbid relationship of the Hyperactive-Impulsive subtype with RD.

The family-based association study, including the haplotype analysis for the ‘Predominantly Inattentive RD’ and ‘Combined RD’ latent classes showed significant genetic associations to three SNPs from COMT gene (rs4680, rs165599, rs737865) and one SNP from TTRAB (rs2143340) and one SNP from the KIAA0319 gene (rs4504469) of the 6p.22 region. Two SNPs of the COMT gene (rs4680 and rs165599) exhibited associations with the two comorbid latent classes. In addition, there were six significant associations for both the ‘Predominant Inattentive RD’ and ‘Combined RD’ latent classes; whereas the *DSM-IV* Combined subtype did not show any genetic association. The genes contributing to ADHD can be detected based on the homogeneity found among the comorbid latent classes. Therefore, identifying genetically informative phenotypes represented by these two comorbid ADHD-RD latent classes could be adequate to detect and identify the susceptible genes for ADHD and RD.

The pattern of the genetic association among the ‘Predominantly Inattentive RD’ and ‘Combine RD’ latent classes showed that both latent classes exhibited genetic associations with one ADHD candidate gene represented by the COMT gene, and two RD candidate genes, represented by the TTRAP and KIAA0319 genes. There were previous association studies that found an association of ADHD with the COMT gene (Eisenberg et al., 1999; Qian et al., 2003; Thapar et al., 2005). Recent molecular genetic studies also found genetic associations of TTRAP and KIAA0319 genes with RD (Cope et al., 2005; Francks et al., 2004; Paracchini et al., 2006). However, observing particular SNPs of the COMT, TTRAP, KIAA0319 genes and their association with the comorbid ADHD-RD subtype is without precedent. Therefore, the current study considers these findings significant but speculative until further studies confirm them.

Both latent classes exhibited genetic association with the COMT gene: ‘Predominantly Inattentive with RD’ exhibited an association with two SNPs (rs4689 and rs165599), whereas ‘Combined RD’ exhibited an association with three SNPs (rs4689 and rs165599, and rs737865). Moreover, although the latent classes exhibited association with the 6p.22 region, the former latent class demonstrated association with rs2143340 SNP from the TTRAP gene, while the latter latent class demonstrated association with a different gene, i.e., rs4504469 from the KAAI0319 gene on the 6p.22 region. These results cannot confirm whether both comorbid latent classes have the same the genetic components or each one has different genetic components, due to the low number of genes and SNPs examined for these latent classes. Nevertheless, the current findings can reveal to some extent the presence of partial and similar genetic components between the Inattentive and Combined subtypes comorbid with RD, due to unequal shared additive genetic components found between the two latent classes.

The current study strongly supports the Willcutt, Pennington, Olson, and DeFries (2007) study that confirmed the importance of molecular genetic studies for refining the phenotypic characteristics of the comorbidity between ADHD and RD. This evidence came from the haplotype analysis which showed haplotype block contained two ht SNPs of the COMT gene (rs4680 and rs165599), including the presence of three risk alleles (‘AA’, ‘GC’, and ‘AC’). Both the ‘AA’ and ‘GC’ alleles exhibited an association with the ‘Predominantly Inattentive RD’ and ‘Combined RD’ latent class.

As this study did not examine the heritability of RD phenotypic components, a recent ILTS study by Willcutt and his colleagues (2007) can be used to indicate the presence of heritable factors among the RD phenotypic components. Their study showed that the phenotypic correlation between Inattentive ADHD and pre-reading performance, including phonological awareness, rapid naming, verbal memory, vocabulary, grammar/morphology, and print knowledge in school-age children, has common genetic influences. These findings might support the assertion that the ‘AA’ and ‘GC’ alleles of the haplotype block that contained two ht SNPs of the COMT gene are causal or risk alleles that contribute to RD phenotypic components found in the ‘Predominantly Inattentive RD’ and ‘Combined RD’ latent classes. However, further investigations are

still required to determine the risk ‘AA’ and ‘GC’ alleles with which RD phenotypic component(s) correspond.

LCA was used to look for more specific phenotypes of ADHD and RD in order to identify the shared genetic components between them. This study concluded that the utilisation of the comorbid latent classes in the genetic association studies and haplotype analyses might identify more candidate genes for ADHD-RD comorbidity, and also may identify the haplotype blocks contributing to this comorbidity, by finding the high risk alleles of responsible individual RD phenotypic components that are comorbid with a particular ADHD phenotype.

8.2 *Study limitations*

8.2.1 *Limitations with DSM-IV ADHD items*

Certain limitations became apparent with regards to the 18 *DSM-IV* ADHD items, since there are certain ADHD symptom variants which are not included in the *DSM-IV* criteria, and thus cannot be taken into account when detecting ADHD and/or classifying expressions of the disorder into the *DSM-IV* subtypes. As Rasmussen et al. (2004) noted, the 18 *DSM-IV* ADHD items do not cover the entire spectrum of ADHD phenotypic variance. Accordingly, should this classification system be expanded to include additional ADHD symptoms or measures, the basic structure of the latent classes may be altered, which may in turn have implications for the accuracy of genetic findings related to ADHD subtype clustering (Rasmussen et al., 2004). The study found that the existing 18 items are limited in their ability to adequately describe phenotypes which are of interest from a genetic point of view. In support, after performing factor analysis on the 18 items, Rasmussen et al. (2002a) reported they could only account for approximately one-third of the phenotypic variation seen in ADHD sufferers.

A second limitation in this study was the use of the same raters; only the parental responses were used for the 18 *DSM-IV* ADHD items and the seven RD items. Additional sources such as teachers and self-reporting were not used in assessing the child’s symptoms. Thus, the potential for rater bias was perhaps higher than if multiple informants had been used. It is possible that this study may have produced different

ADHD/RD classes had multiple raters been involved. Rasmussen et al. (2002b) found that the eight-class model derived from parent reports only did not suit the adolescent-report ADHD information collected as the basis of their study.

8.2.2 Limitations with the seven RD items

According to Willcutt et al. (2005), cognitive tests are the most appropriate tests to use in diagnosing RD, as distinct from the behavioural assessments used to diagnose ADHD. In assessing a child for RD using the *DSM-IV* criteria, there is no overlap between the ADHD and RD items. It is therefore impossible to determine the relationship between the two disorders based on these conventional criteria, since a different approach is taken to testing for each disorder. This was a significant factor in the decision to use a seven item parent-rated behavioural assessment to detect RD in this study, in order to allow the RD results to be included in Latent Class Analysis. Other factors which influenced this decision were the study sample size, along with budget, time and geographical constraints – it was not considered efficient or economical to administer complex, time-consuming cognitive tests to a large number of children dispersed all over Australia.

It is important to note, however, that the RD criteria used do not give the same precision of results as the cognitive tests. In particular, the criteria used were potentially inadequate in identifying the specific RD components present in cases of RD. Due to these inadequacies, it is possible that the links between the RD components and the latent classes are not as strong as they would have been had cognitive testing been used to detect RD components. Additionally, no prior studies have been conducted using the seven RD items mentioned earlier as the basis for latent class analysis. As this study is the first to use LCA on these seven RD items, there are no studies with which the results obtained can be compared or verified.

8.2.3 Limitations of genetic fit modelling

In performing genetic modelling, this study faced three major limitations. Firstly, the modelling was done using univariate and bivariate models only, and was not designed to take into account modelling specific to comorbidity. There exist certain designs for comorbidity that can be used for investigating ADHD-RD comorbidity (Rijsdijk & Sham,

2002). Secondly, due to time constraints, it was not possible to carry out extensive modelling to investigate the genetic and environmental contributions among siblings (sibling designs) using either the *DSM-IV* ADHD/RD criteria or the nine ADHD/RD latent classes. The third and final limitation was that there was not an adequate number of MZ and DZ twins per latent class to perform genetic modelling on the nine latent classes. To effectively perform such modelling, a sample size of at least 200 individuals in each class would be required to determine whether there are significant genetic or environmental influences on a heritable trait (Rijsdijk & Sham, 2002).

8.2.4 Limitations of genotyping analysis

The genotyping analysis was significantly hampered by a lack of funding, which was only sufficient for recruiting 190 individuals from 37 twin families, a figure which represents a low power sample size for detecting genetic association. There was also a restriction in the number of ADHD and RD candidate genes that could be included, which further limited the number of genetic markers (SNPs) that could be examined for the candidate genes included. Examining a broader range of SNPs for each candidate gene will facilitate greater understanding of each gene's involvement in both the aetiology and comorbidity of ADHD and RD.

8.3 Implications, recommendations and future directions

This study concluded that using LCA-defined ADHD and RD subtypes in genetic association studies and haplotype-block analysis is more efficient than the use of *DSM-IV*-defined ADHD subtype individuals. This conclusion, ironically, is supported by two studies that found evidence of aetiology but which did not specify the uniqueness of the phenotypes. The evidence of Reading Disability association with the KIAA0319 gene found by Cope et al. (2005), which was replicated in this study, did not specify the phenotypic components for RD (e.g. spelling, verbal learning and memory, phonological awareness, rapid memory etc.). Second, Feng et al. (2005 a,b) specified which ADHD subtypes (Inattentive, Hyperactive-Impulsive, or Combined) showed associations with RD but only made assertions about association at the level of phenotype, not individual characteristics. The LCA approach is believed to be the most robust and successful factor in reducing the genetic heterogeneity among ADHD and RD phenotypes, allowing them

to be grouped homogeneously, based on symptoms. LCA can add validity to genetic association analysis to detect the unique – if any - genetic contribution for each particular phenotype.

Furthermore, this study attempted to identify some genes of ADHD alone, RD alone, and ADHD-RD comorbidity. Because *DSM-IV* ADHD diagnostic criteria may not be the most informative in composing the phenotypes of ADHD, RD and comorbid ADHD-RD, this study attempted to decompose and refine the phenotypes of ADHD, RD, and comorbid ADHD-RD using LCA, in order to obtain genetically informative homogeneous phenotypes that would be useful for performing genetic analyses. This approach has great potential as the basis of a new strategy to find susceptible genes for other disorders comorbid with ADHD such ODD, CD, and others. As knowledge between the relationships between genes, brain and behaviour is uncertain and incomplete, using this approach in future studies might provide glimpses into possible links between them.

The comorbidity of ADHD and RD still needs further investigation. Latent Class Analysis can enable the identification of groups which display similar symptom clusters in both ADHD and RD symptomatology. While the seven RD items, corresponded to only to small number of phenotypic RD components, including more RD items in LCA may produce further specific heritable latent classes, which may in turn demonstrate genetic association between particular ADHD subtypes and specific RD phenotypic components. For example, it may be found that a latent class exists which shows that the spelling component of RD is comorbid with the Inattentive ADHD subtype, or that the phonological awareness RD component is comorbid with the Combined ADHD subtype. Such further analysis would also facilitate more accurate identification of susceptibility genes, due to the biological and genetic homogeneity of the latent classes.

This study has several implications: the study obtained well defined and heritable phenotypes for ADHD alone, RD alone, and comorbid ADHD-RD, enabling identification of some putative loci containing variations associated with these phenotypes. This also has an additional implication as it might help to offer biological evidence for a functional allele, and assist in the development of pharmaceutical and diagnostic tests based on

genetic results. Moreover, the current study might offer a better understanding to clinicians about ADHD-RD comorbidity. LCA might help in zygosity assignment, because LCA can strongly detect if a particular latent class is heritable or not in the absence of genotyping information. LCA also can be considered as having greater utility than more traditional approaches using questionnaire data (Heath et al., 2003).

This study was able to some extent, to identify a particular haplotype block contributing to ADHD subtypes, RD, and ADHD-RD comorbidity, suggesting the use of this approach can be used to detect genes with their SNPs and more specific phenotypes. For instance, the quantitative genetic research by Byrne and his colleagues (2006a,b) reported that some of RD phenotypic components such as memory, verbal, phonological etc. have genetic bases. This implies that RD needs further molecular genetic studies investigating the phenotypic components of RD, especially given that previous quantitative genetic studies have showed the presence of heritable evidence.

The current study also noted that many of the genes active in RD contribute to some extent to ADHD subtypes and vice versa; however, these results need to be validated through replication in future studies. As with ADHD, there are questions about RD that need answers such as: Are the diagnostic categories of RD valid? Are there different components of the disorder that can classify RD into more than one subtype? It is known that the understanding of the aetiology of ADHD-RD comorbidity will not necessarily lead to a cure, however, the identification of the genes involved in this comorbidity, such as the COMT gene, with several other promising candidates already under study, might assist future studies to address the biological and biochemical deficits that lead to this comorbidity, or to identify children at risk of this comorbidity before school age. This might facilitate the design and planning of intervention programs and help to minimize social, educational, and personal problems that the child might be exposed to from this comorbidity. The untangling of this comorbidity might also assist in the development of more treatment strategies. From this population-based sample, this study found two ADHD-RD comorbid latent classes which are genetically informative. This can help our understanding of the comorbidity more and also assist in the development of appropriate treatment for ADHD and RD comorbidity. In addition, future investigations could utilise

the 'severe' latent classes to identify more extreme phenotypes to identify more susceptible genes and haplotype blocks.

Recent advances in the endophenotypes of ADHD alone, RD alone, and ADHD-RD comorbidity might provide support for the identification of their susceptible genes. It could be one or some of the susceptible genes may play a fundamental role in Executive Functions (EF) such as response inhibition, working memory and others. It appeared from several neuropsychological and endophenotype studies (de Jong, Oosterlaan, & Sergeant, 2006; Doyle et al., 2005; Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005; Sonuga-Barke, 2005; Willcutt, Pennington, Olson, Chhabildas, & Hulslander, 2005) that EF components might be good candidate endophenotypes to understand the aetiological factors for ADHD alone, RD alone, ADHD-RD comorbidity. According to the above studies, endophenotype studies might be a promising tool to distinguish which ADHD subtype(s) and RD show overlap on EF deficits, and which subtypes of ADHD and RD differ concerning the contribution of EF.

Future studies could determine whether COMT variants are causally related to RD alone or comorbid ADHD-RD, and identify additional causal variants contributing to these phenotypes, which might lead to a better understanding of these disorders. This study also recommends refining and decomposing the phenotypes of ADHD subtypes, RD and comorbid ADHD-RD when conducting quantitative and molecular genetic studies. The current study recommends applying this approach with other candidate genes including other genetic variants, for different psychiatric and behavioural disorders. Because several molecular genetic studies showed associations of DRD4, DAT, COMT, SNAP25, HTR1B, and KIAA0319 with ADHD and RD, future studies should use haplotype analysis, rather than focusing on single markers, in order to identify informative SNPs that might help to understand the two disorders and their comorbidity. In addition, finding informative SNPs and haplotype blocks for these phenotypes might be useful in pharmacogenomics.

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APPENDIXES

Appendix 1: Twin and Sibling Questionnaire

D. Hay
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PERTH WA 6845

Twin and Sibling Questionnaire
(TO BE COMPLETED BY PARENT)

ZYGOSITY

The purpose of these questions is to determine whether your twins are genetically identical (formed from the splitting of one fertilised egg) or genetically non-identical (formed from the fertilisation of two eggs). If your

twins are **not** the same sex as each other, please go to the medication questions (**Question 25**).

22. I believe the twins to be:

- Genetically identical (one egg, monozygotic)
- Genetically non-identical (two eggs, dizygotic)
- Not sure

If your twins are of the same sex, and you have had their zygosity determined by blood or DNA test, please answer the following questions: (if they have not been tested, please go to Question 24)

- 23 A. The test found the twins to
 - genetically identical
 - genetically non-identical
- B. What was the test used?

24. To what extent are the twins similar at this time for the following features	Not at all similar	Somewhat similar	Exactly similar
A. Height	<input type="text"/>	<input type="text"/>	<input type="text"/>
B. Weight	<input type="text"/>	<input type="text"/>	<input type="text"/>
C. Facial Appearance	<input type="text"/>	<input type="text"/>	<input type="text"/>
D. Natural Hair Colour	<input type="text"/>	<input type="text"/>	<input type="text"/>
E. Eye Colour	<input type="text"/>	<input type="text"/>	<input type="text"/>
F. Complexion	<input type="text"/>	<input type="text"/>	<input type="text"/>

	Yes	No	Don't Know
G. Do they look as alike as two peas in a pod?	<input type="text"/>	<input type="text"/>	
H. Does their mother ever confuse them in appearance?	<input type="text"/>	<input type="text"/>	
I. Does their father ever confuse them in appearance?	<input type="text"/>	<input type="text"/>	
J. Are they sometimes confused in appearance by other people in the family?	<input type="text"/>	<input type="text"/>	
K. Is it hard for strangers to tell them apart?	<input type="text"/>	<input type="text"/>	
L. Do they have very similar personalities?	<input type="text"/>	<input type="text"/>	

M. Did they have the same placenta?

N. Do they have the same blood group?

For the following questions, please choose the best alternative that applies to your children by circling one response.

Circle the 0 if the item does not apply to your child at all. Circle the 1 if the item applies just a little or sometimes. Circle the 2 if the item applies pretty much or often. Circle the 3 if the item applies very much or very often.

		0=Not at all		1=Just a little/Sometimes		2=Pretty much/Often		3=Very much/Very Often	
		Twin A	Twin B	Sibling 1	Sibling 2	Twin A	Twin B	Sibling 1	Sibling 2
50	Does this child have difficulty with spelling	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
51	Did this child have difficulty learning letter names	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
52	Did this child have difficulty learning phonics (sounding out words)	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
53	Does this child read more slowly than other children of the same age	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
54	Does this child read below grade or expectancy level	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
55	Did this child have difficulty learning the days of the week or the months of the year	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
56	Has this child required extra help in school because of problems in reading or spelling	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3

BEHAVIOUR QUESTIONS

Listed below are descriptions of children's behaviour or the problems that they sometimes have. Please indicate how applicable you think each item is for each child now or within the time period specified (e.g. 12 months) when compared to other children of the same age. Choose the best alternative that applies to your children by circling one response.

Circle the 0 if the item does not apply to your child at all. Circle the 1 if the item applies just a little or sometimes. Circle the 2 if the item applies pretty much or often. Circle the 3 if the item applies very much or very often.

The questionnaires were designed for children in the age range of 6 - 18 years. Because of this age range, there will be questions that are not relevant to your child, because they are applicable either to older or younger children. *Complete these questions by answering "Not at all" (circling '0')*

Compared to other children of the same age, how applicable are the following items (142-234) for each child now or within the past 12 months? *Please circle the appropriate response.*

0=Not at all 1=Just a little/Sometimes 2=Pretty much/Often 3=Very much/Very Often

		Twin A		Twin B		Sibling 1		Sibling 2	
142	Has difficulty keeping attention on work or games	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
147	Has difficulty organising tasks or activities	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
148	Talks excessively	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
153	Fidgets with hands or feet or squirms in seat	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
156	Has difficulty playing or engaging in leisure activities quietly	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
164	Makes careless mistakes in schoolwork, work or other activities	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
168	Interrupts or intrudes on others (e.g. butts into conversations or games)	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
170	Loses things needed for tasks or activities at home or school (e.g. pencils, toys, or tools)	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
171	Is easily distracted by things happening around him/her (e.g. noise or people talking)	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
175	Listens when spoken to directly	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
178	Runs around or climbs on things in situations where this is inappropriate	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
180	Has trouble following through on instructions	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
184	Forgets things in day to day activities	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
185	Completes schoolwork, chores, or duties	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3
186	Is always 'on the go' or acts as if 'driven by a motor'	0	1	0	1	0	1	0	1
			2		2		2		2
			3		3		3		3

187	Avoids, dislikes or is reluctant to engage in tasks that require prolonged concentration (e.g. schoolwork or homework)	0	1 2 3	0	1 2 3	0	1 2 3	0	1 2 3
188	Blurts out answers to questions before they have been completed	0	1 2 3	0	1 2 3	0	1 2 3	0	1 2 3
190	Finds it hard to stay seated in the classroom or other situations in which sitting is expected	0	1 2 3	0	1 2 3	0	1 2 3	0	1 2 3
234	Has difficulty awaiting his/her turn	0	1 2 3	0	1 2 3	0	1 2 3	0	1 2 3

Appendix 2: Assumption Testing for Age Limitation

2.1 Monozygotic and Dizygotic univariate correlations for ADHD subtypes and Reading Disability by age variable (Younger age)

Table A2.1

Monozygotic and dizygotic univariate correlations for ADHD subtypes and Reading Disability for 13 years old and younger.

<u>Phenotype</u>	<u>Monozygotic Twin</u>	<u>Dizygotic Twin</u>
5. Inattentive Subtype	0.853**	0.459**
6. Hyperactive-Impulsive Subtype	0.888**	0.554**
7. Combined Subtype	0.881**	0.550**
8. Reading Disability	0.879**	0.342**

** Correlation is significant at the 0.01 level (2-tailed).

Table A2.2

The MZ and DZ bivariate correlations between Inattentive subtype and Reading Disability for 13 years old and younger.

			Twin 1 RD score	Twin 2 RD score
MZ	t1DSMin	t2DSMin		
In T1	1	.853(**)	.436(**)	.400(**)
In T2	.853(**)	1	.339(**)	.380(**)
RD T1	.436(**)	.339(**)	1	.879(**)
RD T2	.400(**)	.380(**)	.879(**)	1
DZ				
In T1	1	.459(**)	.416(**)	.180(**)
In T2	.459(**)	1	.164(**)	.436(**)
RD T1	.416(**)	.164(**)	1	.342(**)
RD T2	.180(**)	.436(**)	.342(**)	1

** Correlation is significant at the 0.01 level (2-tailed)

T1= Twin 1; T2= Twin 2; MZ=Monozygotic; DZ=Dizygotic; In=Inattention; RD= Reading Disability

Table A2.3
The MZ and DZ Bivariate Correlations between Hyperactive-Impulsive Subtype and Reading Disability for Younger Age

MZ	t1DSMhi	t2DSMhi	Twin 1 RD score	Twin 2 RD score
t1DSMhi	1	.888(**)	.229(**)	.217(**)
t2DSMhi	.888(**)	1	.223(**)	.246(**)
Twin 1 RD score	.229(**)	.223(**)	1	.879(**)
Twin 2 RD score	.217(**)	.246(**)	.879(**)	1

DZ	t1DSMhi	t2DSMhi	Twin 1 RD score	Twin 2 RD score
t1DSMhi	1	.554(**)	.210(**)	.147(**)
t2DSMhi	.554(**)	1	.116(**)	.265(**)
Twin 1 RD score	.210	.116(**)	1	.342(**)
Twin 2 RD score	.147(**)	.265(**)	.342(**)	1

** Correlation is significant at the 0.01 level (2-tailed)

T1= Twin 1; T2= Twin 2; MZ=Monozygotic; DZ=Dizygotic; hi=Hyperactive-Impulsive; RD= Reading Disability

Table A2.4
The MZ and DZ Bivariate Correlations between Combined Subtype and Reading Disability for 13 years old and younger

MZ	t1DSMc	t2DSMc	Twin 1 RD score	Twin 2 RD score
t1DSMc	1	.881(**)	.367(**)	.343(**)
t2DSMc	.881(**)	1	.302(**)	.339(**)
Twin 1 RD score	.367(**)	.302(**)	1	.879(**)
Twin 2 RD score	.343(**)	.339(**)	.879(**)	1

DZ	t1DSMc	t2DSMc	Twin 1 RD score	Twin 2 RD score
t1DSMc	1	.550(**)	.354(**)	.180(**)
t2DSMc	.550(**)	1	.165(**)	.393(**)
Twin 1 RD score	.354(**)	.165(**)	1	.342(**)
Twin 2 RD score	.180(**)	.393(**)	.342(**)	1

** Correlation is significant at the 0.01 level (2-tailed)

T1= Twin 1; T2= Twin 2; MZ=Monozygotic; DZ=Dizygotic; C=Combined; RD= Reading Disability

2.2 Monozygotic and Dizygotic univariate correlations for ADHD subtypes and Reading Disability by age variable (Older age)

Table A2.5
Monozygotic and Dizygotic Univariate Correlations for ADHD Subtypes and Reading Disability for 13 years old and older

<u>Phenotype</u>	<u>Monozygotic Twin</u>	<u>Dizygotic Twin</u>
9. Inattentive Subtype	0.877**	0.439**
10. Hyperactive-Impulsive Subtype	0.888**	0.507**
11. Combined Subtype	0.913**	0.510**
12. Reading Disability	0.922**	0.215**

** Correlation is significant at the 0.01 level (2-tailed).

Table A2.6
The MZ and DZ bivariate correlations between Inattentive subtype and Reading Disability for 13 years and older

				Twin 1 RD score	Twin 2 RD score
MZ	t1DSMin	t2DSMin			
In T1	1	.877(**)	.317(**)	.278(**)	
In T2	.877(**)	1	.301(**)	.318(**)	
RD T1	.317(**)	.301(**)	1	.922(**)	
RD T2	.278(**)	.318(**)	.922(**)	1	
DZ				Twin 1 RD score	Twin 2 RD score
	t1DSMin	t2DSMin			
I T1	1	.439(**)	.362(**)	.169(**)	
I T2	.439(**)	1	.106(**)	.343(**)	
RD T1	.362(**)	.106(**)	1	.215(**)	
RD T2	.169(**)	.343(**)	.215(**)	1	

** Correlation is significant at the 0.01 level (2-tailed)

T1= Twin 1; T2= Twin 2; MZ=Monozygotic; DZ=Dizygotic; In=Inattention; RD= Reading Disability

Table A2.7

The MZ and DZ bivariate correlations between Hyperactive-Impulsive subtype and Reading Disability for 13 years and older

MZ	t1DSMhi	t2DSMhi	Twin 1 RD score	Twin 2 RD score
t1DSMhi	1	.888(**)	.113(**)	.108(**)
t2DSMhi	.888(**)	1	.109(**)	.118(**)
Twin 1 RD score	.113(**)	.109(**)	1	.922(**)
Twin 2 RD score	.108(**)	.118(**)	.922(**)	1
DZ	t1DSMhi	t2DSMhi	Twin 1 RD score	Twin 2 RD score
t1DSMhi	1	.507(**)	.221(**)	.145(**)
t2DSMhi	.507(**)	1	.150(**)	.214(**)
Twin 1 RD score	.221(**)	.150(**)	1	.215(**)
Twin 2 RD score	.147(**)	.214(**)	.215(**)	1

** Correlation is significant at the 0.01 level (2-tailed)

T1= Twin 1; T2= Twin 2; MZ=Monozygotic; DZ=Dizygotic; hi=Hyperactive-Impulsive; RD= Reading Disability

Table A2.8

The MZ and DZ bivariate correlations between Combined subtype and Reading Disability for 13 years and older

MZ	t1DSMc	t2DSMc	Twin 1 RD score	Twin 2 RD score
t1DSMc	1	.913(**)	.257(**)	.234(**)
t2DSMc	.913(**)	1	.250(**)	.268(**)
Twin 1 RD score	.257(**)	.250(**)	1	.922(**)
Twin 2 RD score	.234(**)	.268(**)	.922(**)	1
DZ	t1DSMc	t2DSMc	Twin 1 RD score	Twin 2 RD score
t1DSMc	1	.510(**)	.337(**)	.179(**)
t2DSMc	.510(**)	1	.134(**)	.324(**)
Twin 1 RD score	.337(**)	.134(**)	1	.215(**)
Twin 2 RD score	.179(**)	.324(**)	.215(**)	1

** Correlation is significant at the 0.01 level (2-tailed)

T1= Twin 1; T2= Twin 2; MZ=Monozygotic; DZ=Dizygotic; C=Combined; RD= Reading Disability

Appendix 3

A3.1 DNA collections and extractions protocol by Oragene Saliva Kit DNA

As QIMR has performed the protocol extraction of DNA from the saliva kit, they have provided me their procedure. The following method (Oragene, 2006) was obtained from Anthony Caracella from QIMR:

Table A3.1 *The apparatus and consumables used in DNA extraction*

<u>Apparatus</u>	<u>Consumables</u>
<ul style="list-style-type: none">• flipper rack• 10mL tube rack• P200 pipette• P1000 pipette• waterbath @ 50°C• microfuge• Bench top centrifuge	<ul style="list-style-type: none">• 60 x 1.5mL autoclaved Axygen conical base tubes (RBC) with appropriate coloured looped lids• 12 x 10mL yellow capped Sarstedt tubes• 4 x rows barcode per sample• 36 x sterile plastic transfer pipettes• yellow 200µL pipette tips• blue 1000µL pipette tips• 200µL filter tips• 12 x 1000 µL filter tips• 1720 µLs Oragene purifier (aliquot directly from original tube)• 60 mLs 100% Ethanol

3.1 Procedure

Pick 11 Oragene Collection Kits and number the saliva kit lids from 1 to 11, then scan barcodes in this order and print 4x rows per kit.

Incubate the Oragene saliva samples at 50°C in a water bath for a minimum of 1 hour or overnight if more convenient.

Fill in an Extraction Worksheet for each saliva sample Kit set up and label 4x 1.5mL (RBC) tubes, 1x Sarstedt tube plus an extra 1.5mL (RBC) tube (for the final DNA storage tube). Add further set of tubes for the negative control and rotate on tethered lids with a colour suitable to the sample study.

Label lids with numbers in order on the Sarstedt tubes and each set of 4x 1.5mL (RBC) tubes. This illustrates that for first participant there is a '1' on the tubes, for second participant there is a '2' on the tubes, and so on.

DAY 1: *Follow through STEPS 4 and 5 in batches of 6 Collection Kits*

1. Perform a sequence check and DEO and date the Extraction Worksheet after incubating the samples.
2. Put in 40 μ L of Oragene Purifier to each of the four 1.5mL tubes for each participant and negative control. Record the lot number of the purifier on the Extraction Worksheet.
3. Divide the contents of every Oragene saliva sample across the four tubes (approximately 1mL per tube) and gently overturn guaranteeing that the Purifier completely mixes with the saliva sample. For the negative control add 1mL of 'Filtered & Autoclaved MilliQ water' to each of the four tubes and then treat precisely the same as all other samples.
4. Put saliva samples on ice for 10 minutes and then centrifuge in the microfuge at 10,000 rpm for 3 minutes. (Start processing the next batch during the incubation on ice.)
5. Immediately after centrifugation pool the supernatant from the 4 tubes per individual into one 10mL Sarstedt tube using a sterile transfer pipette without disturbing the pellet.
6. Add ~ 4mLs of 100% Ethanol to each tube and gently invert several times before leaving tubes on bench for 10 minutes at room temperature to precipitate DNA. (don't incubate at <5°C as impurities may co-precipitate)
7. Centrifuge tubes at 3,000 - 3,500rpm for 10 minutes in the Blood Room centrifuge.
8. Discard supernatant and remove as much Ethanol as possible using a transfer pipette without touching the DNA pellet.
9. Leave pellet to dry in an undisturbed place covered loosely with a kim wipe for a minimum of 2hrs and no longer than overnight before resuspending.
10. Add 400 μ L of 1xTE and leave on rocker overnight to allow DNA to resuspend.

DAY 2

11. Vortex and zip spin Sarstedt tubes before transferring DNA solution to a pre-labelled DNA storage tube (1.5mL RBC) using a P1000 pipette and filter tips. Ensure that the lid has an O-ring intact as this will minimise dehydration of the sample during storage.

12. Put a green “Saliva” cryo dot on the lid of each storage tube.
13. Log extractions into BL_Episode by opening each samples record in the Bleeding Episode form (in the database “O:\client\xpgenepi.mde”) and entering the extraction date and DEO into the ‘DNA Extraction Details’ section. Also select the ‘Link to BLSTOCK’ button and reduce the BUCCAL field by one (should be reduced from 1 to 0). Check that the extractions were logged correctly by running the ‘aa Saliva Extr Check’ query from “G:\GeneticEpiLab\BloodLab on L\xpBLEpisode copy.mdb”
14. Quantitate DNA using the spectrophotometer or Picogreen conjugated assay on the Fluoroskan and adjust an aliquot to 50ng/ul in 1 x TE (pH 8.0).

Formulas

$$\text{Vol of TE to add} = \frac{\text{Conc} \times \text{Vol}}{50\text{ng/uL}} - \text{Vol}$$

Where: Conc = initial concentration of sample (ng/uL)
 Vol = initial volume of sample (uL) (usually 400uL)

$$\text{Vol of Stock to Add} = \frac{(\text{Dil Vol} \times 50\text{ng/uL}) - (\text{Dil Vol} \times \text{Dil Conc})}{(\text{Stock Conc} - 50\text{ng/uL})}$$

Where: Dil Vol = the initial volume of the dilution tube (uL)
 Dil Conc = the initial concentration of the dilution tube (ng/uL)
 Stock Conc = the measured concentration of the Stock tube (ng/uL)

Source: ‘Anthony Caracella’ from QIMR

A3.2 Genotyping Analysis

Genotyping SNPs was performed using a MassARRAY MALDI-TOF MS (Sequenom Inc, San Diego CA). Assays were designed to type 25 SNPs using the Sequenom MassARRAY Assay Design software (version 3.0) and typed using iPLEX™ chemistry on a MALDI-TOF Mass Spectrometer (Sequenom Inc, San Diego CA). Four SNPs (rs10535985, rs2038137, rs6039806, and rs6911855) failed during the design and testing stage and were excluded. The remaining twenty-one SNPs came from nine ADHD RD candidate genes (DAT1 ‘SLC6A3’, DRD4, HTR1B, COMT, SNAP25, KIAA0319, MRS2L, and THEM2). Polymerase Chain Reaction (PCR) was carried out in each of 2.5 µL reaction in standard 384-well plates. PCR was performed with 12.5 ng genomic DNA, 0.5 units of *Taq* polymerase (HotStarTaq, Qiagen, Valencia, CA), 500 µmol of each dNTP, and 100 nmol of each PCR primer. PCR thermo-cycling was performed using an ABI Dual 384-Well GeneAmp® PCR System 9700 cycler (Applied Biosystems, Foster City, CA) and cycling conditions as follows: an initial denaturation stage at 94°C for 15 min, followed by 40 cycles of 20 sec at 94°C, 30 sec at 56°C, 60 sec at 72°C. One µL of solution containing 0.15 units Shrimp Alkaline Phosphatase was added to the completed PCR reaction mix, which was incubated for 20 min at 37°C, followed by inactivation for 5 minutes at 85°C. After adjusting the concentrations of extension primers to equilibrate signal-to-noise ratios, the post-PCR primer extension reaction of the iPLEX assay was performed in a final volume of 5.5 µL containing 0.122 µL termination mix, 0.025 µl DNA polymerase (Sequenom) and 600 nM to 1200 nM extension primers. A two-step 200 short cycles program was used during iPLEX thermo-cycling: initial denaturation was for 30 sec at 94°C followed by 5 cycles of 5 sec at 52°C and 5 sec at 80°C. An additional 40 annealing and extension cycles were then looped back to 5 sec at 94°C, 5 sec at 52°C and 5 sec at 80°C. A final extension at 72°C for three minutes was followed by cooling to 20°C. The iPLEX reaction products were desalted by diluting samples with 18 µL of water and 3 µL of resin to optimize mass spectrometric analysis and then spotted on a SpectroChip (Sequenom), processed and analysed in a Compact Mass Spectrometer by MassARRAY Workstation software (version 3.3) (Sequenom). Assay quality and genotype calls were assessed in the SpectroTYPER software (Sequenom). Detailed PCR conditions, genotyping conditions,

and fragment lengths for the individual SNPs are shown in Table A2.2.

Table A3.2 *PCR and Genotyping Conditions for the ADHD RD Candidate Genes*

SNP ID	Gene	Primer Pair	Extension primer
SNP 1	DRD4 (rs3758653)	F: ACGTTGGATGGAGAAAGTGCTTGCAAAGCG R: ACGTTGGATGAATACCTCTCAGGTCACAG	ttTGCAAAGCGCAGCAGAGA
SNP 2	DAT1 (rs27072)	F: ACGTTGGATGTCTACAAGGATCGTGATCCC R: ACGTTGGATGTTCATGCTGTCTGTGTGAGG	GGGGCAGCCTCAGAGC
SNP 3	DAT1 (rs6347)	F: ACGTTGGATGTCATCTACCCGGAAGCCATC R: ACGTTGGATGATACCCAGGGTGAGCAGCAT	CGCTCCCTCTGTCCTC
SNP 4	SNAP25 (rs362549)	F: ACGTTGGATGGGACTTCCCTTGGTGACAAA R: ACGTTGGATGACTGAGCATGACAACAGAC	gAAATTTTCTAGAGGAATGAAAGT
SNP 5	SNAP25 (rs362987)	F: ACGTTGGATGGCAAGCTCTCAACAATTGTC R: ACGTTGGATGCTGTTTGGGTCTGGATTATG	TTAAGTCGAGGCATTAGA
SNP 6	SNAP25 (rs362998)	F: ACGTTGGATGTTCGTCCTACTACAGCAGCAG R: ACGTTGGATGTCCTCACCCTCTCTTTTGC	cGGCTGGCCACCACTCC
SNP 7	SNAP25 (rs1051312)	F: ACGTTGGATGATTCAGCAAATGCCACCGAG R: ACGTTGGATGTAGTGGTCATTTGGTGGCTC	GAGAAAATGAAAAATGAAACTCA
SNP 8	COMT (rs737865)	F: ACGTTGGATGTCTACGGTCCCTCAGGCTT R: ACGTTGGATGCTAACAGACCTGCTTTTTGG	gaacAGCAACAGGACACAAAAA
SNP 9	COMT (rs4680)	F: ACGTTGGATGTTTTCCAGGTCTGACAACGG R: ACGTTGGATGATCACCATCGAGATCAACCC	aTGCACACCTTGTCTTCA
SNP 10	COMT (rs165599)	F: ACGTTGGATGGGCTGACTCCTCTTCGTTTC R: ACGTTGGATGACAGTGGTGCAGAGGTCAG	TCTTCGTTTCCCAGGC
SNP 11	MRS2L (rs2793422)	F: ACGTTGGATGTGCATTGGAGAGATCGTCAG R: ACGTTGGATGAAAGAGCAGTGCTGGGATTG	cttgTTCCAACAGCAACTCCAT
SNP 12	KIAA0319 (rs4504469)	F: ACGTTGGATGAGAGCACAGCATCCCAACAC R: ACGTTGGATGTGGTAGGATATGGGTAGC	ccctAACACCTCCCCTAGC
SNP 13	KIAA0319 (rs2179515)	F: ACGTTGGATGTTACTCAGTTCATTTTGCCC R: ACGTTGGATGCATGTCCTGAATGTAGGAGC	ccgCAGTTCATTTGCCCCTAGAATA
SNP 14	TTRAP (rs6935076)	F: ACGTTGGATGAACCGAAGCCAGAGAAAAAC R: ACGTTGGATGAAAAAATTCCTGGCCAGGG	agagCAGACATGAGGAGAATGA
SNP 15	TTRAP (rs2143340)	F: ACGTTGGATGAACCTGGCATGCAATTCTTG R: ACGTTGGATGGTAGCCCTCATTTTACAGAC	aCCTGTAAGGACAGTGTCACTT
SNP 16	THEME2 (rs3777664)	F: ACGTTGGATGCTGTTATTAGGCAGCCCTTC R: ACGTTGGATGACACTCCACATGACACAGAG	gCCTTCTCCCTATCTATCTTT
SNP 17	HTR1B (rs2000292)	F: ACGTTGGATGGTTACGTTATAGGAGAATGGC R: ACGTTGGATGCAGCATCAGAAAATGGATCAC	TATAATCTCACTCGTTTTTCTC
SNP 18	HTR1B (rs6297)	F: ACGTTGGATGAACTTGGTCCCCAAAGGTCG R: ACGTTGGATGGCATTCCATAAACTGATACG	GCGACCCCACTGCAAA
SNP 19	HTR1B (rs6296)	F: ACGTTGGATGTCGGAGACTCGCACTTTGAC R: ACGTTGGATGCTCTATTAACCTCGCGGGTTC	gCTCGCACTTTGACTTGGTT
SNP 20	HTR1B (rs6298)	F: ACGTTGGATGCGCCAAGGACTACATTTACC R: ACGTTGGATGAAGGTGATGAGCGCCAATAG	ACTACATTTACCAGGACTC
SNP 21	HTR1B (rs130058)	F: ACGTTGGATGTCAATTATTCTCCGCCAG R: ACGTTGGATGTTAGCTAGGCGCTCTGGAAG	ACAGCTGAAACTAGAGGTCA

F, Forward; R, Reverse; PCR, Polymerase Chain Reaction; SNP, Single Nucleotide Polymerase; A, Adenine; T, Thymidine; C, Cytidine; G, Guanine.

Appendix 4
4.1 ATR Family Approach Letter

«PARENTAD1»
«PARENTAD2» «PARENTAD3»

11th October 2005

Dear «PARENT_SURNAME»,

Thank you for your family's continued membership of the Australian Twin Registry. Enclosed is a letter inviting you and your twins to continue participating in research on the behaviour of school-age twins which is being conducted by Abdullah Sheikhi (PhD Candidate), Professor David Hay, Associate Professor Jan Piek of the Department of Psychology at Curtin University (Perth) and Professor Florence Levy of the Avoca Clinic at the Prince of Wales Children's Hospital (Sydney). As part of investigating the behaviour of twins and their siblings, this study will investigate genes that affect children's level of attention and activity. Therefore, the researchers need your genetic material (DNA) to look at some genes thought to be associated with behaviour. The aim of the study is to compare genes among twins and siblings with different levels of activity and attention. The enclosed letter from investigators provides you with a general description of the study and explains in detail what is involved in participating.

Briefly, your participation in this study will involve donating a DNA sample for analysis by providing a saliva sample or buccal (cheek) cells:

That involves nothing more than spitting into a tube OR rubbing a swab inside the mouth (the researchers will send you the kit for this) and takes less time than reading all this information!

significant scientific value and as satisfying the necessary ethical requirements for such research. Legislation around DNA research does mean the Consent Forms are more complex than usual. The researchers provide a Free call number if you want more information.

Participation in this or any Twin Registry study is voluntary. So, if you are willing to take part in the study, please fill out the enclosed response form and return it in the reply paid envelope provided. Alternatively, if you are not interested in participating, or you are unable or feel your family is ineligible, please indicate this on the enclosed form and return it anyway, as this will let people know that you have received this letter and have made a decision about your involvement.

If you need changes made to your address and/or other details, please note these on the form or phone us on Free call 1800 037 021 and we will update our records.

We greatly appreciate your taking the time to consider this request and thank you in anticipation of the researchers receiving your response.

Yours sincerely,



Professor John Hopper
Director

4.2 Study Information Sheet

Australian Twin and Sibling Behavioural Gene Study

«PARENTAD1»

«PARENTAD2» «PARENTAD3»

11th October 2005

Dear «PARENT_SURNAME»,

We would like to thank you very much for taking the time to contribute to our research by completing the TWIN AND SIBLING questionnaires. We would like to invite you to take part in a follow-up behavioural gene study in twins and siblings.

- **What is this about?**

As part of investigating the behaviour of twins and their siblings, we are currently trying to find genes that affect children's level of attention, activity, and school performance. We know that both your genes and your environment are important in shaping your behaviour. Therefore, we need your genetic material (DNA) to look at some genes thought to be associated with behaviour. You are being asked to participate in this research study titled "**Australian Twin and Sibling Behavioural Gene Study**". Before you can decide whether or not to volunteer for this study, you should understand enough about the purpose of the study, its risks and benefits to make an informed judgment about whether or not you want to be part of the study.

Please read the information provided in this sheet, and if you are happy to be involved, complete and sign the DNA consent form. If your family decide definitely not to participate, **please complete question 1 in the Parent's DNA Consent Form** about non-participants, and return it in the reply paid envelope to: *Australian Twin and sibling Behavioural Gene Study, School of Psychology, Curtin University of Technology, GPO Box U1987, Perth WA, 6845*. This will let us know that you have received this letter and have made a decision about your involvement. Once you understand the study, you are being asked to explain the study to your children and help them to fill and sign the **Child's DNA consent form** too. Although it is not possible for young children to fully consent to this study, children can and should have the study explained to them and agree to the taking of their samples.

If you want to talk to us about the study, phone numbers are at the end of this letter.

- **Why your family has been chosen?**

The reason for choosing you/your family is we need families whose children vary a lot in their attention, activity, and school performance based on the Twin and Sibling Questionnaire you had completed.

- **What is involved in this study?**

This study involves Australian twin families including Parents, Identical (MZ), Fraternal (DZ) twins and brothers and sisters from 6 years old upwards. Your family's participation in the study should only take a little time. In order to examine the **GENETIC MATERIAL (DNA)**, you/your family are being asked to participate in this research by giving a **SALIVA SAMPLE or CHEEK "Buccal" SWAB CELLS** from your mouth.

You/your family can collect the sample by spitting into a tube or by rubbing the inside (including the cheeks, lip and gums) of your mouth with sterile cotton wool buds.

AFTER YOU RETURN TO US THE SIGNED DNA CONSENT FORMS, WE WILL SEND YOU THE SALIVA KIT OR THE BUCCAL SWAB KIT WITH COLLECTION PROTOCOL.

- **What are Risks associated with saliva and buccal swab cells collection?**

Both these two methods are home-based rather than being done in hospitals and clinics. They are considered SAFE, WITHOUT RISK, and can even BE DONE BY CHILDREN themselves.

- *Consent to unspecified future attention and activity genes:*

The new genetic ethics regulations and guidelines state that **ONLY** genes relevant to the study are permitted to be investigated. This study aims to investigate the current known genes that contribute to attention and activity and other genes that may show a significant role in behaviour in the future. Both current genes and genes that may appear in the future will be targeted, as genetic advances are happening so fast in this area. You/ your family have the right to **GIVE** or **NOT GIVE** consent to only the current genes related to attention and activity and/or genes that they might show involvement with attention and activity in the future. You/your family have the choice to authorise us to study both current and future genes by ticking ‘**YES**’ on the DNA consent form or alternatively if you choose not to participate in the study tick ‘**NO**’ in DNA consent form. For your confidentiality and privacy, we will make sure that your/your family’s names will be separate from their genetic samples so that no link can be made between you/your family’s names and the samples to which you relate. **Your/your family DNA will ONLY be used for the one purpose of investigating the genes that are involved with attention and activity.** At present most of these genes involve the brain chemical dopamine that helps get information from one nerve to the next.

- *What are the Potential Benefits from this study?*

Your/ your family’s participation is essential to this work and will provide valuable information about understanding the behavioural differences among twins and siblings and genes that are involved with attention, activity, and school performance. Internationally this is a very active area of research and the co-operation of families such as yours does mean Australia is at the forefront. However, we cannot be sure that this study will benefit you/ your family directly.

- **What happens to the information you gave us?**

The information you/your family provide is **STRICTLY CONFIDENTIAL** and will be protected. Samples and genetic information obtained for this research study will be accessible **ONLY** by the researchers directly involved in this study. No information about you/your family will be available to any other person including insurance companies. So you can answer ‘**NO**’ if a medical insurance company asks if you have ever had genetic testing. This also means that we cannot give you any specific information about your sample beyond zygosity if you wish. We will let you know about the results of the whole study in the future.

Your/ your family’s DNA samples and data information will have numerical codes instead of names. These will be stored separately from identifying information. All identifying information will be kept confidential through the use of a secure computer database. The database will be password protected and accessible **ONLY** to researchers directly involved in this study. You/ your family’s names will be removed from all lab tubes prior to storage and replaced by an ID code to maintain confidentiality. As a result, general laboratory personnel will have access only to ID numbers. Reporting in any format, including scientific meetings or journals will never contain names, or other identifying information.

- Your family's participation is voluntary:

You are under **NO** obligation to take part in this study and can withdraw from it at any stage.

- **If you/your family are willing to take part in the study**, please fill out the enclosed DNA consent form and return it in the enclosed reply paid envelope.
- **If you/ your family are not willing, or you/ your family are unable or feel you are ineligible, to participate**, please indicate this on the enclosed DNA consent form and return it to us anyway, as this will let us know that you/ your family have received this letter and have made a decision about your/ your family involvement.
- *Who do I contact if I have any further questions?*

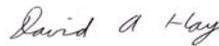
If you would like to know more about this study before deciding whether or not you want to participate, or you have a question regarding the eligibility of your family, please call **Abdullah Sheikhi** on (08) 9266 2758 (email: a.sheikhi@curtin.edu.au) or **Professor Jan Piek** on (08) 9266 7990 (email: J.Piek@curtin.edu.au). Alternatively, if you would like to talk to an independent person, you can contact **Ms Sinead Darley** (S.Darley@curtin.edu.au) on (08) 9266 2784. For those calling long distance, you can call us on the **Toll Free Number 1800 850 739**.

We greatly appreciate your participation and thank you in anticipation for your co-operation and assistance with this study.

Abdullah Sheikhi
(*PhD Candidate*)



Professor David Hay
(*Supervisor*)



Professor Jan Piek
(*Co-Supervisor*)



4.3 Parent's DNA Consent Form

Australian Twin and Sibling Behavioural Gene Study

«PARENTAD1»
«PARENTAD2» «PARENTAD3»

11th October 2005

Dear «PARENT_SURNAME»,

Please read the information sheet carefully, fill in this form, and then return it to the address provided below:

1. Our family is definitely interested in participating in this study

YES

NO

*If you answered 'NO' you do not need to complete the rest of the form.

2. I _____
(First name) (Surname)

as a parent/ guardian give permission for myself and for my family to have DNA collected/tested as indicated on the Information Sheet:

	BIOLOGICAL MOTHER	BIOLOGICAL FATHER
<i>First Name</i>	_____	_____
<i>Surname</i>	_____	_____
<i>Date of Birth</i>	_____	_____

3.

	TWIN A	TWIN B	SIBLING 1	SIBLING 2
--	--------	--------	-----------	-----------

First Name

Date of Birth

Gender

Please, let us know if your details are not correct. These are based on our existing records.

Please answer the following questions in order to comply with the increasingly complex legislation in genetic testing:

4. I/ my family give permission for the testing of:

Only those genes currently thought to be involved with attention and activity

Twin 1	Twin 2	Sibling 1	Sibling 2	Biological Mother	Biological Father
<input type="checkbox"/>					

AND / OR

Genes involved with attention and activity that are found at a future date

Twin 1	Twin 2	Sibling 1	Sibling 2	Biological Mother	Biological Father
<input type="checkbox"/>					

5. If your twins are of the same sex, our DNA testing will determine if they are identical (MZ) or not (DZ). Do you wish us to give you this information?

YES NO

My family have discussed the information and all of their questions have been satisfactorily answered and I/ my family understand that:

Our participation in this study is entirely voluntary and that me/ my family may refuse to participate without any consequence.

I/ my family also appreciate that at any time I/ my family have the right to withdraw from the study and request that I/ my family's biological samples, or DNA samples be returned or destroyed. In this instance, you/your family need simply sign a letter stating, " I/ my family wish to withdraw from the study "Australian Twin and Sibling Behavioural Gene Study", and request that my/ my family biological samples or DNA be destroyed". The letter should also include the name and signature of a witness, and be mailed directly to the address below.

My/ my family's DNA will be used ONLY in relation to genes contributing to attention and activity.

My/ my family's confidentiality will be respected and no information that discloses our identity will be released or published without our specific consent to the disclosure.

The Investigators of this study, as well as Curtin University of Technology, will not be liable for any loss or damage to biological samples taken or used in accordance with this form.

Thank you in anticipation for your co-operation and assistance with this study

Parent's / Legal guardian's name Signature Date

Witness's name Signature Date

Please return it in Reply Paid Envelope to (no stamp needed):
Australian Twin and Sibling Behavioural Gene Study
School of Psychology
Curtin University of Technology
Reply Paid GPO Box U1987, Perth WA, 6845

4.4 Child's DNA Consent Form

Australian Twin and Sibling Behavioural Gene Study

«PARENTAD1»
«PARENTAD2» «PARENTAD3»

11th October 2005

Dear «CHILD_SURNAME»,

Your Mum and Dad have just read about a study involving twins and their brothers and sisters that is being done at Curtin University. The study is trying to discover if some ingredients in people's saliva or cheek cells might affect how well they can pay attention.

To find these ingredients we just need you to get spit into a tube or use a cotton bud to wipe some spit from inside your mouth.

Before deciding whether you want to be a part of this study, you can ask your parent to tell you more and explain what you should do. If you agree to be part of this study, you will need to sign your name at the bottom of the page.

I understand what this study is about and any questions I may have been satisfactorily answered in language I can understand.

I _____ willingly consent to participate in this study

(Child name)

YES

NO

(Child signature)

(Date)

To Parents: If in your opinion, your child understands what their signature above means, please sign here.

(Parent/ legal guardian signature)

(Date)

Thank you for your co-operation and assistance with this study.

4.5 Reminder Letter

«PARENTAD1»
«PARENTAD2» «PARENTAD3»

11th October 2005

Dear «PARENT_SURNAME»,

We would like to thank you for your past participation in the twin and sibling project. Recently you received a letter from Curtin University asking if you would be interested in participating in a research project titled *The Australian Twin and Sibling Behavioural Gene Study*, which aims to investigate genes that contribute to attention, and activity.

We know it is a very busy time of year for all families but it would be great if you could please return the completed consent form to Curtin University of Technology in the reply-paid envelope. We have asked families to return the consent form even if they do not wish to participate further, as this will let us know that you have received the information letter and have made a decision about your involvement.

We would like to bring your attention to **Q4** in the **Parent's DNA Consent Form**. The original consent form contained a misprint which has caused confusion for some families and we apologise for this. When asked if you/your family would like to be involved in the testing of currently known genes '**OR**' genes discovered at a future date, the letter should have read '**AND/OR**' to allow you to have the opportunity of selecting either the first option or both options. If you require further clarification of this, please do not hesitate to contact us on the Toll Free Number supplied below.

If you require another consent form and/or envelope to be sent to you, or if you wish to discuss the research before giving consent to participate, please call **Abdullah Sheikhi** on (08) 9266 2758 (email: a.sheikhi@curtin.edu.au).. For those calling long distance, you can call **Grant Baynam** on the **Toll Free Number 1800 850 739**.

Please ignore this letter if you have recently returned your consent form or if you have received a phone call asking about your consent form.

Thank you for considering this request.

Kind Regards,

Abdullah Sheikhi
(*PhD Candidate*)



Professor David Hay
(*Supervisor*)



Professor Jan Piek
(*Co-Supervisor*)



4.6 Saliva Kit Information Sheet

August, 2006

Dear Family,

Thank you for agreeing to participate in the “*Australian Twin and Sibling Behavioural Gene Study*”. We are sending you this Saliva Kit Protocol in order to collect your DNA. Your package contains the following (*PLEASE READ THE INSTRUCTIONS CAREFULLY*):

Your package contains:

- a) An Information Sheet with instructions for collecting, packaging and returning your saliva sample to us.
- b) A cardboard box in which to package your Saliva Kits for safe transport
- c) Saliva Kit containers labelled ORA gene™ DNA Self-Collection Kit.
- d) A large TNT plastic bag with a **Pre-Paid**, addressed consignment note (attached to the TNT plastic bag in which the box is to be placed) for overnight delivery by TNT Courier Service. The contact number for **TNT is 131 150** to make the arrangements for the collection of the Courier Pack.

What is the ORA gene™ DNA Self-Collection Kit?

The ORAgene™ DNA Self-Collection Kit is a non-invasive system (no needles!) for collecting DNA from saliva. It is an easy to use, reliable method of self-administered DNA collection.

Please carefully notice that each ORA gene™ DNA Self-Collection Kit is labelled by name for each family member. **CAREFULLY MAKE SURE THAT YOU HAVE YOUR OWN CONTAINER WITH YOUR OWN NAME WRITTEN ON IT.**

When can your saliva samples be collected?

Any convenient time, but before spitting, please rinse your mouth with water to get rid of food particles. Then wait at least one minute before spitting your sample to ensure that any residual water is completely swallowed. This is because the residual water in the mouth can dilute saliva. **Some people may find it hard to spit so much saliva. It is easier to spit more if you place ¼ teaspoon of plain white sugar on your tongue.**

How to use the ORA gene™ DNA Self-Collection Kit? (*Please read the Pamphlet inside the ORA gene kit*)

Please follow the steps below to collect your Saliva Sample:

STEP ONE: **Collect the recommended volume of saliva**

Spit your saliva into the ORA gene Container. The recommended volume of saliva is 2mL, about a teaspoonful. Less saliva means proportionately less DNA yield. Please make sure that **there are no bits of food** in the mouth when spitting

STEP TWO: **Finish spitting within 30 minutes**

Keep spitting until the amount of liquid saliva (not counting foam) reaches the top of the white label. The full saliva sample should be collected within 30 minutes and the ORA gene vial

should be capped immediately. Waiting longer than 30 minutes may decrease the yield and quality of the DNA.

STEP THREE: Tighten the cap very firmly

Capping the container releases the DNA-preserving solution which mixes with the saliva.

STEP FOUR: Gently mix your saliva by shaking your container.

How you can store your saliva?

Please DO NOT put your saliva samples in the fridge or expose them to direct sunlight. Your saliva samples are stable and **CAN BE KEPT AT ROOM TEMPERATURE**. **Please record the date and time of collection on the label on your saliva container.**

How to post your Saliva samples?

When all of the ORAgene™ DNA Self-Collection Kit containers have been filled, please put them in the cardboard box, along with enough packaging material to stop them from moving around. Put the box inside the TNT plastic bag. **Sign the consignment note (attached to the plastic bag) in the section for Sender's Signature. Telephone your local TNT Courier on 131 150 to arrange collection of the Courier Pack.** Please give them the address of where they can collect the pack, and most importantly, advise TNT that the Courier Pack is to be delivered to **QIMR (Queensland Institute of Medical Research) in BRISBANE, PAID** overnight delivery.

NOTE: Please do not allow the TNT operator to upgrade your package. It should only be sent **PAID Overnight Delivery. All costs are covered and you must not pay them anything!**

It is critical that the samples MUST get back to us AS SOON AS POSSIBLE before they deteriorate. Bear in mind that cells start dying as soon as they leave the body and if a sample deteriorates then the amount of DNA we can get ranges from very little to none.

Once again, thank you for participating in this research study. If you do have any questions, have any difficulty with **the ORAgene™ DNA Self-Collection Kit**, please do not hesitate to contact **Abdullah Sheikhi** on **(08) 9266 2758** or email him on 'a.sheikhi@curtin.edu.au'. For those calling long distance, you can call us on the **Toll Free Number 1800 850 739**.

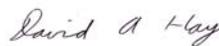
Thank you for your co-operation and assistance with this study.

With Best Regards,

Abdullah Sheikhi
(PhD Candidate)



Professor David Hay
(Supervisor)



Professor Jan Piek
(Co-Supervisor)



Appendix 5: ATR Ethics Application

AUSTRALIAN TWIN REGISTRY
RESEARCH APPLICATION COVER SHEET

Registry Study Number:					-			
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AUSTRALIAN TWIN REGISTRY
RESEARCH APPLICATION

Registry Study Number:					-			
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BIOGRAPHICAL DATA

Name of Principal Investigator Abdullah R. SHEIKHI
Appointment Held: PhD Candidate
Department and Institution: School Of Psychology Curtin University of Technology
Degrees/Professional Qualifications: BSc, MSc. in Biology

RESEARCH PROGRAM

Short Title of Project: Two Approaches To The Molecular Genetic Analysis Of ADHD Subtypes In Australian Twins
Aims: Identifying genes contributing to Attention-deficit hyperactivity disorder (ADHD) Estimating significant Single Nucleotide Polymorphisms (SNPs) that contribute to ADHD Performing Quantitative Trait Loci (QTLs) linkage and association mapping for The Australian Twin ADHD Project (ATAP) samples. Exploring whether 6p21.3 region is susceptible for ADHD as it has been confirmed it is susceptible to Reading Disability.

Summary of Previous Work in This Field:

Bsc, MA (Biology & Education) – Principles of Human Genetics, Human Molecular Genetics, Population Genetics.

Completed Workshops:

2002: Molecular Genetics (Australian Neuromuscular Research Institute, Queen Elisabeth II Hospital) – Nucleic Acid Extraction (DNA, RNA); Setting up a Southern Blot; Polymerase Chain Reaction (PCR) Protocols [Primer design and ordering]; *in situ* PCR and DNA Sequence Analysis.

2003: Genetic Data Analysis & Association Mapping (La Trobe University, Melbourne) **Quantitative Trait Locus Mapping** (Southern Institute in Statistical Genetics, North Carolina State University)

My supervisor Professor David Hay has extensive experience in research involving twins and is the patron of the Australian Multiple Birth's Association (AMBA)

RESOURCES

Personnel:

INVESTIGATOR:

1. PhD Candidate Abdullah Sheikhi, School of Psychology, Curtin University of Technology
2. Professor David Hay, School of Psychology, Curtin University of Technology

CO-INVESTIGATORS:

3. Professor Florence Levy, School of Psychiatry University of New South Wales
4. Professor Jan Piek, School of Psychology, Curtin University of Technology

Funding Support:

This study is funded by two grants from both the National Health and Medical Research Council (NHMRC) and the National Institute of Mental Health (NIMH) in USA.

Facilities:

Twin Recruitment:

MZ and DZ twin families will be recruited from the current 4th wave of ATAP study. Twin recruitment for this study will be based on the current 4th wave of the Australian Twin ADHD project (ATAP), held at Curtin University under Professor David Hay's supervision. Assessment of twins having ADHD and reading problems will be based on the Australian Disruptive Behaviours Questionnaire (ADBQ) (or The Twin and Sibling questionnaire) for twin and siblings in which 6000 families are aiming to recruit and assess. Participants will involve MZ and DZ twins and their siblings from 6- 17 years old and their parents

Sample collections:

Blood and/or cheek 'buccal' cells will be collected from participants based on their choice. For blood collection participants will be provided labmailer package containing blood collection tubes, DNA consent forms, and the the study information sheet. A convenient pathology service will withdraw blood from participants and they will not charge any fees. Also, for buccal cells collections, a package containing cotton wool buds will be sent to participants which they can collect cells from cheeks by themselves at any convenient time to them. Cotton wool buds can be placed in a tube containing a storage liquid. The cotton wool buds then can be put in the padded envelope, stamped, addressed, and post to us.

Behaviour assessment:

The Twin and Sibling questionnaire will be used to assesst twin families (MZ and DZ) who have ADHD from ongoing ATAP 4th wave study. The criteria of measuring ADHD is based on DSM-IV.

For QTL association mapping study

The association study will take place at either QIMR (Queensland Institute of Medical Research) in Queensland or AGRF (Australian Genome Research Facility) in Melbourne based on their costing, since they both have the Matrix-Assisted Laser Desorption Ionization-Time-of-Flight- Mass Spectrometry (MALDI-TOF MS) machine. This stage will include DNA extractions, pool constructions and SNPs allele frequencies by MALDI-TOF MS. The PCR primers and the primer extension assays will be created and designed by using the SPECTRODESIGN software (Applied Biosystems or Sequenom, Inc.). In addition, the detection of SNPs will be based upon analysis of primer extension products generated from amplified genomic DNA using a chip-based MALDI-TOF mass spectrometry platform (Applied Biosystems or Sequenom).

For QTL linkage mapping study

The linkage analysis will be held at Graylands Hospital and at Royal Perth Hospital with the co-operation of Prof Assen Jablensky. Furthermore, Dr. Neilson Martin from UK Wellcome Trust Centre for Human Genetics, Bioinformatics and Statistical Genetics will participate in this study. He joined Curtin School of Psychology in October. 2003.

APPROACH AND FOLLOW UP PROCEDURES FOR RECRUITING TWINS

Participants will be MZ and DZ twins and their siblings (if any) aged 6-17 years old and their parents. The participants will be recruited and identified from the current study by the Australian Twin ADHD Project (ATAP) titled "Genetic comparison of two measures of Attention Deficit Hyperactivity Disorder (ADHD)" held at Curtin University of Technology. The ATAP study is using the Australian Disruptive Behaviours Questionnaire (ADBQ) (or The Twin and Sibling questionnaire) investigating the behaviour of school age twins and their siblings titled "Twin and Sibling Questionnaire". I am planning to recruit 500 out of 6000 families based on the questionnaire assessment which will showed the complete range of attention and activity that children fit in.

Six categories are aimed to target from The Twin and Sibling questionnaire:

Category	Measured by DSM-IV ADHD
Inattention Only	(Inat=>6, Hyp-imp<=2, Reading=0)
Combined Only	(Inat=>6, Hyp-imp=>6, Reading=0)
Inattention + Reading	(Inat=>6, Hyp-imp<=2, Reading=1)
Combined + Reading	(Inat=>6, Hyp-imp=>6, Reading=1)
No ADHD + No Reading	(Inat<=2, Hyp-imp<=2, Reading=0)
No ADHD + Reading	(Inat<=2, Hyp-imp<=2, Reading=1)

Each twin family will be sent the study Information Sheet and the DNA consent forms. In the consent forms, participants have the choice either to give venous blood or buccal swab cells. The information sheet with the title "Australian Twin and Sibling Behavioural Gene Study" provides information about aims, what is involved, risks associated with blood drawing and buccal swabs, privacy, potential benefits and information about the procedure will be used to collect DNA from venous blood and buccal cavity. In case participants agree to participate, they will be sent labmailer package containing blood collections tubes and/or cotton wool buds, parent's information sheet providing blood collection protocol and/or cheek 'buccal swab' protocol, and an information sheet for participants' pathology service. Telephone calls will be made to participants to follow up how the procedures are going.

SELECTION CRITERIA FOR TWIN SAMPLE

JUNIOR TWINS:

Nominate groups broken down according to: **Zygoty** (identical/ MZ or fraternal/ DZ or Unknown), **Sex Combination** (Male/male; female/female; or male/female), **Age range** (please stipulate an upper and lower age limit), **Place of Residence** (whether for at least one twin or both twins; nominated by state or postcode). **List any other relevant criteria and the number of pairs required for each group.**

I am aiming to recruit approximately from 500 to 600 families selected from the umbrella project. These families will be chosen because they fit in the complete range of attention and activity score, which we assessed in the ABDQ questionnaire.

Zygoty: Both MZ , DZ twins are required for this study .

Age Range: **The targeting age of twins are children aged from 6 to 17 years of age.**

Place of Residence: All DNA Samples will be collected by mail from all parts of Australia.

Adult Twins:

Nominate groups broken down according to: **Zygoty** (identical/ MZ or fraternal/ DZ or Unknown), **Sex Combination** (Male/male; female/female; or male/female), **Age range** (please stipulate an upper and lower age limit), **Place of Residence** (whether for at least one twin or both twins; nominated by state or postcode). **List any other relevant criteria and the number of pairs required for each group.**

ADULT TWINS WILL NOT BE INCLUDED IN THIS STUDY

Please tick

ATTACHMENTS

Outline of research plan or Copy of the research grant application	Attached	<input checked="" type="checkbox"/>
	Attached	<input type="checkbox"/>
A list of all the tests and procedures you will be asking twins to undergo, including the risks associated with them and an indication of which procedures, if any, are invasive.	Attached	<input type="checkbox"/>
All questionnaires which are to be administered to twins.	Attached	<input type="checkbox"/>
Consent Forms where written consent is appropriate.	Attached	<input type="checkbox"/>
Plain Language Statement A copy of the plain language statement that has been or will be submitted to the Institutional Ethics Committee (See Australian Twin Registry Guidelines for Users)	Attached	<input type="checkbox"/>

Draft Approach Letter(s) to Twins or Their Parents; including a draft Response Form where appropriate. (See Australian Twin Registry Guidelines for Users)	Attached <input type="checkbox"/>
Ethical Clearance(s) for this project from properly constituted Ethics Committees for all institutions involved in the research proposal.	Attached <input type="checkbox"/>

SIGNATURES

Principal Investigator

I certify that the above information is correct and that my proposed research will not vary from that outlined above without prior approval from the Australian Twin Registry.

Signature

Date: *20 August, 2004*

Head of Department / Institution

I certify that:

- i. this project is appropriate given the general facilities in my department
- ii. the proposed research conforms to the general principles set out in the *National Statement on Ethical Conduct in Research Involving Humans*.

Signature

Date:
