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Cover Page

Title: The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy

Running title: The CDKL5 disorder

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ABSTRACT

The clinical understanding of the CDKL5 disorder remains limited, with most information being derived from small patient groups seen at individual centres. This study uses a large international data collection to describe the clinical profile of the CDKL5 disorder and compare with Rett syndrome. Information on individuals with *CDKL5* mutations (n=86) and females with *MECP2* mutations (n=920) was sourced from the InterRett database. Available photographs of CDKL5 patients were examined for dysmorphic features. The proportion of CDKL5 patients meeting the recent Neul criteria for atypical Rett syndrome was determined. Logistic regression and time-to–event analyses were used to compare the occurrence of Rett-like features in those with *MECP2* and *CDKL5* mutations. Most individuals with *CDKL5* mutations had severe developmental delay from birth, seizure onset before the age of three months and similar non-dysmorphic features. Less than one quarter met the criteria for early-onset seizure variant Rett syndrome. Seizures and sleep disturbances were more common than in those with *MECP2* mutations while features of regression and spinal curvature were less common. The CDKL5 disorder presents with a distinct clinical profile and a subtle facial, limb and hand phenotype that may assist in differentiation from other early-onset encephalopathies. Although mutations in the *CDKL5* gene have been described in association with the early onset variant of Rett syndrome, in our study the majority did not meet these criteria. Therefore, the CDKL5 disorder should be considered separate to Rett syndrome, rather than another variant.

Key words: CDKL5, Rett syndrome, Dysmorphology, Natural history, Phenotype

INTRODUCTION

Mutations in the c*yclin-dependent kinase-like 5 (CDKL5)* gene have been found in individuals diagnosed with the early-onset seizure variant of Rett syndrome (ESV RTT), infantile spasms or West syndrome. In 2003, chromosomal rearrangements involving the *CDKL5* gene were identified in two females with severe intellectual disability and early-onset seizures[1] and the following year in four females with Rett syndrome (RTT).[2,3] To date, 141 individuals (127 females and 14 males) with a *CDKL5* mutation have been described, mostly within small case series,[1-38] the largest including 20 patients.[11] Therefore the understanding of the CDKL5 disorder profile is still limited and a larger series is required to further understand its presentation and natural history.

The clinical characteristics commonly associated with a *CDKL5* mutation include early-onset seizures and severe intellectual and gross motor impairment. The ESV RTT was originally described in a girl with RTT who developed seizures by one year of age.[39] The recently published Neul criteria[40] for atypical RTT included five specific items (early-onset seizures before 5 months of age, infantile spasms, refractory myoclonic epilepsy, seizure onset before regression and decreased frequency of typical RTT features) for differentiating the ESV from other atypical forms. The number of distinguishing criteria required for a diagnosis of ESV RTT was not clear although testing for a *CDKL5* mutation was recommended.

Various dysmorphic features have also been described in individuals with a *CDKL5* mutation including large deep-set eyes, strabismus, high forehead, full lips, wide mouth and widely spaced teeth.[1,4,5,7,13,18,20,22,25,26,28-30,35] The presence of typical facial or other

features could provide additional assistance in the clinical identification of individuals with a *CDKL5* mutation.

Here we describe the phenotype of females and males with a pathogenic or potentially pathogenic *CDKL5* mutation, identified through the International Rett Syndrome Phenotype Database (InterRett).[41] We assessed whether individuals with a *CDKL5* mutation shared similar characteristics of the face, hands and feet. We determined whether these individuals met the latest criteria for ESV RTT[40] and compared the presence of RTT features in females who have a *CDKL5* mutation with females who have a pathogenic *MECP2* mutation.

METHODS

InterRett is an international Rett syndrome database which has ascertained over 2000 RTT cases worldwide since 2003.[41] Since 2007, partly through the advocacy of the family support group, families of an individual with a *CDKL5* mutation have been invited to complete an InterRett questionnaire. Families also provided photographs of their son or daughter's face (frontal and side), hands (dorsal and palmar) and feet (dorsal and plantar). A comparison group of females with RTT and a pathogenic *MECP2* mutation was also obtained from the InterRett database.

Data from family and clinician questionnaires, concerning acquisition of gross motor milestones, purposeful hand function and speech, presence of hand stereotypies, seizures, gastrointestinal problems, spinal curvature (scoliosis or kyphosis), autonomic problems and sleep problems were examined. All photographs of patients with CDKL5

mutations were reviewed by a clinical geneticist (MW) and the characteristics noted were tabulated.

Approval for this study was provided by the Princess Margaret Hospital for Children Ethics Committee, Perth, Western Australia.

Statistical analysis

The phenotypical characteristics of the CDKL5 disorder and the difference between females and males were determined using descriptive statistics. The proportion of CDKL5 disorder individuals that met each of the criteria for ESV RTT, by sex and age (aged five years and under or over five years) was determined. Clinical features known to be associated with RTT were compared between females with a *CDKL5* mutation and females with a *MECP2* mutation. Odds ratios adjusting for age were estimated. Survival analysis was used to investigate the timing of onset of seizures, stereotypies and spinal curvature.

RESULTS

Seventy-seven females and nine males with a *CDKL5* mutation were included in this study (supplementary table 1 contains genetic information). Age at ascertainment ranged from 6 months to 22.4 years (mean 6.1 years, median 4.7 years) for females and 1.1 to 14.9 years (mean 5.2 years, median 4.6 years) for males.

Seizures and motor delay

Seizures occurred in all except one female, and in all males, at a mean age of 7.3 weeks (range 0.3 to 34.8) and 6.4 weeks (2.1 to 13.0 weeks) respectively. Seizures occurred by three months of age in 90%. At ascertainment, 52/72 females and 8/9 males had daily seizures, 5/72 females had weekly seizures, 10/72 females and 1/9 males had monthly seizures, while the remaining five females had no seizures in the last year. Nine families reported that their child was no longer treated with anticonvulsant medications.

Severe gross motor delay was reported in all but three females. The ability to sit was acquired by just over half (41/75, 54.7%) of the females (mean age 25.2 months, median 18.2 months, range 6 months to 14 years) and by one male at 8 months. Eight (10.8%) females achieved independent walking at a mean age of 39.6 months (95% CI 28.2-51.1, and median 38 months, range 1.4-5.5 years). A further nine (13.6%) females walked with assistance (mean age 41.2 months, 95% CI 26.4-55.9, range 1.6-5 years). No males were able to walk, with or without assistance.

Hand function and speech

Functional hand use was acquired by just over half (43/72) of females and 1/8 males, of whom 11/37 (29.8%) females and no males lost the ability. Early speech skills (ranging from babble to single words) were acquired by 30/76 (39.5%) females and 2/9 (22.2%) males, of whom 14/29 (48.3%) females and no males subsequently lost speech. Families of 57/72 females and 6/8 males felt that their child could communicate non-verbally (e.g. facial expressions, sign language and/or eye pointing) and families of 56/72

females and 6/8 males felt their child was able to follow simple commands and respond to changes in tone of voice.

RTT-like features and co-morbidities

Hand stereotypies developed in 61/76 (80.3%) females (mean age 13.1 months, median 1 year, range 3 months to 5.5 years). In comparison only 3/9 (33.3%) males developed stereotypies at 3, 12 and 24 months (p=0.004). In the majority (41/56, 73.2%) more than one type of hand stereotypy was present, the most frequently described being mouthing (n=36/56), wringing (n=18/56), clapping (n=18/56), biting (n=12/56) and clasping (n=11/56). Regression in skills occurred in 25/77 (32.5%) females (mean age of 18.5 months) and no males.

Gastrointestinal problems, such as constipation and reflux, were present in 59/75 (78.7%) of females and in 8 males. A spinal curvature was reported in 20.5% (15/73) of females (mean age of onset (where known) 7.6 years, range 2 to 14 years) and one third of the males (age range 4 to 13 years). Autonomic problems, such as cold feet, were present in just under half of females (30/73, 41.1%) and 3/8 of males, whilst sleep problems occurred in 89.9% (62/69) of females and 87.5% (7/8) males. Head growth deceleration occurred in 25/42, however only 3 of these would be classified as having microcephaly.

Dysmorphism

Initial review of photographs (60 females and 7 males) indicated that “dysmorphism” was subtle. Frequently observed facial features included: a prominent and/or broad

forehead; high hairline; relative mid-face hypoplasia; deep-set but "large"-appearing eyes and infra-orbital shadowing (Figure 1). Eyebrows were usually well defined and tended to be straight rather than arched. Synophrys was present in 13.4% overall but was more prevalent in males (42.9%). Lips were full in most, often with eversion of the lower lip. Ears were normally placed and of normal configuration. Young children tended to have a low nasal bridge, mildly anteverted nares, prominent nasal tip and a well-defined (normal) philtrum (Table 1). With age the facial appearance tended to coarsen, but the nasal profile remained notably straight in nearly all individuals.

The fingers in young children were often tapered, some with puffy proximal and narrowed distal phalanges. Some had a puffy dorsum of the hands and/or feet. In older individuals, the fingers tended to be slender, with prominent proximal interphalangeal (IP) joints and narrow distal IP joints. Hallux valgus was present in 25%. Some had relatively long great toes, with slightly broad toenails. Toes 2-5 were usually very regular in shape and position, with mild brachydactyly (Table 1).

The spectrum of features was similar overall in females and males, but 5/7 males had a distinctly anteverted nasal tip, two of whom had a short philtrum and retracted, everted upper lip. Only one female had anteverted nares; she also had a receding forehead and laterally upswept eyebrows; she had a 2.7Mb deletion including the *CDKL5* and *NHS* genes. The mutation type and features present in non-typical individuals is shown in Table 2.

Fulfilment of criteria for ESV RTT

Overall 19/75 (23.7%) females and no males fulfilled all criteria for ESV RTT (Table 3). This was largely due to the absence of regression in all males and 67.5% of females. Hand function was lost by only 16.7% and spoken language by only 18.7% of females. No male lost either hand function or spoken language. Gait abnormalities were the most widely met main criterion. Bruxism, impaired sleep and abnormal muscle tone were the most widely met supportive criteria.

More females aged over 5 years (12/36) met all criteria in comparison to those aged 5 years and under (7/39) (Table 3). Just under half of the females aged over 5 years had developed a spinal curvature, compared to less than 5% of those aged 5 years and under. In males all those aged over 5 years had a spinal curvature compared to none of those less than 5 years. Peripheral vasomotor disturbances were also reported more frequently in the older group in both females and males.

Comparison between females with the CDKL5 disorder and females with RTT

There were a total of 920 females with a clinical diagnosis of RTT and a pathogenic *MECP2* mutation. Data was available from both families and clinicians (n=574) and from clinicians only (in de-identified form, n=346). The mean age of the females with RTT was 10.5 years (range 1.3 to 54.2 years). After adjusting for age, females with the CDKL5 disorder were more likely to have seizures (OR 54.01, 95% CI 7.45-391.33) and sleep disturbances (OR 4.89, 95% CI 2.20-10.89) than females with RTT (Table 4). They were less likely to have breathing disturbances (OR 0.43 95%, CI 0.26-0.71), a spinal curvature (OR 0.34, 95% CI 0.17-0.65) and gastrointestinal problems (OR 0.52,

95% CI 0.27-0.98); to develop hand stereotypies (OR 0.12, 95% CI 0.06-0.25) and to lose hand (OR 0.06, 95% CI 0.03-0.12) and speech skills (OR 0.13, 95% CI 0.07- 0.23).

Time to event analysis (Figures 2-4) showed that females with the CDKL5 disorder were more likely to develop seizures earlier, with three quarters of females having developed epilepsy before 12 months of age compared to 10 years for RTT (HR 24.8, 95% CI 18.6-32.9). Hand stereotypies also appeared earlier in females with the CDKL5 disorder, with 75% having developed them by two years of age compared to 75% by three years for females with RTT (HR 1.1, 95% CI 0.86-1.5). A spinal curvature was less likely in females with the CDKL5 disorder compared to females with RTT (HR 0.51, 95% CI 0.28-0.90).

The most characteristic features of females and males with the CDKL5 disorder are shown in Table 5. These features can be used as a guide to assist decision-making where targeted *CDKL5* mutation analysis is being considered.

DISCUSSION

Individuals with mutations in the *CDKL5* gene have been variably classified as having early infantile epileptic encephalopathy, X-linked dominant infantile spasm syndrome, the ESV RTT or diagnosed with other epileptic disorders such as West syndrome.[1,3] We described the clinical presentation and physical appearance of individuals with the CDKL5 disorder, providing information from a large international dataset. The CDKL5 disorder appears to be characterised by seizure onset in the majority before three months of age, severely impaired gross motor, language and hand function skills, and subtle but

shared physical characteristics such as a prominent/broad forehead, deep-set but large-appearing eyes, full lips and tapered fingers. Our findings suggest that the CDKL5 disorder is an independent entity and should not be considered part of the RTT spectrum, as less than 25% of the cases in our study would meet the clinical criteria for ESV RTT. Therefore we propose that females and males with a mutation in the *CDKL5* gene be given a specific diagnosis of the CDKL5 disorder.

Our study found that early-onset epilepsy was one of the hallmark features of the CDKL5 disorder. In the literature many seizure types and EEG changes have been described. These include infantile spasms, multifocal and generalised seizures with myoclonic, tonic (tonic vibratory) and clonic features. Reported EEG patterns include a normal background, background slowing and a burst-suppression pattern.[10,12] Whilst no specific seizure semiology has been described, two reports have noted a seizure pattern that may be unique to this disorder.[31,34] The first (n=4) reported a seizure pattern of tonic-tonic/vibratory contraction, followed by a clonic phase with spasms and finishing with distal myoclonus.[34] The second (n=4) reported an initial hypermotor phase, then tonic extension, followed by spasms. Severe developmental delay was typical of the CDKL5 disorder. Learning to sit and walk was usually considerably delayed or not achieved, more so in males.[42] Communication was usually restricted to non-verbal strategies. It would be important to further investigate the influences of genotype, seizure severity or therapy utilization on early development in those with the CDKL5 disorder. Consistent with other literature[1-38] our large case series confirms that early-onset of epilepsy and severe developmental delay are key features.

Males with the CDKL5 disorder in this study tended to be more severely affected. None learned to walk, with or without assistance, and few acquired spoken communication and hand function. Seizure onset was slightly earlier than in females, and all males were having either daily or monthly seizures. Hand stereotypies were reported in only a third of males compared to 80% of females. Some of these differences may be due to amelioration of the phenotype in females because of X-inactivation resulting in a mosaic expression of normal and mutant CDKL5 protein. In RTT, males tend to be either extremely affected, often dying at an early age due to severe infantile encephalopathy, or more mildly affected than females.[43] Those who are more mildly affected tend to have hypomorphic *MECP2* mutations that are not found in females with RTT. It would be of interest to further investigate the genetic variability between males and females with the CDKL5 disorder to determine whether a similar phenomenon exists.

We observed a shared physical resemblance between females with the CDKL5 disorder, with the most consistent features being a prominent/broad forehead, deep-set but "large-appearing" eyes, full lips and tapered fingers. Most affected males were more obviously dysmorphic, with distinctly anteverted nares and several males also had a short philtrum and everted upper lip. Previous studies of the CDKL5 disorder have also reported dysmorphic features including: large deep-set eyes, strabismus, high forehead, full lips, wide mouth, widely spaced teeth and a high palate.[1,4,5,7,13,18,20,22,25,26,28-30,35] Dysmorphic features have been described in other conditions presenting with early-onset encephalopathy, such as those with *FOXG1* mutations and in Pitt-Hopkins syndrome (PHS). In those with *FOXG1* mutations, subtle, non-specific dysmorphic features have

been reported, along with severe microcephaly, which is typical of *FOXG1* syndrome but not common in individuals with the CDKL5 disorder.[44] The facial features in PHS include a high nasal bridge and prominent, beaked nose, different from the consistently straight nose and normal or low nasal bridge seen in the CDKL5 disorder. While both can have prominent lips, the lips in PHS have a more marked cupid's-bow contour. Individuals with PHS may have clubbing of the fingertips whereas in the CDKL5 disorder the distal phalanges are relatively narrow and/or short.[45] In classical RTT no typical facial gestalt has been described, although some clinicians have suggested a facial similarity to Angelman syndrome.[46] A recent study using a combination of measurement and subjective impression found the only distinctive facial profile difference in RTT was a relatively broad upper face, especially in girls less than three years old.[47] Whilst subtle, the features that we have described appear to be characteristic of the CDKL5 disorder population, and could therefore be useful in determining whether the patient warranted *CDKL5* specific mutation testing.

The majority of patients in this study with the CDKL5 disorder did not meet the new criteria for the ESV RTT, mainly due to the absence of regression in over two-thirds. Several of the specific supportive criteria, such as diminished response to pain, a spinal curvature and intense eye pointing, were also infrequently reported. Some of these supportive characteristics appear age-dependent and were observed more commonly in those aged over five years. Longitudinal monitoring of these features would contribute to a better understanding of the evolution of this disorder.

Considering only the females with the CDKL5 disorder, the differences in comparison to RTT persisted. Early development was more severely impaired in those with the CDKL5 disorder, with approximately half learning to sit and 10% learning to walk in comparison to approximately 80% learning to sit and just under half learning to walk in a recent study of 293 females with RTT.[48] Those with the CDKL5 disorder were less likely to use words whereas use of some words was acquired by nearly 90% of females with RTT before regression.[48] Females with the CDKL5 disorder were also less likely to develop hand stereotypies, breathing disturbances, gastrointestinal problems and a spinal curvature, but were much more likely to develop seizures and have sleep disturbances. These findings again suggest that the CDKL5 disorder is clinically separate to RTT, indicating the need to develop independent clinical criteria for the identification of the CDKL5 disorder.

The two largest studies conducted to date (n=9 and n=20) found that the classical period of regression was rarely present in females with the CDKL5 disorder and that autonomic, gastrointestinal, breathing and spinal problems were rare and lack of eye contact, hypotonia and epilepsy common.[11,22] Our study also found that regression, autonomic disturbances, breathing disturbances and a spinal curvature occurred less frequently than in RTT, but that the prevalence increased with age. Gastrointestinal problems were quite common, although occurring less frequently than in females with RTT. In contrast to other CDKL5 studies[1-38] we found that sleep problems were reported frequently. Small case series may be less likely to accurately identify some of the patterns detectable by a larger study, which could account for this difference. Clearly large sample sizes, preferably population-based, are needed to provide accurate

representation of the clinical features of such a rare condition. Our study is the first to have had the capacity to compare the characteristics of males and females with the CDKL5 disorder. Further investigation of the natural history is still required, and there are likely other features that have not been captured by existing studies.

This study is the first international case collection investigating the clinical presentation of the CDKL5 disorder and includes more than four times the number of individuals than the largest previously reported case series. It is also the first to examine whether these individuals meet the criteria set forth for the ESV RTT. However, our study is not population-based, and we suggest the development of a population-based register of this disorder, as has occurred in RTT.[49] Because we used the infrastructure of InterRett, our data collection tools were initially designed for RTT and therefore we may not have been able to capture the presence of features that are unique to the CDKL5 disorder. Our study population was also weighted towards younger children, and some clinical features are likely to evolve with age. With regard to the identification of "dysmorphic" features, our study was limited by the lack of a photographic control group, the variable quality of images used, and the subjective nature of the assessments (although the latter has been a traditional approach to dysmorphology). Acknowledging these limitations, and that the features noted were variable, we felt the combined gestalt in a child with early onset encephalopathy, without major malformations, could suggest a *CDKL5* mutation as a likely underlying aetiology.

Many of the early-onset encephalopathies have overlapping phenotypes, including severe developmental compromise with or without prior regression, early-onset

seizures, abnormal movements (stereotypies or dyskinesias) and respiratory irregularities. Some are distinctive either by dysmorphism (PHS), or typical neurodevelopmental profile (classical RTT). Others may be less easily recognised, which is why classifications evolve using terms like “Rett variant” or “PHS-like”. While use of such terms in some conditions (e.g. Noonan or Noonan-like) has been borne out by discovering mutations in genes in shared pathways, this type of classification should be approached with some caution. Although mutations in the *CDKL5* gene have been found in association with a clinical picture similar to RTT in some instances, the majority of cases are different. It may be more accurate and beneficial for families and clinicians if the CDKL5 disorder is considered independent of RTT, rather than another variant. Therefore we suggest that when describing the clinical picture of females and males with the CDKL5 disorder, researchers should not only concentrate on features which should be present in RTT, but rather the features which are present in the CDKL5 disorder. Only then will it be possible to develop accurate clinical diagnostic and management guidelines.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Titles and legends to figures

Figure 1. Examples of identified facial, hand and feet features in males and females with the CDKL5 disorder (n=67).

A. Female aged one year 10 months: high/prominent forehead, deep-set eyes, prominent lips, well-defined philtrum, proximal puffy phalanges and tapered fingers.

B. Female aged two years: high/prominent forehead, deep-set eyes, prominent lips, well-defined philtrum, tapered fingers, slightly broad hallux, hallux valgus and "regular" toes.

C. Female aged four years: deep-set eyes, a well-defined philtrum, laterally orientated nasal apertures, prominent lips, slightly tapered fingers, small distal phalanges and a broad hallux.

D. Female aged three years two months: prominent forehead, mild synophrys, deep-set eyes, prominent lips, puffy proximal phalanges, tapered fingers, slightly broad hallux, and "regular" other toes.

E. Female aged seven years 11 months: high/prominent forehead, deep-set eyes, everted lower lip, puffy proximal phalanges, prominent proximal IP joints and tapered fingers.

F. Female aged 14 years: broad forehead, deep-set eyes, epicanthic folds, well-defined philtrum, prominent lips, tapered fingers and prominent proximal IP joints.

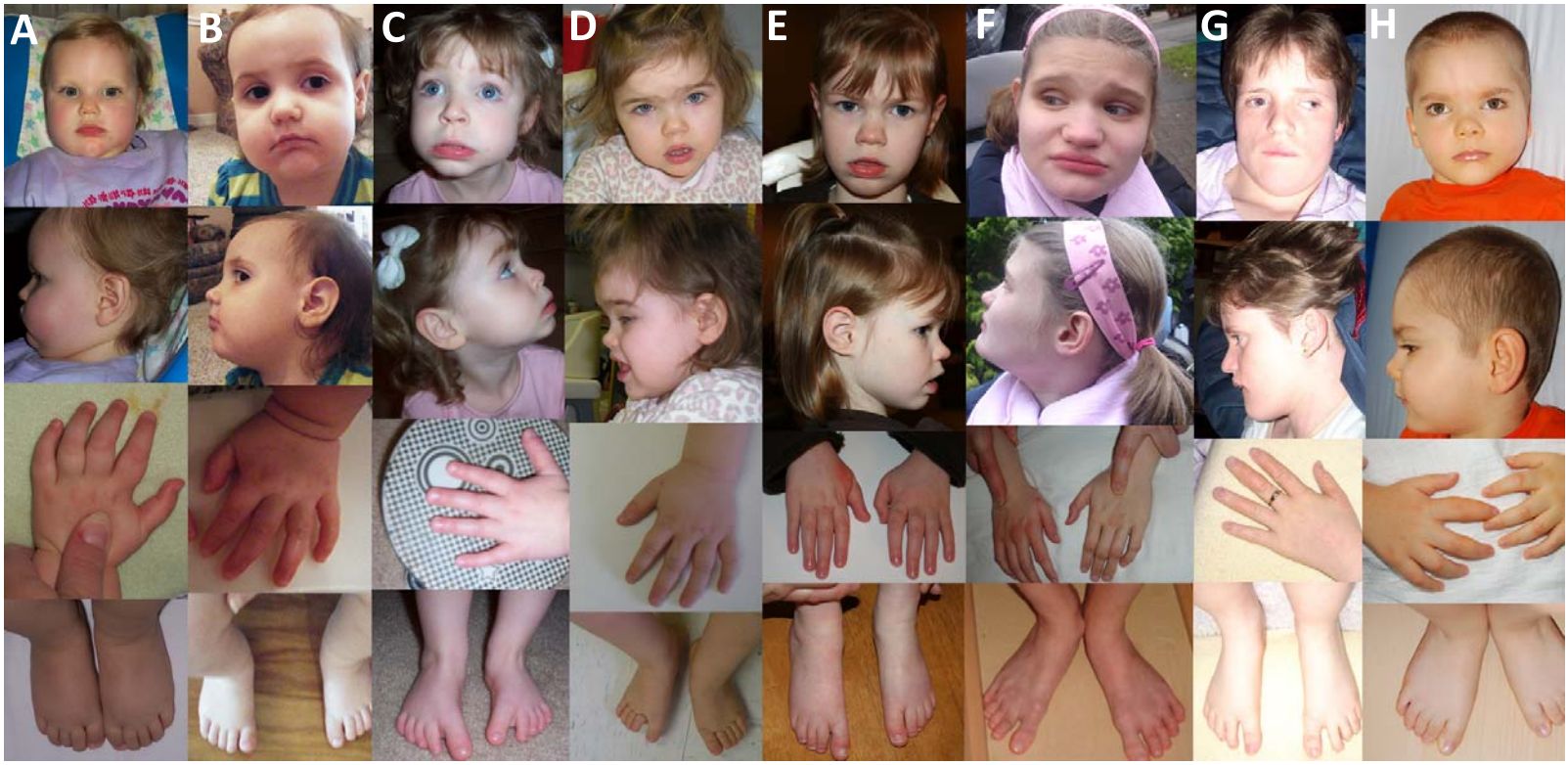
G. Female aged 24 years and two months: high forehead, deep-set eyes, well defined philtrum and prominent lips.

H. Male aged four years four months: high/prominent forehead, well-defined philtrum, prominent lips, tapered fingers and "regular" toes.

Figure 2. Kaplan-Meier survival curve for the risk of developing seizures by single year of age.

Figure 3. Kaplan-Meir survival curve of the risk of developing hand stereotypies by single year of age.

Figure 4. Kaplan-Meier survival curve for the risk of developing a spinal curvature by single year of age.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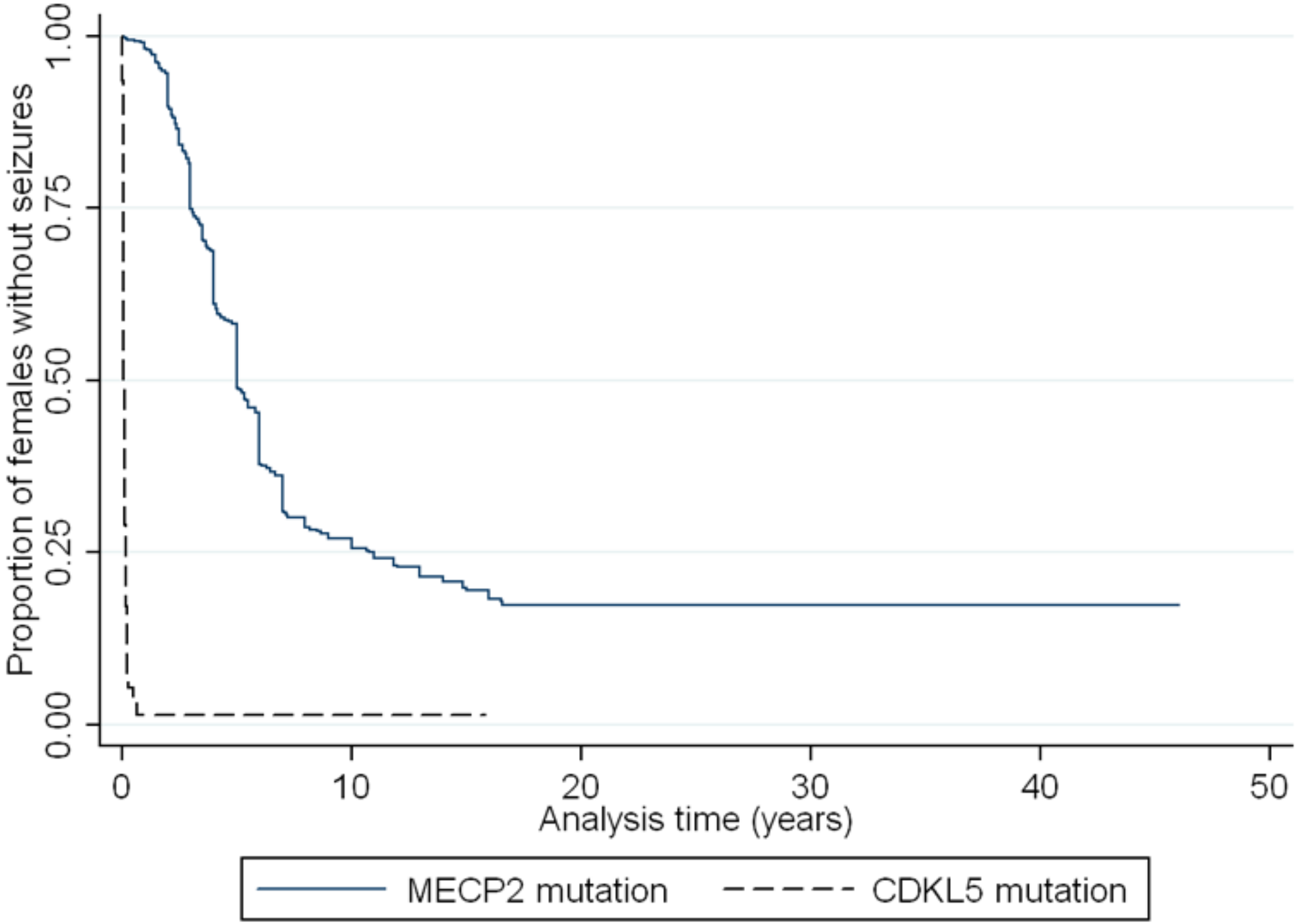
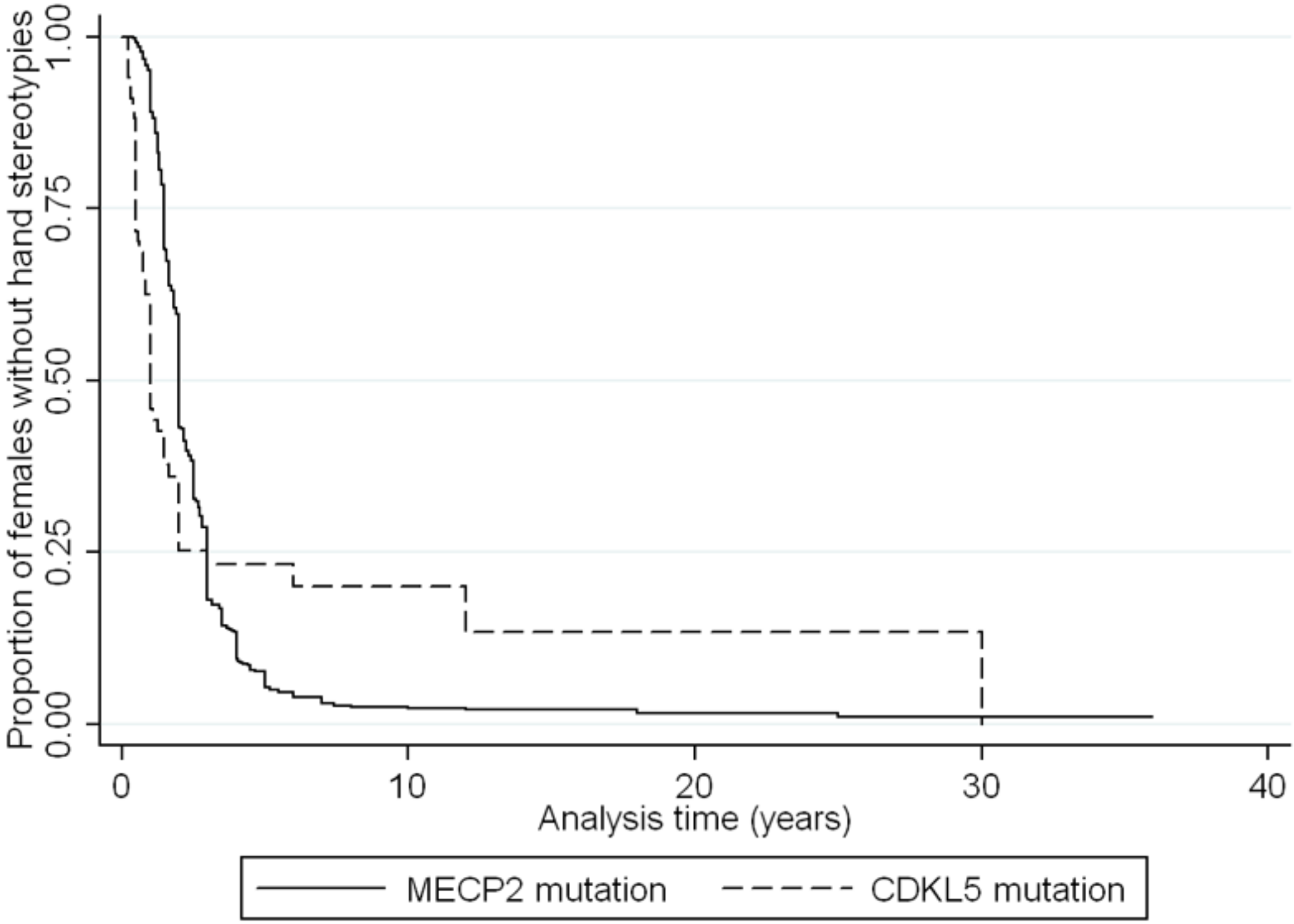

Proportion of females without seizures
0.00
0.25
0.50
0.75
1.00
0
10
20
30
40
50
Analysis time (years)
MECP2 mutation
CDKL5 mutation


Proportion of females without hand stereotypies
0.00
0.25
0.50
0.75
1.00
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40
Analysis time (years)
MECP2 mutation
CDKL5 mutation

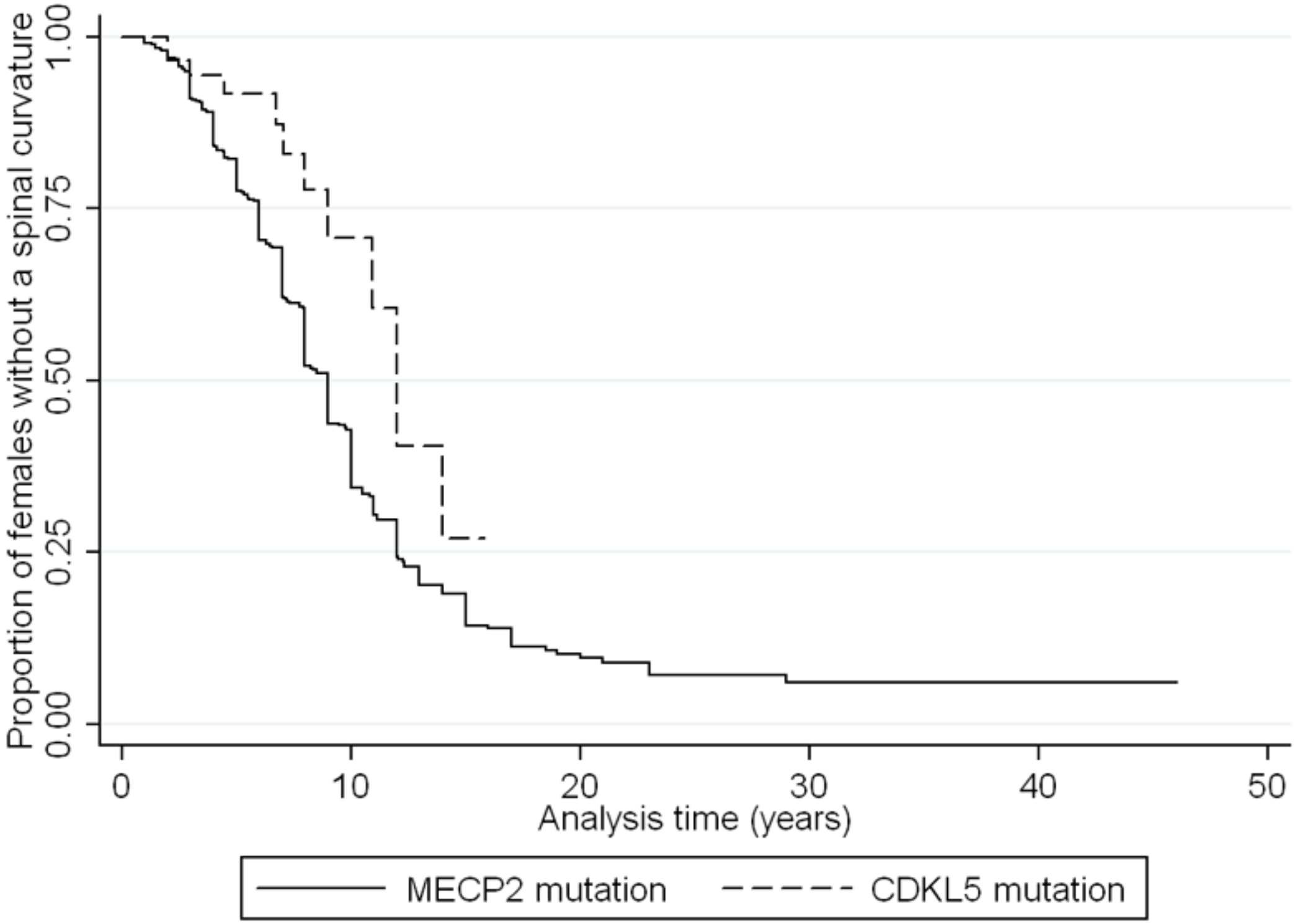
Proportion of females without a spinal curvature
0.00
0.25
0.50
0.75
1.00
0
10
20
30
40
50
Analysis time (years)
MECP2 mutation
CDKL5 mutation

Table 1. Distribution of observed dysmorphic features identified in 60 females and 7 males with the CDKL5 disorder.

Feature	**Number of cases (%)**
Broad/prominent forehead	46/62 (74.2)
High forehead (not broad)	5/62 (8.1)
Receding or narrow forehead	3/62 (4.8)
Deep set eyes	49/67 (73.1)
Synophrys (5 females, 3 males)	8/67 (13.4)
Well-defined philtrum	40/66 (60.6)
Full lips/everted lower	45/67 (67.2)
Fingers proximally puffy/prominent proximal IP joint	26/62 (41.9)
Hallux valgus	16/64 (25.0)