This is the accepted version of the following article: Clifford, H.D. and Hayden, C.M. and Khoo, S.-K. and Daniche, D. and Mandomando, I.M. and Zhang, G. and Richmond, P. and Le Souef, P.N. 2013. Impact of CD14 Promoter Variants on Measles Vaccine Responses and Vaccine Failure in Children from Australia and Mozambique. Tissue Antigens. 82 (6): pp. 420-422., which has been published in final form at http://doi.org/10.1111/tan.12226

LETTER TO THE EDITOR

Impact of *CD14* promoter variants on measles vaccine responses and vaccine failure in children from Australia and Mozambique

Holly D. Clifford,[a,b] Catherine M. Hayden,[a] Siew-Kim Khoo,,[a,b] Denise Naniche,[c,d] Inacio M. Mandomando,[c,e] Guicheng Zhang,[a,b] Peter Richmond,[a,b] Peter N. Le Souëf,[a]

[a] School of Paediatrics and Child Health, University of Western Australia, Perth, Western Australia

[b] Telethon Institute for Child Health Research and the Centre for Child Health Research, Perth, Western Australia

[c] Centro de Investigação em Saúde da Manhiça, Manhiça, Mozambique

[d] Barcelona Centre for International Health Research (CRESIB), Universtitat de Barcelona, Barcelona, Spain

[e] Instituto Nacional de Saúde (INS), Ministério da Saúde, Maputo, Mozambique

Corresponding author: Holly D. Clifford

School of Paediatrics and Child Health, University of Western Australia,

100 Roberts Road, Subiaco, WA, 6008

Tel: +61 8 9489 7787 Fax: +61 8 9489 7700 Email: holly.clifford@gmail.com

Short title: *CD14* polymorphisms and measles vaccine responses

Keywords: Africa; CD14; Measles; Polymorphism; Vaccine; Vaccine failure

Conflict of interest

P. Richmond declares that he has received part funding for other studies from GlaxoSmithKline Australia and Merck. All other authors declare that they have no potential conflicts of interest.

Considering the high burden of measles disease, including the increase in measles incidence and outbreaks over the last few years (1), the global annual mortality rates of ~158,000 (1), and the measles vaccine failure rates of > 30% in underdeveloped countries (2), it is important to identify the factors that cause poor responses to measles vaccine. Host genetics are likely to play a crucial role (3), particularly variants that alter key innate immune response genes.

The immune development of infants may be affected by early life exposure to bacterial antigens, as Th1 maturation is enhanced by microbial stimulation (4). This priming of the immune system may be affected by the CD14 receptor, a critical component in the response to bacterial lipopolysaccharide (LPS), via the toll-like receptor-4 (TLR4) pathway (5). There are several common variations in the *CD14* gene, and these *CD14* variants can functionally affect soluble (s)CD14 levels, which in turn can alter the Th1/Th2 cytokine balance (6). Whether these *CD14* variants impact early innate immune responses and the development of primary vaccine responses, and contribute to vaccine failure, is not yet known.

We investigated common *CD14* single nucleotide polymorphisms (SNPs), their functional effects on sCD14 levels, and their associations with measles IgG responses and measles vaccine failure. We assessed these outcomes in two differing cohorts of measles-vaccinated children from Perth, Western Australia and Manhiça, Mozambique, Africa. To our knowledge, this is the first study to investigate *CD14* variants with regards to measles vaccine responses. This study is also unique with regards to our resident African population, some of whom displayed true measles vaccine failure.

Healthy 12-14 month old Caucasian children were recruited from *Perth, Western Australia* (n=137), and each received a single dose of measles-mumps-rubella (MMR) vaccine (Priorix™; GlaxoSmithKline, Belgium) (7,8). Children aged 6 months-14 years were also recruited from *Manhiça, Mozambique, Africa* (9,10), some of whom presented with the clinical symptoms of measles despite vaccination (vaccine failure cases n=66), and were compared with age-matched controls (n=172). Children were designated as

measles vaccine failure cases if they had been vaccinated (recorded by vaccination card) and they presented with the clinical symptoms of measles (presence of fever, rash and one or more of the following signs: cough, coryza or conjunctivitis) (9,10).

DNA was extracted from blood using Qiagen QIAamp DNA Mini kits. Three polymorphisms in *CD14* (-159C/T, -550G/A, -1619C/T) were genotyped using an iPLEX assay on a MALDI-TOF MassARRAY platform (Sequenom Inc., San Diego, CA, USA). Haplotypes were inferred using the Bayesian statistical based program PHASE (11). Soluble (s)CD14 levels were quantified in plasma in pre- and (6-8 weeks) post-vaccination samples of the Australian cohort using Quantikine Human sCD14 Immunoassay (R&D Systems). Post-vaccination measles-specific IgG and IgM titres were measured by Enzygnost Immunoassay kits (Dade Behring, Marburg, Germany) and in-house ELISA, respectively (7-10). All statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Chi-squared tests were used to ensure that genotype frequencies did not deviate from the Hardy-Weinberg equilibrium, to compare allele frequencies between the two populations, and to determine the proportion of genotypes in the Mozambique vaccine failure cases compared with controls. Differences between antibody titres with genotype were analysed by one-way ANOVA or nonparametric Kruskal-Wallis tests, with Pearson's correlations and linear regression used when correcting for confounders (age, gender, time since vaccine, age when vaccinated).

All *CD14* polymorphisms had significantly different allele frequencies between the two populations ($P<0.0001$; Table 1). Post-vaccination sCD14 levels were significantly lower (mean 1.63µg/ml, 95% CI: 1.54 – 1.73) compared with pre-vaccination sCD14 levels (2.12µg/ml, 2.02 – 2.22; $P<0.001$) (Figure 1A). *CD14* -159C/T and -550G/A were not associated with either pre- or post-vaccination sCD14 (Figure 1B). However, *CD14* -1619TT had significantly lower post-vaccination sCD14 (1.98µg/ml, 1.82 – 2.13) compared with CC (2.39µg/ml, 2.08 – 2.69) and CT (2.18ug/ml, 2.02 – 2.33; $P=0.029$) (Figure 1B). *CD14*

polymorphisms were not significantly associated with measles-specific IgG production in either cohort (Table 1). Haplotype analysis also showed no significant associations (data not shown).

A subset of Mozambique children were designated as "clinically-defined" measles vaccine failure cases (n=66 total, n=18 with samples) as they presented with measles symptoms and had documented vaccination history. Measles IgG was not different between these cases compared with age-matched controls (n=71) (P=0.111). When taking into account laboratory measles confirmation via IgM, subjects were also grouped into "laboratory-defined" cases (n=9) and controls (n=165) and measles IgG was significantly lower in these cases compared with controls (P=0.004) (10). *CD14* polymorphisms did not show different distributions between clinically- or laboratory-defined vaccine failure cases and controls (Table 1).

In this study, we report that although *CD14* polymorphisms can functionally affect sCD14 levels and therefore can presumably influence early life immune responses and immune maturation, there was no evidence to suggest that these variants have a significant impact on measles vaccine responses or vaccine failure in children from Australia or Mozambique.

CD14 has previously been shown to be involved in the antiviral response to respiratory syncytial virus, a single stranded RNA paramyxovirus closely related to measles (12), and *CD14* polymorphisms have also previously been linked with pneumococcal vaccine responses (13), however this is the first time this gene has been investigated with regards to measles vaccine. Our results with sCD14 levels both contrast and agree with previous studies. *CD14* -159C/T has been linked with levels of sCD14, and sCD14 in turn has been associated with IFN-γ and IL-4 cytokine responses (6), while *CD14* -550T has been associated with higher sCD14 in breast milk and lower sCD14 in plasma (11). We however found no associations between these variants and sCD14 levels. Our results do agree with Guerra *et al.* (11) in that we also show a significant link between sCD14 and -1619C/T. Replication of CD14 genetic studies has proved

controversial and likely depends on a number of factors including environmental exposures and ethnicity (14). *CD14* -159 TT homozygotes have been shown to have higher anti-pneumococcal vaccine antibody levels in children (13). We however did not find any associations with *CD14* genetic variants and measles vaccine IgG responses. Therefore, we suggest that alternative immunogenetic factors are likely to have stronger effects on measles-specific antibody responses, and thus the occurrence of measles vaccine failure.

Genetic association studies with measles vaccine responses have predominantly been in Caucasian populations, a strength of our study is that we are the first to evaluate genetic determinants of measles responses in children from a developing country where measles is endemic. We were therefore able to include actual cases of vaccine failure (compared with just low seronegative antibody levels like in our Australian cohort). However, small numbers of cases necessitate that our findings be replicated in future studies. Our study also emphasizes the difficulty in diagnosing measles cases, with clinical (symptoms only) and laboratory (symptoms and IgM confirmation) cases definitions yielding different results.

Determining the factors that drive inadequate immune responses and the development of vaccine failure are particularly important in developing countries, where measles is endemic and children suffer more severe disease and death. Knowledge in this area may inform as to which children are likely to be at high risk, and this may lead to the initiation of more effective vaccine strategies for these children. Elucidating all of the contributing immunogenetic factors is also important for the future of vaccine research as we aim towards a more targeted, personalised approach to vaccinology (15), as well as moving toward the ultimate goal of worldwide measles elimination.

Acknowledgements

We thank the children and their families who were involved in these studies. We thank the staff of the Vaccine Trials Group for the clinical conduct of the Perth study and Barbara Holt and Jenny Tizard for sample processing. We also acknowledge Marcela Pasetti from Applied Immunology Section, Center for Vaccine Development (CVD), University of Maryland and Professor Pedro Alonso for their involvement in the Mozambique study. This study was supported by funding from the National Health and Medical Research Council (NHMRC) of Australia and the Bill and Melinda Gates Foundation.

References

(1) Centers for Disease Control and Prevention (CDC). Global control and regional elimination of measles, 2000 - 2011 Wkly Epidemiol Rec 2013: 62 (02): 27-31.

(2) Akande TM. A review of measles vaccine failure in developing countries. Nigerian Medical Practitioner 2007: 52(5-6): 112-6.

(3) Tan PL, Jacobson RM, Poland GA, Jacobsen SJ, Pankratz VS. Twin studies of immunogenicity--determining the genetic contribution to vaccine failure. Vaccine 2001: 19: 2434-9.

(4) Holt P, Sly P, Björkstén B. Atopic versus infectious disease in childhood: a question of balance? Pediatr Allergy Immunol 1997: 8: 1–5.

(5) Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990: 249(4975): 1431-3.

(6) Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. Am J Respir Cell Mol Biol 1999: 20(5): 976-83.

(7) Yerkovich ST, Rowe J, Richmond P, et al. Assessment of the potency and potential immunomodulatory effects of the measles mumps rubella and varicella vaccine in infants. Vaccine 2007: 25: 1764-70.

(8) Clifford HD, Richmond P, Khoo S-K, et al. SLAM and DC-SIGN measles receptor polymorphisms and their impact on antibody and cellular responses to measles vaccine. Vaccine 2011: 29(33): 5407-5413.

(9) Mandomando I, Naniche D, Pasetti MF, et al. Assessment of the epidemiology and burden of measles in Southern Mozambique. Am J Trop Med Hyg 2011: 85(1): 146-51.

(10) Clifford HD, Hayden CM, Khoo S-K, et al. Polymorphisms in key innate immune genes and their effects on measles vaccine responses and vaccine failure in children from Mozambique. Vaccine 2012 2012: 30(43): 6180-5.

(11) Guerra S, Lohman IC, LeVan TD, Wright AL, Martinez FD, Halonen M. The differential effect of genetic variation on soluble CD14 levels in human plasma and milk. Am J Reprod Immunol 2004: 52(3): 204-11.

(12) Kurt-Jones EA, Popova L, Kwinn L, et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nat Immunol 2000: 1(5): 398-401.

(13) Wiertsema SP, Khoo SK, Baynam G, et al. Association of CD14 promoter polymorphism with otitis media and pneumococcal vaccine responses. Clin Vaccine Immunol 2006: 13(8): 892-7.

(14) Zhang G, Goldblatt J, LeSouëf PN. Does the relationship between IgE and the CD14 gene depend on ethnicity? Allergy 2008: 63(11): 1411-7.

(15) Poland GA, Ovsyannikova IG, Jacobson RM. Personalized vaccines: the emerging field of vaccinomics. Expert Opin Biol Ther 2008: 8(11): 1659-67.