ABSTRACT

Plasmodium falciparum severe malaria anaemia [SMA, haemoglobin (Hb) <5.0 g/dL or Hb<6.0 g/dL with any parasite density] remains the most life-threatening malaria. With its immuno-genetic basis partially understood, efficacious therapeutics’ development remains unachieved. Genetic susceptibility factors offer tools for unravelling molecular mechanisms involved. Cellular receptors pathways are crucial in immunological reactions leading to parasite clearance. However, contributions of genetic variations within immune cell receptors to SMA pathogenesis is partially understood. This study determined the role of polymorphisms in immune cell receptors i.e. Cluster of differentiation 40 (CD40), Interleukin-23 receptor (IL-23R) and Fc gamma receptors and their pathway genes; Nuclear factor of kappa light enhancer in B-cells (NFκB1) and its inhibitor IkappaB alpha (NFκBIA) on susceptibility to paediatric SMA in Siaya County, Western Kenya. Moreover, the in-vitro effects of P. falciparum haemozoin (PfHz) on expression of inflammatory mediators via CD40 pathway was also determined. Laboratory measures were determined in children (N=1,128, aged 6-36 months) at Siaya County Referral Hospital. Genotyping and gene expression was done using TaqMan® assays while inflammatory mediators’ levels were determined by human cytokine 25-plex Ab Bead Kit. Odds ratios were computed by regression analysis controlling for confounders (age, HIV-1, bacteraemia, α-thalassemia and sickle-cell trait). Differences in the levels of inflammatory mediators between carriage and non-carriage of haplotypes were determined by Man-Whitney U-test/student’s t-test. CD40 -508G/173C/-1C (GCC) haplotype carriage had 69% protection against SMA [OR=0.31, 95% CI=0.14-0.67, P=0.003] while the carriage of GCT haplotype was associated with susceptibility to SMA [OR=5.24, 95% CI=3.31-8.82, P<0.001]. Carriage of GCC haplotype had increased IL-1β, IL-2 and MIP-1α (P=0.002, P=0.029 and P=0.048, respectively) while the carriage of GCT haplotypes had lower levels of IL-1β and IL-17, (P=0.017 and P=0.003, respectively). Analyses of NFκB1 revealed that carriage of AT (-8079A/-3297T) haplotype was associated with risk of SMA (OR=1.58, 95% CI=1.13-2.33, P=0.008) while GC (-8079G/-3297C) was associated with 40% reduced risk of SMA (OR=0.60, 95% CI=0.42-0.86, P=0.005). The NFκBIA (-826A/-310A) AA haplotype carriage was associated with SMA risk (OR=1.60, 95% CI=1.02-2.47, P=0.042) while AG (-826A/-310G) conferred 43% protection from SMA (OR=0.57, 95% CI: 0.33-0.98, P=0.037). The carriage of NFκBIA (AG) (-826A/-310G) haplotype had increased levels of IL-10 and IP-10 compared to non-AG (P=0.050 and P=0.016, respectively). Combined genotypes showed that NFκB1-8079AA/NFκBIA-826GA was associated with susceptibility to SMA (OR=2.31, 95% CI=1.30-4.08, P=0.007). NFκB1I-3279CC/NFκBIA-826GG had 31% protection against SMA (OR=0.69, 95% CI=0.48-0.96, P=0.033) while the NFκB1I-3279TT/NFκBIA-826GA was associated with increased risk of SMA (OR=2.77, 95% CI=1.10-6.97, P=0.031). Carriage of the NFκB1I-3279CC/NFκBIA-310GG was associated with protection against SMA (OR=0.64, 95% CI=0.44-0.92, P=0.016) whereas NFκB1I-3279TT/NFκBIA-310G combination were two-fold susceptible to SMA (OR=2.10, 95% CI=1.32-4.10, P=0.002). Further IL-17 levels were positively correlated with haemoglobin levels (r=0.151, P=0.027). Expression levels of IL-1β, TNF-α and IL-6 where increased by PfHz (P<0.05). Both IL-23R rs1884444T/rs7530511T (TT) and FcyRIIA-131Arg/FcyRIIAA-176F/FcyRIIB-NA2 haplotypes were associated with increased susceptibility to SMA (OR=1.12, 95% CI=1.07-4.19, P=0.030) and (OR=1.70; 95% CI=1.02-2.93; P=0.036, respectively). Results demonstrate that polymorphisms in immune cell receptors and their associated pathway genes condition SMA susceptibility and that PfHz is important modulator of expression of pro-inflammation in-vitro. Polymorphisms associated with malaria risk identified here are important markers of genetic risk factors to disease severity. Future genetic studies should investigate a panel of cell receptor help explicitly unravel the molecular mechanisms involved in cell signalling leading to disease phenotypes and therapeutics with the ability to counter the deregulatory effects of PfHz during infection.