Association between Interferon-gamma Promoter -183 (G/T), -1616 (A/G) and Intronic +2200 (A/G) Variants and Malarial Disease Outcomes in Children Exposed to *Plasmodium falciparum*

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ABSTRACT

Infection with *Plasmodium falciparum*, the most common human plasmodial parasite causes significant global morbidity and mortality. Interferon-gamma (IFN-γ) is a type-1 cytokine with T helper (Th) 1-biased immune responses for intracellular pathogen control. Elevated IFN-γ levels contribute to protective immunity against clinical and cerebral malaria. High IFN-γ responses are associated with reduced asexual parasite multiplication rates following a primary infection with *P. falciparum*. Previous studies have associated IFN-γ single nucleotide polymorphisms (SNPs) with susceptibility to asthma, tuberculosis and functional changes in circulating IFN-γ levels. However, the role of IFN-γ polymorphisms in conditioning malarial disease outcomes in children residing in *P. falciparum* holoendemic transmission areas remains unexplored. The current study, using a hospital-based cross-sectional design, investigated the functional associations between IFN-γ genotypic and haplotypic promoter [-183G/T and -1616A/G] and intronic [+2200A/G] variation in conditioning malarial disease outcomes [(severe malarial anaemia (SMA; Haemoglobin (Hb)<6.0 g/dL), malarial anaemia (MA; Hb<8.0 g/dL and high-density parasitaemia (HDP; ≥10,000 parasites/μL)] in infants and young children (age 3-36 months, n=574) enrolled at Siaya District Hospital, western Kenya. Blood samples (3mL) for malaria diagnosis and complete haematological measurements were obtained upon consent from parent/guardian. In addition, plasma was separated and frozen at -80°C until determination of circulating IFN-γ levels. DNA was extracted from blood spotted on filter paper using Chelex method. Genotyping of the IFN-γ SNPs was performed using a Taqman® 5′ allelic discrimination Assay-By-Design method according to manufacturer's instructions and by PCR followed by restriction fragment length polymorphism (RFLP), IFN-γ levels were measured as part of human

25-cytokine plex assay. Multivariate logistic regression models controlling for the confounders revealed that GT heterozygosity at the IFN-γ -183G/T variants showed a trend of reduced risk of developing SMA (OR, 0.603; 95% CI, 0.286-1.271; *P*=0.183) relative to the wild type (GG homozygotes). Additionally, the IFN-γ -1616A/G variants demonstrated that individuals with GG had a two-fold increased risk of developing MA (OR, 2.306; 95% CI, 1.141-4.660; *P*=0.020) relative to the homozygous A (wild type). The IFN-γ +2200A/G variants were not associated with SMA, though heterozygous individuals (AG) demonstrated a tendency towards reduced risk of developing MA (OR, 0.597; 95% CI, 0.331-1.076; *P*=0.086). In addition, the GAA haplotype tended to reduce risk of developing MA (OR, 0.635; 95% CI, 0.391-1.030; *P*=0.066), while the GAG haplotype tended towards increased risk of developing MA (OR, 1.794; 95% CI, 0.957-3.364; *P*=0.068). However, carriage of these genotypes and haplotypes did not influence the circulating levels of IFN-γ. The results demonstrate that genetic variation in the three IFN-γ genotypes investigated in this study may be important in regulating MA outcomes in paediatric *P. falciparum* infection. These findings provide an enhanced understanding of how variation in IFN-γ conditions malaria pathogenesis and the important role that IFN-γ plays in children with falciparum malaria. From these results it is recommended that future work should focus on comprehensive longitudinal studies to delineate the role of IFN-γ variants in modulating malarial outcomes in this population.