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Effect of IgG_3 Hinge Region Length Polymorphisms on Iron Fortification among Rural Pre-School Ghanaian Children and Its Associated Risk to Malaria and Anaemia

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ABSTRACT

Sub-Saharan African pre-school children have an interdependence among immune response, infection resistance and adaptive effect of the dietary intake. However, the long hinge region of immunoglobulin G3 makes binding easy to malaria antigens and receptors, eliciting a more protective response among subclasses of immunoglobulin G. However, it is unknown if iron-containing multimicronutrients are associated with risk of anaemia and malaria in pre-school children with hinge region G3 immunoglobulin polymorphism living in high malaria transmission areas. We aimed to determine the impact of iron fortification in pre-school children with immunoglobulin G3 hinge region length polymorphisms and their associated risk of malaria and anaemia in rural Ghana. This retrospective controlled, double blind, randomized population study was conducted over six months in rural Ghana among 6 to 35-month-old infants and young children. Participants were randomly allocated either iron-free multimicronutrients powder (placebo group) or iron (intervention group; 12.5 mg iron daily) into clusters where mixed semi-liquid homemade meals were delivered daily for 5 months. Anti-malaria mosquito bed nets and anti-malaria treatment were readily available during the study. Standardized therapeutic and epidemiological questionnaires were administered. Participants' blood samples were assessed for immunoglobulin G3 hinge region length polymorphism, full blood count, anthropometry, and malaria microscopy. Baseline anthropometry, anaemia, iron status, demographic characteristics and dietary consumption were similar ($p > 0.05$). 27.85 % were venerable to clinical malaria at baseline. Homozygote medium polymorphism had the maximum frequency of 48 %, followed by homozygote long polymorphism (43 %), heterozygote long-medium polymorphisms (7.0 %) and relatively few were homozygote short 2.0 % with similarities between the study groups ($p = 0.14$; Hardy-Weinberg equilibrium estimate, $\chi 2$ analysis). However, iron fortification did not influence the risk of aneamia and malaria among participants with immunoglobulin G3 hinge region length polymorphisms but was not detrimental to participants.

Keywords
Malaria, Anaemia, Immunoglobulin G_3 hinge region length polymorphism, Malaria, Micronutrient powder.

Introduction
Malaria and anaemia co-exist as major public health problems among pre-school children in Sub-Saharan Africa [1]. Malaria accounts for about half a million deaths, with morbidity and anaemia cases estimated at 220 million in 2016 [2]. However, malarial immunity depends on the presence of specific but appropriate IgG_3 subclass antibodies to recognize merozoites surface antigens and mitigate the severity of *Plasmodium falciparum* infection among children under five years in order to reduce the burden of anaemia [3].

Previous studies have strongly associated IgG_3 subclass that can pass in utero via the placenta to the fetus with immunity against malaria during infancy [1,4]. Out of the four subclasses of the IgG isotype, the IgG_3 molecules, though short-lived, are more protective against various infections including malaria due to the presence of their long flexible hinge region, which facilitate easier interaction with antigens and fragmented crystallizable receptors (FcR) [5,6]. This allows the IgG_3 hinge region length polymorphism (IgG_3HRLP) to exhibit several immune potentials that are more associated with immunity against clinical malaria and indirectly mitigate the burden of anaemia among pre-school children [7,8]. Other immunogeno-epidemiological studies have also suggested that IgG_3HRLP are associated with malarial immunological processes, like cell adhesion and inflammation that adequately boosts opsonization and complement fixations, which enhances splenic clearance of malaria parasites [9]. Previous iron supplementation studies have shown that infants and young children had their anaemic status improved when provided prophylactic micronutrient powders plus iron fortificant [10-15]. However, research findings associating anaemia and malaria to IgG_3HRLP among pre-school children on micronutrient powder with iron in high malaria transmission setting is not known. Therefore, the aim of this study was to determine the impact of iron fortification on IgG_3 hinge region length polymorphism among pre-school children and assess their associated risk to malaria and anaemia in malaria endemic areas.

Subjects and Methods
Study Site, Design and Population
This study was a community-based double-blinded cluster-randomized controlled trial among children aged 6 to 35 months over a period of 6 months conducted from selected communities at Wenchi Municipal and Tain District of Ghana. Details of the study area and design have been previously reported [16]. About 2200 infants and young children were screened and enrolled into the study once permission was obtained from the opinion leaders, as well as the caregivers from the middle of March to April. Sprinkles (Ped-Med Minimal and Sprinkles Global Health Initiative Inc. India), a powdered multi-mineral and vitamin fortifying substance, was introduced whether participants were consuming weaning foods with or without breastmilk, free of serious disease, afebrile, and residing for the duration of the trial in the research region.

Data and Specimen Collection
The wellbeing of the participant (including axillary temperature), a capillary blood sampling of 500 µL obtained from the finger or heel into a 0.5 mL ethylene diamine tetra-acetic acid (EDTA) tube was measured at baseline (BL) and endline (EL) of MNP intervention. The remaining sample was transported to the KHRC laboratory for haematological, malaria microscopy and polymer reaction chain (PCR) assays.

Processing and Analysis of Specimen
Both thick and thin smears were prepared on the same slide at the laboratory for malaria parasitaemia and speciation. Thin films were fixed with methanol and both smears were stained with Giemsa. Two independent microscopists read each slide while a third microscopy was conducted in case of over 50% discrepancy [16]. Full blood counts (FBC) were determined using a haematology autoanalyzer (Horiba ABX Micros 60-OT-CT-OS-CS, France). Human genomic DNA was purified from blood samples in EDTA tubes stored at 2 – 8°C for six years using the QIAamp® DNA Mini Kit (QIAGEN Sample and Assay Technologies, USA) protocol based on published methods [17,18].

The IgG_3 hinge region length polymorphism was amplified using the primers designed by Adu and Mensah-Opoku [6,7]. The sense (5'–AAAACCCCACTTTGGTGACAC) and the antisense (5'-GGGTCCGGGAAATCATAAGG) primers (DNA Technology, A/S, Denmark) were designed to anneal to specific sequences in exon 2 and exon 5 respectively to amplify the fragment encoding the hinge region of human IgG_3 from genomic DNA. The PCR reaction mixture was made of 10 - 30 ng genomic DNA, 10 mM of primer (sense and antisense), 1.25 mM of each of the dNTPs, 1 unit of HotStarTaq® DNA polymerase and the corresponding 10X HotStar reaction buffer in a total volume of 25 µL. The PCR cycling condition was an initial denaturation at 95 ^{0}C for 15 minutes, followed by 38 cycles consisting of a denaturation step at 95 ^{0}C for 30 seconds, an annealing step at 61 ^{0}C for 30 seconds, an elongation step at 72 ^{0}C for 30 seconds, and then a single final elongation step at 72 ^{0}C for 7 minutes [7].

After PCR, 5 µL of the PCR products were separated on 2% agarose gel (SeaKem® GTG® Agarose, Lonza, Rockland, ME, USA) in 0.5 X Tris-EDTA running buffer (Biopioneer Co, USA) by electrophoresis at 90 volts (Apelex Power station, France) for 60 minutes using 1µL of blue DNA loading dye (Promega Co, USA) and stained with 0.5 µg / mL ethidium bromide (Life Technologies Co, USA). A hundred base pair nucleotide sequence molecular size marker (Ladder IV) (Sigma Mo, USA) was ran alongside the PCR products on the gel. The gel was visualized and pictures taken using UV-illumination (AlphadigiDocTM, Alpha Innotech Corporation, EEC) and analyzed capering the text sample to the ladder.

Statistical analysis
Clinical and epidemiological data were entered into a data processing application for Visual Fox Pro version 9.0 (Microsoft), imported for review into STATA version 14.0 (Statcorp, Texas) and

SigmaPlot version 11.0. Using the Chi-square test, the distribution and contrast of proportions of the IgG_3HRLP was analysed. Statistically meaningful experiments with p-values of 0.05 were considered. To assess if IgG_3HRLP was correlated with the risk of malaria and anaemia in children receiving MNP with or without iron, logistic regression analysis was used. By examining the role of IgG_3HRLP in the pathogenesis of malarial anaemia, modified odds ratios for significant variables were reported for gender and age control. For each study participant, an overall wealth index was calculated using main component analysis [19,20] by including the amount and form of assets present in the household of the study participant (e.g. televisions, vehicles, utilities, toilet facilities, house ownership). Participants in the sample were classified into high or low socioeconomic status by their income indices. The malaria incident happened when a child's axillary temperature was over 37.5 ^{0}C or a febrile background and parasitaemia within the past 48 hours.

Results

Background characteristics of study participants

2220 children 6 – 35 months of age from 22 communities were screened and 871 children who met the inclusion criteria, representing 774 clusters were randomized into the iron and non-iron groups. Only three percent were lost to follow-up and the main reason for lost to follow-up in both groups were moving out of the study area. *Clinical and demographic characteristics* between the groups were similar at BL ($p > 0.05$), however, household heads' *socio-economic status* from the Fe group was significantly lower compared to those in the non-Fe group ($p = 0.02$) (Table 1). There were also similarities ($p = 0.42$) at BL prevalence of moderate anaemia (haemoglobin level of 7 - 10 g / dL) between participants from the Fe groups; 42.9 % (95 % CI, 38.1 % - 47.4 %; 185 / 431) and those from the non-Fe group; 40.3 % (95 % CI, 35.8 % - 45.0 %; 175 / 434). Baseline prevalence *of asymptomatic* malarial parasitaemia among the children from the Fe group; 28.3 % (95 % CI, 24.3 % - 32.8 %; 123 / 434) was similar ($p = 0.75$) to those from the non-Fe group; 27.4 % (95 % CI, 23.4 % - 31.8 %; 118 / 431). After the intervention, adherence for the children receiving MNP with Fe was 88.1 % (95 % CI, 86.9 % - 89.4 %) and MNP without Fe was 88.0 % (95 % CI, 86.7 % - 89.3 %) ($p > 0.05$).

Distribution of IgG_3 hinge region length polymorphism between the study groups

The highest frequency of the IgG_3HRLP were the homozygote medium (MM) variants with allelic frequency of 0.48, followed by the long homozygote hinge region polymorphs (LL) with allelic frequency of 0.43. The long-medium heterozygote (LM) was in the third position while the short homozygote (SS) IgG_3 hinge region variants were the least (2.0 %) among the participants (Figure 1).

Consequently, the heterozygote medium-short (MS) and long short (LS) IgG_3HRLP were absent in this study. The distribution of the polymorphisms among the children between the study groups ($p = 0.14$) with similar prediction of Hardy-Weinberg equilibrium ($p > 0.05$, χ^2 analysis) (Figure 1).

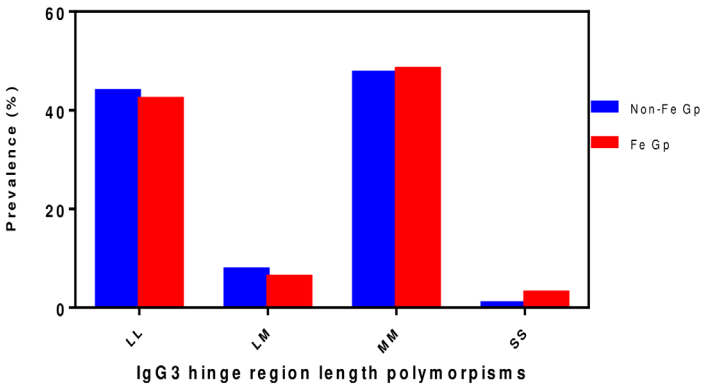

Figure 1: Prevalence of IgG_3 hinge region length polymorphisms between the groups.
% = Percentage, Fe =Iron, Non-Fe = Non-iron, L-long hinge region allele, M-medium hinge region allele, S-short hinge region allele. Fisher's exact tests for p-values.

Effect of IgG_3 hinge region length polymorphism on haemoglobin levels

At endline (EL), haemoglobin (Hb) levels of IgG_3HRLP among the children were similar between the study groups ($p > 0.05$), except that IgG_3 hinge region small (SS) length polymorphic children from the iron group had significantly higher Hb levels compared to children with similar traits in the non-iron group ($p = 0.05$). However, within the two study groups, the Hb levels of IgG_3HRLP among the children recruited at BL were significantly higher than their counterparts at the end of the MNP intervention ($p < 0.05$), except the IgG_3 hinge region small (SS) length polymorphic children from the iron group, that had similar Hb levels between BL and EL ($p = 0.27$) (Table 2).

The prevalence of anaemia among IgG_3 hinge region length polymorphic children

Based on WHO standards, severe anaemia falls below Hb levels of 70 g / L; moderate anaemia has Hb levels ranging from 70 to 90 g / L and non anaemia status above 90 g / L [21]. The recruited children had anaemic status not below moderate anaemia. However, IgG_3 hinge region long (LL) and medium (MM) homozygote length polymorphic participants were significantly more anaemic at the end of the MNP intervention irrespective of the study group ($p < 0.05$). IgG_3 hinge region heterozygote long-medium (LM) and homozygote short (SS) length polymorphic children had similar anaemic prevalence at the end of the MNP intervention with or without iron between the BL and EL within the groups ($p > 0.05$) (Table 3).

IgG_3 hinge region length polymorphism and malaria immunity among study children

The distribution of IgG_3HRLP among the children in the iron and non-iron groups were assessed against protection and susceptibility to clinical malaria. However, the distribution of polymorphisms between children who are protected against and susceptible to clinical malaria were similar whether they received MNP with or without Fe ($p = 0.86$, χ^2 analysis) (Table 4). Nonetheless, there was

Table 1: Baseline background characteristics among study participants.

Characteristics	Iron Group (n=431)	Non-iron Group (n=428)	p-values
Total clusters numbers	388	386	
Cluster Size, median (IQR)	1(1-3)	1(1-3)	0.47
Age, mean [range], mo	19.3 [6-35]	19.7 [6-35]	0.50
Gender (%), Male	51.3	51.4	0.97
Prevalence *of asymptomatic malaria*, n (%)	431 (27.4)	434 (28.3)	0.75
Prevalence of moderate *anaemia*, n (%)	431 (42.7)	434 (40.3)	0.47
Parasitaemia, n, geometric mean, count/µL of blood	130, 2906.5	133, 2511.9	0.88
E*conomic status* of household heads, n (%)	413 (100)	423 (100)	0.02
High	116 (28.1)	152 (35.9)	
Low	297 (71.9)	271 (64.1)	
Educational level of household head, n (%)	413 (100)	422 (100)	0.12
None	138 (33.4)	137 (32.5)	
Basic	250 (60.5)	249 (59.0)	
Advance	25 (6.1)	185 (8.5)	
Use of anti-malarial bednet, n (%)	412 (100)	421 (100)	0.69
Yes	378 (91.8)	378 (89.8)	
No	34 (8.2)	43 (10.2)	

n = Frequency of participants, % = percentage of participants affected, IQR = interquartile range, mo = age in months; Two-sample Wilcoxon rank-sum (Mann-Whitney) and Fisher's exact tests for p-values.

Table 2: Effect of IgG_3 hinge region length polymorphisms on haemoglobin levels.

Haemoglobin levels g / dL										
Baseline						**Endline**				
IgG_3 hinge length polymorphisms	**Iron group**		**Non-iron group**		**p-values**	**Iron group**		**Non-iron group**		
	n (median)	**IQR**	**n (median)**	**IQR**		**n (median)**	**IQR**	**n (median)**	**IQR**	**p-values**
LL	186 (10.4)	9.5-11.1	181 (10.5)	9.5-11.2	0.45	189 (9.5)	8.4-10.4	181 (9.4)	8.1-10.3	0.54
LM	33 (10.1)	9.4-10.8	27 (10.4)	9.7-11.3	0.29	33 (8.9)	8.2-9.6	27 (9.6)	6.9-10.4	0.55
MM	204 (10.3)	9.3-11.1	205 (10.3)	9.4-11.0	0.59	204 (9.6)	8.5-10.6	207 (9.4)	8.3-10.5	0.31
SS	4 (10.2)	9.7-10.4	13 (10.1)	9.8-10.6	1	**4 (10.6)**	**9.5-11.4**	**12 (8.9)**	**7.7-9.7**	**0.05**
Iron group						**Non-iron group**				
	Baseline		**Endline**			**Baseline**		**Endline**		
	n (median)	**IQR**	**n (median)**	**IQR**	**p-values**	**n (median)**	**IQR**	**n (median)**	**IQR**	**p-values**
LL	**186 (10.4)**	**9.5-11.1**	**189 (9.5)**	**8.4-10.4**	**<0.001**	**181 (10.5)**	**9.5-11.2**	**181 (9.4)**	**8.1-10.3**	**<0.001**
LM	**33 (10.1)**	**9.4-10.8**	**33 (8.9)**	**8.2-9.6**	**0.003**	**27 (10.4)**	**9.7-11.3**	**27 (9.6)**	**6.9-10.4**	**<0.001**
MM	**204 (10.3)**	**9.3-11.1**	**204 (9.6)**	**8.5-10.6**	**<0.001**	**205 (10.3)**	**9.4-11.0**	**207 (9.4)**	**8.3-10.5**	**<0.001**
SS	4 (10.2)	9.7-10.4	4 (10.6)	9.5-11.4	0.27	**13 (10.1)**	**9.8-10.6**	**12 (8.9)**	**7.7-9.7**	**0.002**

n = frequency of participants, IQR = interquartile range, L-long hinge region allele, M-medium hinge region allele, S-short hinge region allele; Two-sample Wilcoxon rank-sum (Mann-Whitney) and Fisher's exact tests for p-values.

Table 3: Prevalence of anaemia among IgG_3 hinge region length polymorphic children.

Prevalence of Anaemia n (%)								
Hinge Region Genotype	**Baseline**		**p-values**	**Endline**				
	Iron group	**Non-iron group**		**Iron group**		**Non-iron group**		**p-values**
Aneamia	**Mod.**	**Mod.**		**Mod.**	**Sev.**	**Mod.**	**Sev.**	
LL	70 (16.4)	64 (15.0)	0.65	104 (24.2)	12 (2.8)	91 (21.3)	17 (4.0)	0.49
LM	14 (3.3)	8 (1.9)	0.31	20 (4.7)	5 (1.2)	11 (2.6)	7 (1.6)	0.18
MM	86 (20.1)	76 (17.8)	0.29	111 (25.8)	9 (2.1)	117 (27.4)	18 (4.2)	0.41
SS	1 (0.2)	4 (0.9)	0.83	2 (0.5)	0 (0)	9 (2.1)	2 (0.5)	0.24
	Iron group		**p-values**	**Non-iron group**				**p-values**
	Baseline	**Endline**		**Baseline**	**Endline**			
Aneamia	**Mod.**	**Mod.**	**Sev.**		**Mod.**	**Mod.**	**Sev.**	
LL	**70 (16.4)**	**104 (24.2)**	**12 (2.8)**	**0.016**	**64 (15.0)**	**91 (21.3)**	**17 (4.0)**	**<0.001**
LM	14 (3.3)	20 (4.7)	5 (1.2)	0.25	8 (1.9)	11 (2.6)	7 (1.6)	0.76
MM	**86 (20.1)**	**111 (25.8)**	**9 (2.1)**	**0.003**	**76 (17.8)**	**117 (27.4)**	**18 (4.2)**	**<0.001**
SS	1 (0.2)	2 (0.5)	0 (0)	0.50	4 (0.9)	9 (2.1)	2 (0.5)	1.00

n = Number of participants, % = percentage of participants affected, Mod. = Moderate, Ser. = Severe, L-long hinge region allele, M-medium hinge region allele, S-short hinge region allele; Two-sample Wilcoxon rank-sum (Mann-Whitney) and Kruskal-wallis rank tests for p-values.

Table 4: IgG_3 hinge region length polymorphism and clinical malaria among the children.

Hinge region polymorphs	Frequency and percentage of children n (%) Malaria			p-values
	Susceptible	Protected	Total	
Non-iron LL	96 (21.3)	85 (20.8)	181 (21.1)	
Non-iron LM	15 (3.3)	12 (2.9)	27 (3.1)	
Non-iron MM	111 (24.7)	96 (23.5)	207 (24.1)	
Non-iron SS	7 (1.6)	6 (1.5)	13 (1.5)	
Iron LL	103 (22.9)	86 (21.0)	189 (22.0)	
Iron LM	13 (2.9)	20 (4.9)	33 (3.8)	
Iron MM	103 (22.9)	102 (24.9)	205 (23.9)	
Iron SS	2 (0.4)	2 (0.5)	4 (0.5)	
Chi square				0.86

n = frequency of children, % = percentage of children affected, Fe = iron, non-Fe = non-iron, L-long hinge region allele, M-medium hinge region allele, S-short hinge region allele, Fisher's exact tests for p-values.

Table 5: Association between IgG_3HRLP and anaemia among study groups.

IgG_3HRLP	Study groups	Number (% of children with IgG_3HRLP)	Adjusted odds ratio ɡ (95 % CI)	p-values
MM	Iron	210 (24.2 %)	1	
MM	Non-iron	212 (24.4 %)	1.39 (0.85 – 2.27)	0.19
LL	Iron	207 (23.8 %)	1.28 (0.79 - 2.09)	0.32
LL	Non-iron	165 (19.0 %)	1.38 (0.84 - 2.27)	0.20
LM	Iron	31 (3.6 %)	1.33 (0.47 - 3.75)	0.59
LM	Non-iron	25 (2.9 %)	1.75 (0.65 - 4.74)	0.27
SS	Iron	4 (0.5 %)	1.21 (0.14 - 10.70)	0.87
SS	Non-iron	15 (1.7 %)	12.80 (1.54 - 107.31)	0.02

95 % confidence interval (95 % CI), percentage (%), ɡ: Adjusted for child's age and sex. Further adjusted for baseline anaemia, baseline iron deficiency and baseline iron deficiency anaemia in the model for anaemia, iron deficiency and iron deficiency anaemia respectively.

no association between hinge region polymorphic alleles and the study groups after MNP intervention (Table 4).

Association between IgG_3HRLP and anaemia among study participants

The logistic regression analysis in this study indicated that 1.7 % of IgG_3 hinge region SS allelic children from the non-iron group were more likely to become anaemic ($p = 0.02$) but the children with other IgG_3HRLP were not significantly more likely to get anaemia ($p > 0.05$) compared to 24.2 % of IgG_3 hinge region MM allelic children from the iron group. Therefore, the research participants were associated with IgG_3 hinge area SS hinge length polymorph and anaemia under the control of the prophylactic micronutrient iron fortifier (Table 5).

Association between IgG_3HRLP and malaria among study participants

In this study, the logistic regression analysis indicated that children with other IgG_3HRLP were less likely to get malaria compared to 1.5 % of IgG_3 hinge region SS allelic children from the non-iron group but this was not significant ($p > 0.05$). Therefore, no association existed between IgG_3 hinge region length polymorphs and malaria under the influence of prophylactic micronutrient iron fortificant among the study children (Table 6).

Discussion

Studies have shown that IgG_3 hinge region length polymorphism plays an important role in offering a variable degree of protection among pre-school children [6,7]. Other studies have also substantiated the existence of ethnic or genetic variations through natural selection on immunity that profoundly influence the risk of clinical malaria and anaemia among infants and young children [22-25]. The polymorphic nature of IgG_3HRLP confer naturally acquired or passive immunity against several infections including malaria that indirectly may mitigate anaemia among pre-school children [1]. This study is the first to determine the impact of iron fortification on IgG_3HRLP among Ghanaian children and assessed their associated risk to malaria and anaemia in malaria endemic areas.

The prevalence rate of MM alleles for IgG_3HRLP (48.0 %) were the highest among the children while the SS alleles (2.0 %) were the least prevalent in the study (Figure 1). Nevertheless, the prevalence rates between the groups for IgG_3HRLP were similar. In the same way, another study on maternal passive transfer of antibodies for IgG_3HRLP among Ghanaian infants and its associated risk to malaria by Opoku-Mensah (2014) in a similar geo-political area reported similar prevalence rates for IgG_3HRLP [7]. On the contrary, Adu and his colleagues (2010) reported a higher prevalence of LM alleles for IgG_3HRLP (46.0 %), LL alleles (40.1 %), and MM alleles (13.9 %) being the least when he investigated the immuno-genetic correlates among Ghanaian pre-school children to malaria in a different geo-political area with moderately low malaria transmission [6]. Other reasons for these variable distribution of IgG_3HRLP among the population maybe attributed to the impact of natural selection on different malaria endemicity [26,27].

Consequently, the Hb levels for the IgG_3HRLP among pre-school children were significantly reduced at end of MNP intervention with or without Fe ($p < 0.05$) (Table 2). This suggested that the MNP intervention did not influence the level of Hb on IgG_3HRLP among the children. Though, the reasons maybe unclear. Other studies have indicated that Fe is hormone-homeostatically controlled to promote its optimal use for various metabolic processes but its regulatory role deprives other parasitic organisms including malaria falciparum of Fe nourishment impeding their survival [28-30]. The prevalence of moderate and severe anaemia were similar between the study groups for the IgG_3HRLP after the intervention ($p > 0.05$) (Table 3).

However, LL and MM alleles for IgG_3HRLP within the groups at the end of the intervention with or with Fe were significantly protected from anaemia ($p < 0.05$) (Table 3). These findings were consistent with studies by Adu and his colleagues [6] and Mensah-Opoku [7] among Ghanaian infants and children in moderate and high malaria endemic areas respectively. Mechanisms explaining the effect of IgG_3 hinge region length polymorphs on anaemia are not known. Other findings have suggested that IgG_3HRLP are positively associated with the risk of having malaria including other infections, thereby improving the anaemic status of children [1,4,25].

The MNP intervention among the IgG_3 hinge region length polymorphs were not associated to risk of malaria in this study ($p = 0.86$) (Table 4). Though studies on the effect of MNP intervention with or without Fe on IgG_3HRLP are not known, there have been few investigators who have conducted related studies without MNP intervention on IgG_3 hinge region length polymorphs [4,6,7,29]. Also studies by Zlotkin and colleagues on MNP supplementation did not investigate the effect of IgG_3HRLP on malaria [16]. The polymorphic characters of the IgG_3 extends the half-life comparable to the other subclasses of the IgG isotype (from 7 to 21 days) and this adequately enhances opsonization potentials and complement fixation functions that leads to the splenic clearance activity of malaria parasites [1,4,31].

Conclusions

This research revealed that IgG_3HRLP is a host gene essential for the protection of anaemia and clinical malaria but iron-containing micronutrient powder were not detrimental to participants in endemic areas of malaria. However, iron containing MNP was not associated with the risk of anaemia and malaria among pre-school children with IgG_3HRLP living in high malaria transmission areas.

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