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Genetic analysis of IgG subclass responses against RESA and MSP2 of *Plasmodium falciparum* in adults in Papua New Guinea

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SUMMARY

Contributions of environmental and genetic factors to IgG subclass responses against *Plasmodium falciparum* antigens RESA and MSP2 were investigated among adults in a highly endemic area of Papua New Guinea. Heritabilities were estimated using variance component analysis. Familial aggregation of several responses was found, including IgG1, IgG2 and IgG3 responses against RESA, IgG1 and IgG3 responses against the 3D7 form of MSP2 and IgG1, IgG2 responses against the FC27 form of MSP2. Allowance for sharing of houses explained some of the non-genetic variance but not the familial aggregation. The variance of IgG3 responses against RESA and IgG1, IgG2 against MSP2 (FC27) was partly explained by sharing of HLA class II genotypes, although heritability was low. Segregation analyses indicated that any genetic regulation was more complex than governed by a single major gene. Such host genetic variation in responses to specific malaria antigens has implications for immuno-epidemiology and vaccine development.

INTRODUCTION

In recent years, several specific malarial antigens have been identified for possible inclusion into a sub-unit vaccine. Attempts to characterize immune responses to these antigens have demonstrated qualitative and quantitative differences. For example, it has been shown that an IgG3 subclass response is predominant against the merozoite surface protein-2 (MSP2) [1, 2], but not to the ring-infected erythrocyte surface antigen (RESA) [3]. Both antigens are being included together with MSP1 in the combination B malaria vaccine currently under trial in Papua New Guinea [4]. In addition, specific IgG subclass responses are associated with protection against *P. falciparum* infection or disease [5–7]. The ability of individuals to mount such responses may be explained by several genetic and non-genetic factors, each of which should ideally be quantified for effective analysis of vaccine efficacy.

In terms of studying genetic factors, most work has focused on the importance of MHC genes in regulating immune responses to antigens such as RESA [8–10], MSP2 [11], and SPf66 [12, 13]. In most of the studies, genes from the MHC region appeared to play minor roles compared to non-MHC mechanisms in the regulation of immune responses to specific malaria antigens [11, 13–15].

More recently, efforts have been made to quantify the relative contribution of genetic and environmental factors. This has involved calculating heritabilities for the immune response against various malaria antigens in twins in The Gambia [15], Liberia and Madagascar [16] and in families in PNG [11]. All studies demonstrated familial aggregation of total IgG anti-

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body and/or proliferation responses against RESA and MSP2. Determining the heritability of IgG subclass responses against specific malaria antigens has not yet been undertaken.

In this study we estimated familial aggregation in IgG subclass responses to RESA and MSP2 in an area highly endemic for *P. falciparum* malaria. The same study population in Papua New Guinea was chosen as for the analysis of total IgG responses, enabling us to assess the relative contribution of subclass responses to the overall heritability. First, variance component analysis was performed to quantify genetic and non-genetic components in each of the subclass responses. The role of HLA class II genotypes was also examined. Finally, for those responses that appeared to be heritable, the mode of inheritance was assessed by complex segregation analysis.

METHODS

Study area and population

Data from the Wosera area in the East Sepik region of Papua New Guinea, collected and processed within the Malaria Vaccine Evaluation and Epidemiology project (MVEEP) of the Institute of Medical Research in Papua New Guinea [4], were analysed. The study area is highly endemic for malaria with perennial transmission and *Plasmodium falciparum* as the most common parasite species. *P. vivax* and *P. malaria* also occur. Details on the epidemiology of malaria in the study area have been described elsewhere [17, 18].

The study population belongs to the Abelam speakers of the Papuan language group [19]. They live from subsistence farming. Village endogamy is common, and the diversity of HLA class I alleles [20] and HLA class II alleles [13] is limited. There is no ovalocytosis [21]. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is found in 10% [21, 22] and α-thalassaemia in 90% [23] of the population.

Data on familiar relationships were collected in 50% of randomly chosen households in two villages, Kunjingini 1 and 2, using structure questionnaires. Questions were asked about first and second-degree relatives, their age and place of residence. Genealogies were constructed and familial data were validated using the HLA genotypes and additional interviews.

Blood samples were collected in 214 adults (125 females and 89 males) as described earlier [21] and used to determine IgG subclass responses against two specific malaria antigens (see below). The age of the studied individuals was in the range 11–72 years.

Table 1. *Number of relative pairs used in analyses*

Types of relatives	Kin*	Frequency of pairs
Parental	0	19
Parent–offspring	0·25	124
Siblings	0·25	119
Half-siblings	0·125	19
Grandparent–grandchild	0·125	14
Avuncular†	0·125	155
Cousins	0·0625	220
Great-grandparent–great-grandchild	0·0625	1
Great-avuncular	0·0625	39
Half-avuncular	0·0625	16
Total number of relative pairs		726

* Kinship coefficient.
† aunt/uncle–niece/nephew.

The 214 individuals could be linked via 35 distinct genealogies as described previously [11]. One genealogy consisted of 405 individuals related via a complex marital structure. For the purpose of this analysis, the large genealogy was broken down into 16 smaller families consisting of 3–57 individuals, because one analysis programme could not handle the large genealogy. The numbers of relative pairs used in the analyses are shown in Table 1.

Malaria antigens

RESA (ring-infected erythrocyte surface antigen) was a recombinant protein expressed in *Escherichia coli* [24–26] and was produced and provided by Saramane Pty Ltd (Melbourne, Australia).

Two different full-length recombinant MSP2 (Merozoite Surface Protein 2) molecules were used [27, 28]. The antigens resembled the MSP-molecule from the FC27 and 3D7 isolates of *P. falciparum*.

IgG subclass responses

IgG1, IgG2 and IgG3 isotype responses were determined by standard ELISA techniques as described previously [3]. Sera for all antigens were diluted 1 : 100. Optical densities (OD) were photometrically determined at wavelength 405 nm.

HLA typing

PCR/oligonucleotide hybridization was used for the genotyping of the blood samples as described previously [13]. Briefly, DQB*1 was typed according to the description of Bugawan and Erlich [29] and

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DRB*1 was typed as described by Titus-Trachtenberg and colleagues [30]. Number and allele frequencies were given elsewhere [13]. HLA class II alleles were determined in 178 individuals.

Statistical methods

Since the antibody responses, measured in optical densities, were not normally distributed age and sex differences were firstly assessed using Spearman rank correlations and Wilcoxon rank sum tests. In order to assure normality for all subsequent analyses, IgG subclass responses were then transformed into normal scores using the procedure described by Blom [31].

Variance component analysis was performed by the FISHER programme [32], in order to partition the total variance of the IgG subclass responses into separate variances attributable to genetic and non-genetic components. The variation in the immune responses due to genetic components was partitioned into additive (AV) and dominance variance (DV). AV describes the variation due to additive effects of individual alleles not necessarily at one locus [33]. DV is the variation due to non-linear interaction effects between alleles at the same locus caused by dominance [33]. In order to see how much of the genetic variance is explained by sharing HLA class II genotypes at DRB1* and DQB1* loci, two other random effect terms were included into the model [4]. Since HLA class II alleles could only be typed for 178 individuals, the model is not directly comparable to previous models. Therefore, we re-estimated the model without additional HLA variance component included with reduced sample size.

Variation due to non-genetic components was partitioned into variation attributable to shared houses (HV) and a term that included all other non-genetic variation (NGV). The HV describes the variation due to living in the same house and the term was included into the model as described by Hopper & Mathews [34].

Where appropriate, sex and age were included in variance component models as fixed effects, whereas a separate term was fitted for each age group. Models were fitted hierarchically.

Heritabilities in the broad sense were calculated as the proportion of the genetic and total phenotypic variance as follows: $(AV+DV)/(AV+DV+HV+NGV)$ [33]. The asymptotic standard errors (S.E.) were estimated using formulae given by Armitage & Berry [35].

If the heritability was greater than zero and not fully explained by sharing HLA class II genotypes, complex segregation analysis was performed using the REGC programme of SAGE [36] to establish the involvement of a major gene. Class A models were used [37] which assume that siblings are dependent only because of common parentage. Sib–sib correlation is dependent on parent–offspring correlation as described in the SAGE manual [36].

Various non-genetic and genetic models were fitted to the data (Table 2). Models were based on the segregation of three ousiotypes (AA, AB, BB), which corresponded to the three genotypes at a single locus with two alleles (A, B) in a genetic model. The overall distribution of the phenotype (immune response) was assumed to be normal with mean μ and population variance σ^2. The hypothetical allele frequency was parameterized as q_A and the mean phenotypic values were parameterized as $\mu_{AA}, \mu_{AB}, \mu_{BB}$. The probabilities of transmitting allele A for individuals AA, AB, BB were parameterized in terms of $\tau_{AA}, \tau_{AB}, \tau_{BB}$. Under the Mendelian hypothesis, these probabilities were fixed to the following values $\tau_{AA} = 1, \tau_{AB} = 0{\cdot}5, \tau_{BB} = 0$. Under the general model they were free to be estimated. Under the environmental model the transmission probabilities were fixed to q_A, or q_A was estimated and transmission probabilities were fixed to be equal. In addition, residual familial correlations (ρ_{SP}: spouse, ρ_{MO}: mother–offspring, ρ_{FO}; father–offspring) was estimated. Sex was included as a covariate in the analyses where appropriate. Separate age terms were fitted for each age class.

Different models were compared using likelihood ratio tests (LRT) in order to select the most parsimonious model. The LRT statistic, calculated as minus twice the difference of the log-Likelihood (lnL) between two models, follows a χ^2 distribution with degrees of freedom (D.F.) equal to the difference in the number of estimated parameters between the two models. Since the LRT can only be used to compare strictly hierarchical models, the Akaike's information criterion [38] was calculated for each model as $AIC = -2\ln L + 2$ (number of parameters estimated). The most parsimonious model is the one with the smallest AIC.

RESULTS

The distribution of IgG subclass responses against RESA and MSP2 antigens are shown in Table 3. IgG1, IgG3 subclass responses against RESA were

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Table 2. *Description of models tested in segregation analysis*

Model	Abbreviation	Parameters estimated				
Sporadic	—	μ	σ^2			
Environmental	Env	$\mu_{AA}, \mu_{AB}, \mu_{BB}$	σ^2	q_A	$q_A = \tau_{AA} = \tau_{AB} = \tau_{BB}$	
Environmental with non-genetic transmission	Env2	$\mu_{AA}, \mu_{AB}, \mu_{BB}$	σ^2	q_A	$\tau_{AA} = \tau_{AB} = \tau_{BB}$	
Familiar correlation	FC	μ	σ^2			ρSP, ρMO, ρFO
Environmental and Familiar correlation	EnvFC	$\mu_{AA}, \mu_{AB}, \mu_{BB}$	σ^2	q_A		ρSP, ρMO, ρFO
Mendelian single locus	MSL	$\mu_{AA}, \mu_{AB}, \mu_{BB}$	σ^2	q_A	$\tau_{AA} = 1, \tau_{AB} = 0{\cdot}5, \tau_{BB} = 0$	
General single locus	GSL	$\mu_{AA}, \mu_{AB}, \mu_{BB}$	σ^2	q_A	$\tau_{AA}, \tau_{AB}, \tau_{BB}$	
Mendelian single locus and Familiar correlation	MSL FC	$\mu_{AA}, \mu_{AB}, \mu_{BB}$	σ^2	q_A	$\tau_{AA} = 1, \tau_{AB} = 0{\cdot}5, \tau_{BB} = 0$	ρSP, ρMO, ρFO
General single locus and Familial correlation	GSL FC	$\mu_{AA}, \mu_{AB}, \mu_{BB}$	σ^2	q_A	$\tau_{AA}, \tau_{AB}, \tau_{BB}$	ρSP, ρMO, ρFO

For IgG1 and IgG3 subclass response against RESA, sex specific μ and σ^2 were estimated.
For further details see SAGE manual [36].

Table 3. *IgG subclass responses* (*measured in OD*) *to different malarial antigens*, *and the effect of age and sex on these responses*, *are described*

	Median	(25, 75% tile)	Sex differences†	Correlation with age‡
IgG1 RESA	0·50	(0·25, 0·83)	2·47*	0·06
IgG2 RESA	0·64	(0·09, 1·42)	0·01	0·07
IgG3 RESA	0·46	(0·25, 1·15)	2·26*	0·17*
IgG1 MSP2 (FC27)	0·37	(0·11, 0·99)	1·53	−0·26**
IgG2 MSP2 (FC27)	0·23	(0·11, 0·40)	0·34	−0·31**
IgG3 MSP2 (FC27)	1·11	(0·42, 1·53)	0·13	0·04
IgG1 MSP2 (3D7)	0·36	(0·15, 0·72)	0·66	−0·11
IgG2 MSP2 (3D7)	0·16	(0·08, 0·35)	0·77	−0·12
IgG3 MSP2 (3D7)	1·12	(0·70, 1·61)	1·90	0·05

Significance levels: * $P < 0{\cdot}05$, ** $P < 0{\cdot}001$.
† Wilcoxon z-statistic.
‡ Spearman rank correlation coefficient.

higher in males than in females (Table 3, [3]). In addition, IgG3 responses against RESA are positively correlated with age, and IgG1 and IgG2 responses against MSP2 (FC27) are negatively correlated with age (Table 3).

Results of the variance component analysis are shown in Table 4. Most of the total variation in IgG1 and IgG2 subclass response against RESA and IgG1 response against MSP2 (3D7) was explained by genetic variance. The majority of the total variation in all other measured responses was due to non-genetic variation. However, for the IgG1 response against MSP2 (3D7) only dominance variance and no additive genetic variance was found. This strongly suggests that the genetic variance was highly confounded by common sib-ship environment. The model with both genetic and non-genetic components included fitted significantly better than the models where the house components were omitted, except for IgG1, IgG2 responses to MSP2 (FC27) and to MSP2 (3D7). In these cases, the house variance was absent or small (Table 4). Overall, heritabilities were greater than zero for most of the IgG subclass responses, except for IgG3 response against MSP2 (FC27) and IgG2 response against MSP2 (3D7). In addition, the amount of variation attributable to living in the same house was smaller than the genetic variation, when the heritability was large.

Where there was any genetic variance present in the IgG subclass responses, we were interested in es-

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Table 4. *Results of variance component analysis of IgG subclass responses to different malarial antigens. For each antigen, the first row represents the full model and the second row represents the model without the house effect included*

	AV (S.E.)	DV (S.E.)	HV (S.E.)	NGV (S.E.)	LnL	χ^2 (D.F. = 2)*	H (S.E.)
IgG1 RESA	0·53 (0·04)	0·00 (0·00)	0·17 (0·02)	0·37 (0·02)	−3270·643	65·79	0·50 (0·04)
	0·66 (0·04)	0·00 (0·00)	— —	0·42 (0·02)	−3303·537		
IgG2 RESA	0·44 (0·04)	0·00 (0·00)	0·13 (0·02)	0·37 (0·02)	−2884·596	53·70	0·47 (0·04)
	0·62 (0·03)	0·00 (0·00)	— —	0·35 (0·02)	−2911·447		
IgG3 RESA	0·05 (0·02)	0·00 (0·00)	0·18 (0·02)	0·64 (0·02)	−2901·519	104·07	0·06 (0·02)
	0·12 (0·02)	0·00 (0·00)	— —	0·75 (0·02)	−2953·552		
IgG1 MSP2 (FC27)	0·06 (0·02)	0·00 (0·00)	0·01 (0·03)	0·83 (0·03)	−3104·789	0·08	0·07 (0·02)
	0·07 (0·02)	0·00 (0·00)	— —	0·84 (0·02)	−3104·830		
IgG2 MSP2 (FC27)	0·18 (0·02)	0·00 (0·00)	0·00 (0·00)	0·67 (0·02)	−2798·125	0·00	0·21 (0·02)
	0·18 (0·02)	0·00 (0·00)	— —	0·67 (0·02)	−2798·125		
IgG3 MSP2 (FC27)	0·00 (0·00)	0·00 (0·00)	0·30 (0·02)	0·70 (0·02)	−3320·442	244·64	0·00 —
	0·07 (0·02)	0·00 (0·00)	— —	0·93 (0·03)	−3442·764		
IgG1 MSP2 (3D7)	0·00 (0·00)	0·85 (0·07)	0·00 (0·00)	0·11 (0·06)	−3203·315	0·00	0·89 (0·07)
	0·00 (0·00)	0·85 (0·07)	— —	0·11 (0·06)	−3203·315		
IgG2 MSP2 (3D7)	0·00 (0·00)	0·00 (0·00)	0·002 (0·02)	0·96 (0·03)	−3331·402	0·01	0·00 —
	0·00 (0·00)	0·00 (0·00)	— —	0·96 (0·02)	−3331·409		
IgG3 MSP2 (3D7)	0·05 (0·04)	0·19 (0·08)	0·12 (0·02)	0·55 (0·07)	−3105·911	72·18	0·27 (0·07)
	0·09 (0·03)	0·11 (0·07)	— —	0·72 (0·06)	−3142.003		

Abbreviations: AV, additive genetic variance; DV, dominance variance; HV, variance due to shared houses; NGV, non-genetic variance; SE, standard error; LnL, log-likelihood; H, broad sense heritability.

* Likelihood ratio test statistic between the full model and the model without HV included.

Table 5. *Results of variance component analysis of IgG subclass responses to different malarial antigens including two further variance components for sharing genotypes at the HLA class II DRB and DQB loci. For each antigen, the first row represents the full model and the second row the model without the HLA class II variance components included*

	AV (S.E.)	DV (S.E.)	DRB (S.E.)	DQB (S.E.)	HV (S.E.)	NGV (S.E.)	LnL	χ^2 (D.F. = 2)*	
IgG3 RESA	0·00 (0·00)	0·00 (0·00)	0·002 (0·02)	0·06 (0·02)	0·15 (0·03)	0·65 (0·02)	−2438·928	84·7	
	0·04 (0·02)	0·00 (0·00)	— —	— —	0·19 (0·02)	0·65 (0·03)	−2481·295		
IgG1 MSP2 (FC27)	0·00 (0·00)	0·00 (0·00)	0·00 (0·00)	0·02 (0·005)	0·00 (0·00)	0·94 (0·02)	−2838·741	38·5	
	0·06 (0·02)	0·00 (0·00)	— —	— —	0·00 (0·00)	0·92 (0·03)	−2857·998		
IgG2 MSP2 (FC27)	0·04 (0·03)	0·00 (0·00)	0·00 (0·00)	0·04 (0·01)	0·00 (0·00)	0·81 (0·03)	−2570·119	57·0	
	0·15 (0·02)	0·00 (0·00)	— —	— —	0·00 (0·00)	0·75 (0·03)	−2598·604		
IgG3 MSP2 (3D7)	0·00 (0·00)	0·63 (0·08)	0·00 (0·00)	0·001 (0·003)	0·21 (0·02)	0·13 (0·08)	−2723·333	0·59 ns	
	0·00 (0·00)	0·62 (0·08)	— —	— —	0·21 (0·02)	0·14 (0·08)	−2723·392		

Abbreviations: AV, additive genetic variance; DV, dominance variance; HLA, variation due to HLA genotypes; HV, variance due to shared houses; NGV, non-genetic variance; S.E., standard error; LnL, log-likelihood.

* Likelihood ratio test statistic between the full model and the model without HLA loci.

timating how much of this could be explained by sharing HLA class II genotypes (Table 5). For most of the IgG subclass responses, none of the total variation could be explained by HLA class II loci. However, although the heritability was small, the genetic variation of IgG3 responses against RESA and IgG1 responses against MSP2 (FC27) was completely explained by sharing genotypes at either both DRB1* and DQB1* loci or by the DQB1* locus, respectively. In addition, part of the genetic variance of IgG2 response against MSP2 (FC27) was explained by the DQB1* locus.

The results of the segregation analyses are summarized in Table 6. The most parsimonious model according to AIC for most of the IgG subclass responses tested, except for IgG2 against RESA and IgG1 against MSP2 (3D7), was the familial correlation model (FC). However, for IgG1 responses against RESA, all of the sub models were rejected when compared to the full model (GSL FC). The most parsimonious model for IgG2 response against RESA was Env2. However, in comparison to the full model, the environmental model, the familial correlation and general single locus model could not be rejected either. For the IgG2 response against MSP2 (FC27) FC, EnvFC and MSL FC could not be rejected. For the IgG1 response against MSP2 (3D7) the most parsimonious model was EnvFC. However, Env2, FC, GSL and MSL FC could not be rejected either. Overall, the regulation for all IgG subclass responses was not dominated by a single major gene and it was not completely random either. These responses appeared to be influenced by major environmental factors aggregating in families and probably multiple genes.

DISCUSSION

Estimating familial aggregation of immune responses is important in understanding the contribution of genetic and environmental factors to disease processes. In this context, we quantified genetic and non-genetic components in the IgG subclass responses against RESA and MSP2 in an area highly endemic for malaria transmission. Using variance component analysis we found substantial heritability for most of the antibody responses except for IgG3 and IgG2 subclass responses against MSP2 (FC27) and (3D7), respectively. Further variance component analysis also indicated that this heritability does not originate from the MHC for most of the IgG subclass responses.

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Table 6. *Segregation analysis of IgG subclass responses against different malaria antigens*

	Model								
	Sporadic	Env	Env2	FC	EnvFC	MSL	GSL	MSL FC	GSL FC
IgG1 RESA*									
AIC	607·047	590·206	584·887	584·350	592·101	598·410	587·390	592·170	585·315
χ^2	43·73***	16·89***	9·57*	15·04*	12·79***	25·10***	8·08**	12·86***	—
D.F.	11-2	6-2	5-2	8-2	3-2	6-2	3-2	3-2	—
IgG2 RESA									
AIC	608·847	596·541	589·532	594·685	601·741	608·291	592·187	599·651	596·575
χ^2	30·27***	11·97	2·96	10·11	11·17*	23·72***	1·61	9·08*	—
D.F.	9	6	5	6	3	6	3	3	—
IgG2 MSP2 (FC27)†									
AIC	616·446	599·798	601·762	590·450	594·834	604·805	606·225	594·935	599·402
χ^2	35·04***	12·40*	12·36**	3·05	1·43	17·40**	12·82***	1·53	—
D.F.	9-2	6-2	5-2	6-2	3-2	6-2	3-2	3-2	—
IgG1 MSP2 (3D7)									
AIC	649·161	607·987	603·111	601·063	599·729	613·310	602·625	601·523	604·357
χ^2	62·80***	15·63*	8·75	8·71	1·37	20·95**	4·27	3·17	—
D.F.	9	6	5	6	3	6	3	3	—
IgG3 MSP2 (3D7)									
AIC	675·00	619·943	617·331	610·786	616·678	629·613	629·478	616·685	614·957
χ^2	78·04***	16·99**	12·37*	7·83	7·72**	26·66***	20·52***	7·73**	—
D.F.	9-2	6-2	5-2	6-2	3-2	6-2	3-2	3-2	—

Abbreviations explained in Table 2; AIC, Akaike's information criterion; D.F. degrees of freedom.

* Sex included into analysis.

† Age covariates were fitted for each age class separately.

* $P < 0{\cdot}5$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

Segregation analysis indicated that a major gene is not involved in the regulation of these responses.

In order to assess the amount of variation in immune response attributable to common environmental factors, a house effect was included in the analysis. This is especially useful when other covariates such as exposure to parasites cannot be assessed. However, the interpretation of a house effect is not straightforward: house variation can confound genetic variation, since genetically related individuals live often in the same household. This was also evident in a previous analysis of the same individuals, where most of the genetic variance in total IgG response to RESA could be explained by the effect of closely related individuals living in the same houses [11].

In the present analysis, living in the same house explained some of the overall variation for each response, except for IgG1 response against MSP2 (3D7) and IgG2 response against MSP2 (FC27). However, genetic variance was much larger than house variance for all responses except for IgG3 subclass response against RESA and MSP2 (FC27). We also found that genetic variance varied among the different subclass responses against the same antigen. These results imply that some subclass responses are under greater genetic influence than others, and emphasize that to fully understand the regulation of the overall antibody responses, it is necessary to analyse its components.

In this context, we can use the results of the present analysis to explain the results of previous analyses in which only total IgG responses were measured. It is likely that the low heritability of total IgG responses against RESA was attributable to the near absence of heritability of IgG3 responses. The lack of additive genetic variance, but presence of dominance variance in the total IgG response to MSP2 (FC27) [11] can be explained by lack of heritability of IgG3 responses in combination with this isotype being the predominant response. In the case of the total IgG response against MSP2 (3D7) we found both additive and dominant genetic variance, which again can be explained by the predominance of the IgG3 response.

In general variance component analysis is not used to identify genetic mechanisms, but it can be used to assess the possible involvement of typed genetic loci [39]. In the present analysis, a limited number of associations between IgG subclass responses and shared HLA class II genotypes on DRB1* and/or DQB1* locus were found. Previous studies showed that the HLA class II contribution to total IgG response against RESA and MSP2 was rather small or absent [8, 10, 11, 15, 16], except for the antibody response against MSP2 (FC27) [15]. The absence of associations in these studies might be due to the fact that only a very small amount of heritability in specific subclasses were attributable to sharing HLA class II genotypes. These results suggest that the absence of associations between genes and total IgG responses does not exclude the presence of an association with IgG subclass responses, but associations might be very small. However, the significance of such a small effect needs still to be evaluated.

Familial correlation may be the result of many loci and/or environmental factors, but no evidence for major gene effects was suggested by segregation analysis for the majority of IgG subclass responses. These findings confirm previous results in the same study population, which suggested multifactorial inheritance for total IgG responses against RESA [11]. However, a major dominant gene previously suggested to be involved in the total IgG response against MSP2 (FC27) [11] could not be explained by any of the subclass responses. Whether environmental and genetic factors have confounded the identification of this major gene [40] or if the complexity of the total IgG response consisting of different subclass response with different properties is responsible, or both, remains to be seen.

Since there is no definite surrogate marker for protection, it is not obvious whether the antigens used in the present study are important in clinical protection. Furthermore, it remains unclear whether the measured immune responses target crucial epitopes in effective immunity against malaria parasites. However, it has been suggested that cytophilic antibodies, such as IgG1 and IgG3, against asexual stage antigens are involved in density dependent parasite clearance [41]. Analyses like these we present here might improve the understanding of the importance of genetic regulation of IgG subclass responses. Since particular subclass responses are known to be associated with protective immunity [6, 42] and the risk of clinical malaria [7], genetic restriction in specific responses might alter the ability of individuals to mount an effective response. This could contribute to observed patterns of susceptibility to malaria infection and disease. As a result, infection and disease phenotypes may be clustered in individuals with similar genetic background, which has been

demonstrated previously for parasite densities [43, 44]. Therefore, genetic regulation in immune responses to vaccine candidates should be taken into account in the interpretation of disease distribution in intervention studies.

In conclusion, we found substantial heritability in the regulation of IgG subclass responses against RESA and MSP2 and showed that the genetic mechanisms involved were not dominated by a major gene. Although we found that sharing the same house was influential in determining similarities of immune responses of related individuals, this was not as important as suggested by a previous analysis of overall responses. These results strengthen the argument that host genetic variation in immune responses to specific malaria antigens should be considered in the interpretation of results of immuno-epidemiological studies of malaria and of malaria vaccine trials. The factors determining subclass responses can differ considerably from those important for the overall IgG levels.

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