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Fc gamma Receptor IIa-H131R Polymorphism and Malaria Susceptibility in Sympatric Ethnic Groups, Fulani and Dogon of Mali

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Abstract

It has been previously shown that there are some interethnic differences in susceptibility to malaria between two sympatric ethnic groups of Mali, the Fulani and the Dogon. The lower susceptibility to *Plasmodium falciparum* malaria seen in the Fulani has not been fully explained by genetic polymorphisms previously known to be associated with malaria resistance, including haemoglobin S (HbS), haemoglobin C (HbC), alpha-thalassaemia and glucose-6-phosphate dehydrogenase (G6PD) deficiency. Given the observed differences in the distribution of FcγRIIa allotypes among different ethnic groups and with malaria susceptibility that have been reported, we analysed the rs1801274-R131H polymorphism in the FcγRIIa gene in a study of Dogon and Fulani in Mali (n = 939). We confirm that the Fulani have less parasite densities, less parasite prevalence, more spleen enlargement and higher levels of total IgG antibodies (anti-CSP, anti-AMA1, anti-MSP1 and anti-MSP2) and more total IgE ($P < 0.05$) compared with the Dogon ethnic group. Furthermore, the Fulani exhibit higher frequencies of the blood group O (56.5%) compared with the Dogon (43.5%) ($P < 0.001$). With regard to the FcγRIIa polymorphism and allele frequency, the Fulani group have a higher frequency of the H allele (Fulani 0.474, Dogon 0.341, $P < 0.0001$), which was associated with greater total IgE production ($P = 0.004$). Our findings show that the FcγRIIa polymorphism might have an implication in the relative protection seen in the Fulani tribe, with confirmatory studies required in other malaria endemic settings.

Introduction

Malaria is a public health problem in at least 90 countries worldwide, putting 40% of the world's population at risk. Each year ~500 million individuals contract malaria leading to ~1 million deaths annually with more than 90% of the worldwide malaria cases and deaths occurring in sub-Saharan Africa [1]. Among the *Plasmodium* species (the causative agents of malaria), *Plasmodium falciparum* is particularly lethal most notably causing cerebral malaria [2]. Although there are control programmes based on chemoprophylaxis, case management and antivector strategies, there is much interest in how humans have evolved to develop resistance strategies. Some observations in particular demonstrated differences in antibody production and resistance between ethnic groups in West Africa [3–11].

During the past decade, there has been increased interest in assessing the role of Fc receptors for IgG (FcγRs) on leucocytes, because these receptors provide an important bridge between the humoral and cellular arms of the immune response [12]. Among the three classes of FcγR (FcγRI, FcγRII and FcγRIII), the low-affinity FcγRII class is the most broadly distributed. A polymorphism in FcγRIIa has been studied extensively [12, 13]. A point mutation [A=>G (reference/alternative)] resulting in an amino acid change at position 131, histidine (His131) to arginine (Arg131), is located in the second extracellular immunoglobulin-like domain of this receptor. This is

critical for the binding of human IgG2 [13]. Human IgG2 binds efficiently to FcγIIa-His/His131, but not to FcγRIIa-Arg/Arg131, although both FcγRIIa allotypes interact with IgG1 and IgG3. FcγRIIa is particularly important in this regard, because none of the other FcγR classes bind human IgG2 efficiently [12, 13].

Considerable differences in the distribution of FcγRIIa allotypes have been identified across different ethnic groups [14]. This difference poses interesting questions concerning the selective pressure that maintains this polymorphism in the FcγRIIa gene in human populations, as well as the impact of the polymorphism on the outcome of infection and clinical manifestation of the disease. The clinical importance of the FcγRIIa polymorphism has been evaluated for encapsulated bacterial infections, in which IgG2 plays a critical role in host defence. Several recent hospital-based case–control studies have shown an association between FcγRIIa-His/His131 and protection from encapsulated bacterial infections, whereas the poorly IgG2-binding allotype FcγRIIa-Arg/Arg131 is associated with increased susceptibility to these pathogens [15–18]. Despite its association with increased susceptibility to encapsulated bacterial infections, the frequency of the FcγRIIa-Arg/Arg131 genotype remains relatively stable in most human populations. This stability suggests that infections that depend on IgG1 and IgG3 but not IgG2 to mediate protective immunity may induce selection advantage for the poorly IgG2-binding FcγRIIa-Arg/Arg131 allotype. However, the potential association of FcγRIIa-Arg/Arg131 with protective immune responses against infectious diseases that do not rely on IgG2, such as *P. falciparum* infection, has not been studied.

Previously, the FcγRIIa-Arg/Arg131 polymorphism has been studied in the neighbouring Fulani and Dogon ethnic groups in Mali and has been related to antibody production [10]. This study was undertaken in asymptomatic subjects belonging to both tribes and showed a marked difference in allelic distribution, with the Fulani and Dogon having more 131H/H and 131R/R genotypes, respectively [10]. Here, we test for an association of the FcγRIIa-Arg/His131 polymorphism with malaria symptoms and severity and antibody production in Fulani and Dogon in a meso-endemic area of Mali.

Materials and methods

Study participants. The study was performed in a rural village of Manteourou, Mali, that lies within the African Sahel – a transition zone stretching east–west across Africa between the Sahara desert and the southern savannah. The region is characterized by a dry season from October to May and a rainy season from June to October. Here, people from the Dogon and Fulani ethnic groups live together in sympatry within 0.5 km of each other. The Dogon (n = 505, 53.8%) are farmers who migrated from Bandiagara (110 km) to their present location 50 years ago, while the Fulani (Fulani, n = 434, 46.2%) are cattle breeders who migrated 200 years ago from the area of Douentza situated 150 km from the study area. There is no intermarriage between these two ethnic groups [7]. Two cross-sectional surveys were performed, the first at the end of the transmission or *rainy* season (October/November 2006) and the second during the *dry* season (March/April 2007). The study included unrelated healthy volunteers, children and adults, males and females, belonging to both ethnic groups. At each survey, we collected clinical (spleen enlargement, axillary temperature, body weight) and parasitological data (malaria parasite densities and species) as well as blood samples.

Clinical information. Axillary temperature and spleen size were measured in all participants. The spleen size was scored by Hackett's method and dichotomized as enlarged or not enlarged [19]. Thick blood smears were collected and stained with 3% Giemsa and examined for malaria parasites. Parasites and leucocytes were counted. Parasite densities were estimated using an assumed leucocyte count of 7500 leucocytes per microlitre of blood [7]. A film was determined to be negative if no parasites were identified in the course of examining sufficient fields for a total of 300 leucocytes to be counted. Quality control through double reading was also conducted on 10% of the slides randomly selected by a separate physician. Parasitaemia was defined as being present or absent. Clinical malaria was defined as the presence of fever (axillary temperature of at least 37.5 °C) plus the presence of *P. falciparum* parasites on the thick blood smear, in the absence of any other known illnesses. Asymptomatic malaria was defined as the presence of *P. falciparum* parasites, but no clinical symptoms. As this is a cross-sectional survey, there are no severe cases of malaria. Volunteers were followed up for malaria incidence by active and passive methods by the research team, which included a physician and biologist based in the health centre of the village of Manteourou.

Genotyping and immunoassays. All Genomic DNA samples (n = 939) underwent whole genome amplification by (PEP) [20] before the *Fc gamma RIIa* rs1801274 SNP (chromosome 1 161479745; H/R alleles, denoted here as rs1801274-R131H) was genotyped [as part of an ongoing project (see Maiga *et al.*)] [21] using the Sequenom iPLEX MassArray platform [22–24]. Serum was separated from clotted blood by centrifugation (12,000 rpm for 8 min) and analysed by ELISA for antibodies against four malarial antigens (AMA1, $MSP1_{19}$, MSP2 and CSP) plus total IgE. The processing of the immunological assays has been described earlier (Maiga *et al.*) [21]. The cut-off value for each assay was determined by calculating the arithmetic mean of the absorbance of negative control samples obtained from European individuals who had never been exposed to malaria and adding three standard deviations to that value (mean OD+3SD). Using standard positive

(Brefet4 pool, from Gambia [25]) and negative controls (European pool), the positive–negative threshold baseline was constructed using OD values obtained upon ELISA and was used for calculation of the observed antibody titres as described previously [25]. The titre values were log10-transformed to symmetrize them for regression analysis.

Ethical clearance. The ethical clearance was obtained through the Institutional Review Board of the University of Mali. Treatment for malaria and other illnesses detected during the course of the study was provided to the study population at no cost to participants. Community permission was obtained according to the procedures described by Diallo *et al.*, in CID 2005 [26]. Individual written consent was then obtained for each exam or blood collection from the adult or from the child's parent or caregivers.

Statistical analysis. The overall comparison between Fulani and Dogon across different backgrounds and phenotypic variables was performed using Pearson's chi-squared independence tests for categorical variables and the Mann–Whitney–Wilcoxon rank-sum tests for continuous quantitative variables. Genotypic deviations from Hardy–Weinberg equilibrium (HWE) were assessed using a chi-squared statistical test. The association analysis was carried out by fitting a series of genetic models (additive, dominant, recessive, heterozygous advantage and general) to each SNP-phenotype data. The association strength of a given SNP in relation to the phenotype was measured by the minimum *P*-value across all tests performed for association. The AMA1, MSP1, MSP2 and CSP assay results were log10-transformed to obtain approximated Gaussian distributions. All association analyses were performed on each ethnic group separately. All analyses were performed using the R statistical software (http://www.r-project.org).

Results

Study population

The study enrolled 939 participants of which 53.8% were Dogon and 46.2% are Fulani (Table 1). Data from each ethnic group were suitably matched for age, gender and seasonal distribution (all *P*-values >0.05). We observed a significant difference in the frequency of the O blood group (known to be protective against malaria [27]), which is higher in the Fulani than Dogon ($P < 0.001$). This difference is in keeping with measures of infection rate, parasite density and parasite positivity, and clinical malaria, which were marginally higher in Dogon compared with Fulani. The proportion of persons with enlarged spleen was significantly ($P < 0.001$) higher in the Fulani (30.7%) than in the Dogon (8.8%) (see Table 1). Similar results were obtained after adjusting for age, gender and season (data not shown). The frequency of the sickle HbS-S allele was low in both ethnic groups (Dogon 1.8%, Fulani 1.0%), and the HbC-A allele was rare in the Fulani (0.6% versus Dogon 3.8%, $P < 0.0001$).

FcγRIIa R131H genotyping

All individual of the two study groups were genotyped for the G494A single-nucleotide polymorphism (R131H) in the fcγRIIa gene (rs1801274). There was no evidence of deviation from HWE in Dogon ($P = 0.719$) or Fulani ($P = 0.443$) ethnic groups at the polymorphism. As expected, allele frequencies at the rs1801274-R131H SNP were different between the ethnic groups (H frequency: Dogon 0.341, Fulani 0.474, $P < 0.0001$). While both ethnic groups showed a similar frequency of heterozygotes (131R/H), there was a statistical significant difference between them regarding the frequency of homozygotes (Table 1). The 131 R/R genotype was dominating among the Dogon, and the 131 H/H genotype was more common in the Fulani ($P < 0.0001$).

Total IgG antibodies to AMA 1, MSP1, MSP2 and CSP, and total IgE

As observed previously, all immunoassays showed greater median levels in the Fulani (Table 1, $P < 0.02$). Multivariate analysis of immunoassays adjusting for age, gender and season did not change this result (data not presented). There is a high correlation between AMA1, MSP1, MSP2 and CSP (Spearman's rho >0.3), consistent across both ethnic groups (Table S1).

FcγRIIa R131H and malariometric data

An association analysis between the malariometric data (spleen enlargement. parasite positive, fever, asymptomatic and clinical malaria) and rs1801274-R131H revealed a marginal reduced risk on parasite positivity in the Fulani (HH/RH versus RR OR 0.535, 95% CI 0.288–0.993, $P = 0.048$), but not in the Dogon group (OR 1.067, $P = 0.807$) (Table 2). In addition, there is some evidence of reduced risk of asymptomatic malaria in both Dogon (HH versus RH/RR OR, 0.361, 95% CI 0.133–0.981, $P = 0.046$) and Fulani (additive H OR 0.630, 95% CI 0.427–0.929, $P = 0.020$) (Table 2).

FcγRIIa R131H and distribution of antibodies

Comparing the effects of rs1801274-R131H genotypes on (log10) total IgE revealed an effect in the Fulani ethnic group (HH versus RH/RR, adjusted difference 0.129, 95% CI 0.043–0.215, $P = 0.004$), but not Dogon (HH versus RH/RR adjusted difference −0.080, $P = 0.236$) (Table 3 and Fig. 1). We compared the effects of genotypes on the other antibodies. The analysis of CSP was the only one where we obtained a significant result (Dogon HH versus

Table 1 Study characteristics by ethnic group.

	Dogon (n = 505, 53.8%)		Fulani (n = 434, 46.2%)		
	N (median)	% (range)	N (median)	% (Range)	P-value
Age (in months)	(204)	(24–744)	(168)	(24–900)	0.05
0–4 years old	64	12.6	65	15.0	
5–9 years old	85	16.8	78	18.0	0.10
10–15 years old	86	17.0	93	21.4	
>15 years old	270	53.5	198	45.6	
Male	219	43.4	192	44.2	0.84
Rainy season	332	65.7	262	60.4	0.10
ABO blood group O	195	43.5	237	56.5	$<10^{-3}$
Parasitological data					
Parasite positivity	108	21.6	71	16.5	0.06
Pf density	(0)	(0–3,034,000)	(0)	(0–684,400)	0.02
Spleen enlargement	44	8.7	133	30.6	$<10^{-6}$
Fever prevalence	68	13.5	38	8.8	0.03
Immunological data					
AMA1	(1078)	(0–72,770)	(1742)	(2–72,770)	10^{-3}
MSP1	(467)	(0–131,800)	(2063)	(19–356,900)	$<10^{-6}$
MSP2	(1427)	(0–777,500)	(3164)	(49–777,500)	$<10^{-6}$
CSP	(679)	(75–779,700)	(1338)	(0–1,387,000)	$<10^{-6}$
Total IgE	(1403)	(0–21,780)	(1662)	(171–28,960)	0.02
Malaria					
Clinical malaria	63	12.5	30	6.9	
Asymptomatic	91	18.0	79	18.2	
None	351	69.5	325	74.9	0.02
HbS					
AA genotype	446	(96.3)	420	(97.9)	
AS genotype	17	(3.7)	9	(2.1)	0.231
HbC					
GG genotype	341	(92.4)	333	(99.1)	
AG/AA genotypes	28	(7.6)	3	(0.9)	$<10^{-4}$
rs1801274-R131H genotype					
RR	204	(43.8)	123	(28.5)	
RH	206	(44.2)	207	(48.0)	
HH	56	(12.0)	101	(23.4)	$<10^{-6}$
H allele	159	(34.1)	204	(47.4)	$<10^{-4}$

Hyperparasitaemia: parasitemia density >10,000 parasites per microlitre; Parasite positivity: presence of one or more parasite per microlitre; Pf density: number of parasite per microlitre; Spleen enlargement: presence of spleen enlargement; P-value was calculated from a c^2 test for qualitative variables and a Mann–Whitney U-test for continuous variables.

Table 2 The H allele frequency by phenotype. Allele frequencies and association analysis.

	H allele frequency				Association analysis						
	Dogon (n = 505)		Fulani (n = 434)			Dogon			Fulani		
Phenotype	Control	Case	Control	Case	Genetic Model	OR	95% CI	P	OR	95% CI	P
Spleen enlargement	0.349	0.263	0.487	0.447	Additive H	0.672	0.397–1.138	0.139	0.855	0.634–1.155	0.308
Parasite positive	0.339	0.333	0.491	0.401	HH/RH versus RR	1.067	0.633–1.800	0.807	**0.535**	**0.288–0.993**	**0.048**
Fever	0.337	0.363	0.477	0.446	HH/HR versus RR	1.166	0.669–2.032	0.588	1.072[a]	0.492–2.335	0.861
Clinical malaria	0.344	0.385	0.495	0.450	HH/RH versus RR	1.269	0.655–2.460	0.479	0.653	0.270–1.576	0.343
Asymptomatic malaria	0.344	0.300	0.495	0.397	Additive H	0.361[b]	0.133–0.981	**0.046**	0.630	0.427–0.929	**0.020**
Any malaria	0.344	0.336	0.495	0.412	Additive H	0.984	0.706–1.370	0.922	0.686	0.485–0.971	**0.033**

OR, odds ratio; CI, confidence interval, adjusted for age group and season.
[a]OR RH versus other: 0.677 (0.336, 1.365) P = 0.276.
[b]Effect for HH versus other.
Bold values denotes very important results.

RR, adjusted CSP difference −0.298, 95% CI −0.543 to −0.054, $P = 0.017$) (see Table S2).

Discussion

Our study confirms that malaria risk is lower in the Fulani (compared with the Dogon) with observed lower parasite prevalence and density, fewer cases of malaria episodes and a higher prevalence of spleen enlargement. This result supports findings from the Gambia [3], Burkina Faso [4–6] and Sudan [28–30]. We also observed that the Fulani have a higher frequency of blood group O, which is known to be protective in malaria [27]. The Fulani group exhibits increased antibody production compared with the neighbouring ethnic group, also consistent with previous work [7–11]. In general, the lower susceptibility to *P. falciparum* malaria seen in the Fulani has not been fully explained by gene polymorphisms previously known to be associated with malaria resistance, including HbS, HbC, alpha-thalassaemia, G6PD deficiency and HLA [31].

In this study, we have focused on FcγRIIa, a low-affinity Fc receptor (FcR), and an important protein in the host defence against infection. FcRs belong to the family of immune receptors, which includes T cell receptors, B cell receptors and natural killer receptor [32]. The Dogon and Fulani exhibited different frequencies of FcγRIIaR131H (rs1801274-R131H) genotypes and alleles. In particular, the 131H/H genotype and H allele are more frequent in the Fulani (the most protected group to malaria), and the 131R/R genotype and R allele are more frequent in the Dogon (the most susceptible). This result confirms a smaller study from the same geographical area [10], as well as a Sudanese study comparing Fulani with non-Fulani ethnic groups [29, 30]. However, a study performed in neighbouring Burkina–Faso in Fulani and Mossi ethnic groups did not find the same result. They showed that the genotypes are equally distributed across the Fulani and Mossi [33]. This discrepancy between the Malian and Burkina Faso studies could be explained by the age distributions of the study participants. Our study included both children and adults, while in the Burkina study, only adults more than 20 years old were enrolled. Another explanation is the different malaria endemicities, with the Mali study area being meso-endemic and Burkina Faso's hyperendemic.

FcγRIIa has been studied directly in relation to malaria susceptibility with some studies showing a protective effect for the 131R/R genotype (R allele) and reducing the risk of high-density parasitaemia [34, 35]. Similarly, three other studies have demonstrated an increased risk with 131 H/H genotype for severe, cerebral or placental malaria [36–38]. Our data show a potentially marginal 46.5% protective effect of parasite positivity of the 131 R/R genotype in the Fulani group, while there was no significant effect in the Dogon. However, other studies have shown an opposite effect in malaria phenotypes. A Ghanaian study performed in a seasonal malaria transmission area, observed that the 131 R/R genotype was associated with susceptibility to severe malarial anaemia and cerebral malaria (severe malaria) [39]. These findings have been supported by another study from the same country [40]. A study performed in Sudan found that the 131 R/R genotype (R allele) was significantly associated with the odds of severe malarial disease when compared with that of mild malaria [28]. Some of the differences in the results of these studies may be due to study design, where some, like us, are comparing the effect between ethnic groups while others compare disease severity. All agree that this marker is associated with malaria and even though the FcγRIIa 131 polymorphism is known to be functional, the heterogeneity

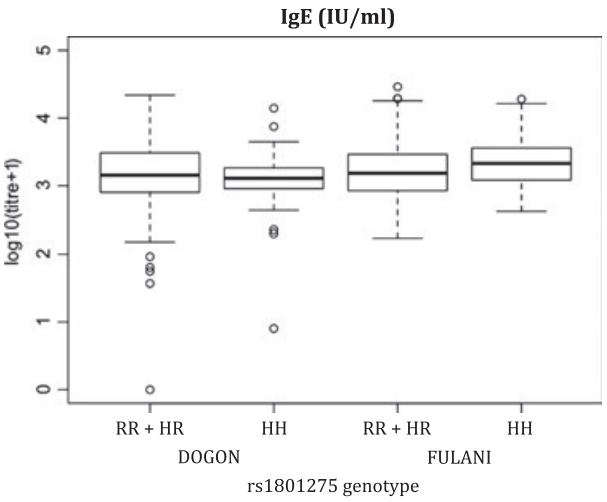

Figure 1 The distribution of total IgE by rs1801274-R131H genotypes.

Table 3 Association analysis between rs1801274-R131H and total IgE (after log10 transformation) in each ethnic group.

		Mean Log10 total IgE				
Population	*N*	HH genotype	RR/RH genotypes	Adjusted difference[a]	95% CI	*P*-value
Dogon	505	3.083	3.171	−0.080	−0.213 to 0.052	0.236
Fulani	434	3.343	3.213	0.129	0.043–0.215	0.004

[a]after adjusting for age group and season, further adjustment for blood group and asymptomatic malaria does not change the interpretation of the results.

of results suggests that there may be other polymorphisms within the gene that modify the response. Alternatively, as this receptor is responsive to different IgG subtypes, part of the heterogeneity may be down to what antibodies are being produced by individuals and they may also be under some level of genetic control.

It is well established that the asexual blood stages of malaria parasites are responsible for the pathology of the disease and that antibodies play an important role in malaria protection [41]. Cytophilic antibodies are thought to be instrumental in protective immunity. Several sero-epidemiological studies have indicated an association between protection from malarial infection or disease with antibodies of IgG1 and IgG3 subclasses [42, 43]. In this study, we have shown that Fulani have greater total IgG antibodies against AMA-1, MSP-1, MSP-2 and CSP antigens and also higher total IgE. In addition, we demonstrated that the 131 H/H is associated with increased total IgE levels in the Fulani ethnic group. In the same area, we have previously shown that the Fulani have higher levels of all IgG antimalarial subclasses except for IgG4, which was very low in both ethnic groups [10]. The 131 H/H genotype and the H allele have been shown to be associated with higher IgG1, IgG2 and IgG3 antibodies. IgG2 that is known to efficiently bind to the FcγRIIa 131H allele and has been suggest to be considered as cytophilic in this setting [10, 44]. Another study has found evidence of increased antibody (IgG1 and IgG3) production in a non-Fulani ethnic group [28]. This discrepancy could be due to the transmission period or the immune status of the subjects at the time of enrolment. At the same time, this study [28] found raised levels of IgG2 in Fulani associated with the FcγRIIa131HH genotype, as in a previous study in Mali [10]. A case–control study performed in India found that the high-affinity IgG2-binding 131 H/H genotype was associated with protection from malaria [45]. In a longitudinal study of the Fulani population resident in eastern Sudan, it has been shown that the FcγRIIa (CD32), HbAS polymorphisms as well as GM and KM allotypes of IgG differ significantly between the Fulani and the others ethnic group [28, 46, 47]. On the basis on these observations, it has been hypothesized that the FcγRIIa genotype and IgG subclass pattern may contribute to the interethnic differences in the malaria susceptibility observed in Fulani and non-Fulani.

The Fulani showed higher levels of antimalaria IgG1, IgG2 and IgG3 antibodies, while IgG4 level was low and similar in both ethnic group [10]. In the same area and under similar conditions, another smaller study comparing the impact of IL-4-590C/T transition on the levels of *P. falciparum*-specific IgE, IgG, and IgG subclasses and total IgE have shown significant higher antibody levels in the Fulani than in the Dogon ethnic group [11]. Here also, IgG4 antibody level was low and similar in both ethnic groups [11].

Some studies have looked at the FcγRIIa131 polymorphism in relation to pregnancy in malaria. For example, a recent study performed in Saudi Arabia has shown that the 131 H/H genotype and H allele are associated with protection in malaria-free controls, compared with asymptomatic malaria infection in pregnant women who showed an increase in the RR genotype and R allele [48]. Collecting such information in our setting would allow us to see whether this effect could be replicated in both our ethnic groups, which would help in implementing and monitoring malaria research strategies in endemic areas.

Conclusion

This study confirms that the Fulani ethnic group compared with the Dogon is less susceptible to malaria. Our work extends the previous finding that the FcγRIIa HH131 genotype and the H131 allele are more prevalent in the Fulani compared with the Dogon. We find that the FcγRIIa polymorphism is associated with differential IgE response and reduced risk of mild malaria in the Fulani ethnic group. We suggest that this polymorphism should be investigated in follow-up studies in Mali, as well as in other ethnic groups in alternative settings, to confirm our findings.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1 Spearman correlations between antibody titres.

Table S2 Association between rs1801274-R231H genotypes and different antibody levels.