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BRIEF COMMUNICATION

Erythrocyte CR1 expression level does not correlate with a HindIII restriction fragment length polymorphism in Africans; implications for studies on malaria susceptibility

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Complement receptor 1 (CR1) expression level on erythrocytes is genetically determined, and in Caucasian populations is linked to high (H) and low (L) expression alleles identified by a HindIII restriction fragment length polymorphism (RFLP). Erythrocyte CR1 may be an important factor in determining malaria susceptibility, as low expression of CR1 reduces the rosetting of uninfected erythrocytes with Plasmodium falciparum-infected cells, a process that contributes to malaria pathogenesis. Prior to studying CR1 expression and malaria susceptibility, we have investigated whether the quantity of erythrocyte CR1 correlates with the H and L alleles in an African population. Mean erythrocyte CR1 in 149 Malian adults was 415 molecules per cell, which is comparable to Caucasian populations; however, there was no relationship between erythrocyte CR1 level and genotype for the HindIII RFLP (mean CR1 per erythrocyte HH = 414, HL = 419 and LL = 403, P > 0.1, Student's t-test). The conclusions of a previous study of erythrocyte CR1 expression level and malaria susceptibility in West Africa that was based on HindIII RFLP genotyping may therefore need to be re-evaluated.
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Complement receptor 1 (CR1) on erythrocytes plays an important role in the clearance of immune complexes and the control of complement activation.[1] Erythrocyte CR1 is also important in malaria, as *Plasmodium falciparum*-infected erythrocytes bind to CR1 on uninfected erythrocytes to form clumps of cells known as rosettes.[2] Rosetting is a parasite adhesion phenotype associated with severe malaria in African children,[3,4] and is thought to contribute to malaria pathogenesis by causing obstruction to blood flow in the microvasculature[5] or by enhancing parasite growth leading to high parasitaemias *in vivo*.[6] We have shown previously that erythrocytes with low expression of CR1 (less than 100 molecules per cell) show greatly reduced rosetting, and we have hypothesised that CR1 polymorphisms affecting interaction with *P. falciparum*-infected erythrocytes may influence susceptibility to severe malaria.[2]

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In Caucasian populations, erythrocyte CR1 levels vary between individuals in the range of about 100–1000 molecules per cell.[7] An individual's erythrocyte CR1 level is a stable phenotype that is genetically determined and correlates with a *Hin*dIII RFLP of the CR1 gene.[8] Homozygotes for a 7.4 kilobase (kb) *Hin*dIII genomic fragment (the H allele) have high erythrocyte CR1, whereas homozygotes for a 6.9 kb *Hin*dIII genomic fragment (the L allele) have low expression, with HL heterozygotes having intermediate CR1 levels.[8,9] The *Hin*dIII RFLP is due to a point mutation in intron 27 of the CR1 gene[10], and is linked in a haplotype with several other point mutations within the CR1 coding sequence.[11] It remains unknown as to which, if any, of these polymorphisms is functionally important in determining the expression level of erythrocyte CR1. One of the point mutations linked to the *Hin*dIII RFLP is a C to G substitution in exon 33 of the CR1 coding sequence that gives a *Mnl*I RFLP.[9,11] This point mutation results in a proline (P) to arginine (A) substitution that introduces a new tryptic protease cleavage site that could reduce CR1 stability and therefore lead to low erythrocyte CR1 levels.[9] In Caucasians, the *Mnl*I and *Hin*dIII point mutations are in linkage disequilibrium, with high/low erythrocyte CR1 expression being linked to the PP/AA *Mnl*I genotypes in addition to the HH/LL *Hin*dIII genotypes.[9]

Although the correlation between erythrocyte CR1 level and H and L alleles has been demonstrated in

several populations in Europe, North America and Asia,[9,10,12] an initial study in African-Americans showed no correlation between CR1 expression level and the H and L alleles[9]. There are no reports to date of the correlation between erythrocyte CR1 expression levels and H and L alleles in any purely African population. Therefore, in preparation for studies on erythrocyte CR1 expression levels and susceptibility to malaria in Mali, we set out to study the relationship between the *Hin*dIII RFLP and erythrocyte CR1 expression level. We also studied the *Mnl*I RFLP because of the suggestion outlined above that this polymorphism may be functionally important in determining erythrocyte CR1 expression levels.

DNA samples were collected from 59 African-American adults from Houston, Texas, and 149 adults from Mali, West Africa. The samples were analysed for the *Hin*dIII and *Mnl*I RFLPs by PCR and restriction digest, and an example of the results obtained for each genotype is shown in Figure 1. Erythrocyte CR1 levels were measured on fresh blood samples from each individual and the relationship between CR1 level and genotype was assessed. As shown in Figures 2 and 3 the erythrocyte CR1 expression level varied between 100 and 1000 molecules per cell in different individuals as described in other populations. In the African-American population, the mean CR1 level of HH homozygotes for the *Hin*dIII RFLP was significantly higher than the mean CR1 level of HL heterozygotes (Figure 2a, Student's *t*-test, $P<0.01$). The same was also true of PP homozygotes for the *Mnl*I RFLP, who had significantly higher CR1 levels than PA heterozygotes (Figure 2b, Student's *t*-test, $P<0.01$). There were no statistically significant differences between mean CR1 levels of LL and HH ($P=0.2$) and LL and HL individuals ($P=0.7$). No homozygous AA individuals for the *Mnl*I RFLP were detected. The *Mnl*1 genotype matched the *Hin*dIII genotype in 79% of the cases. These data suggest that there is some association between the *Hin*dIII and *Mnl*I RFLPs and erythrocyte CR1 expression level in this African-American population; however, as pointed out by Herrera *et al*,[9] this could be due to the relatively large component of Caucasian genes in the population, estimated to be approximately 25%.[13] To examine further the relationship between genotype and erythrocyte CR1 expression levels in Africans, we therefore proceeded to study an indigenous African population from Mali in West Africa.

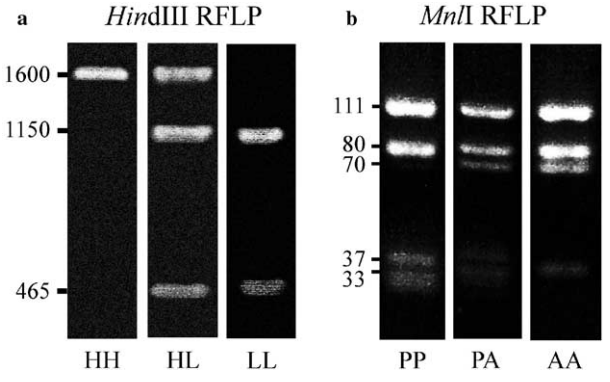

Figure 1 *Hin*dIII and *Mnl*1 RFLP genotyping. Blood samples were collected from adult volunteers after informed consent and all protocols were approved by institutional review boards in Mali and the USA. DNA was extracted using a commercial reagent (Genepure®, Gentra, Minneapolis, MN, USA) and the *Hin*dIII (**a**) and *Mnl*I (**b**) RFLPs were assessed by PCR and restriction digest using primers and conditions described previously.[11] *Hin*dIII RFLP products were electrophoresed on a 1.5% agarose gel and *Mnl*I products on a 20% polyacrylamide gel (Invitrogen, The Netherlands). An example of each genotype is shown and the fragment sizes obtained are indicated in base pairs (bp). For the *Hin*dIII RFLP fragment sizes are HH 1600 bp; HL 1600 bp, 1150 bp and 465 bp; LL 1150 bp and 465 bp. For the *Mnl*I RFLP fragment sizes are HH 111 bp, 80 bp, 37 bp and 33 bp; HL 111 bp, 80 bp, 70 bp, 37 bp and 33 bp; LL 111 bp, 80 bp, 70 bp, and 33 bp. An 11 bp fragment present in all *Mnl*I genotypes is not visible.

Erythrocyte CR1 levels and *Hin*dIII and *Mnl*I genotypes were determined for 149 adults from four locations in Mali populated by different ethnic groups: Bamako (mixed ethnicity, $n=22$), Tienequebougou (Bambaran, $n=55$), Bancoumana (Malinke, $n=47$) and Bandiagara (Dogon, $n=25$). The mean CR1 level in Bancoumana (458 molecules per cell) was significantly higher than the mean CR1 level in Tienequebougou (390 molecules per cell, $P<0.05$, Student's *t*-test). The mean CR1 levels in Bamako (387 molecules per cell) and Bandiagara (390 molecules per cell) were not significantly different from the mean CR1 level in any of the other locations. The relationship between erythrocyte CR1 level and genotype was assessed for each location separately and for all the Malian samples grouped together. The mean CR1 expression level did not differ between individuals genotyped as HH, HL and LL for the *Hin*dIII RFLP for any of the locations (data not shown) or for the samples grouped together (Figure 3a, Student's *t*-test, $P>0.8$ for each comparison). The gene frequency of the L allele in Mali (L 0.14, H 0.86) was slightly lower than has been described in other populations,[14] but is similar to that of the Houston African-American population (L 0.15, H 0.85). We also found no statistically significant difference in the mean erythrocyte CR1 level of individuals with different *Mnl*I RFLP genotypes (Figure 3B, Student's *t*-test, $P>0.1$). The *Mnl*I genotype was identical to the *Hin*dIII genotype in 71% of the cases, indicating that these two RFLPs are not as tightly linked in this African population as they are in Caucasians.[11]

Previous work has suggested that erythrocyte CR1 levels may be modified in malaria-infected children[15]. therefore, we examined Giemsa-stained blood smears from the Malian adults to determine if *P. falciparum* infection could be influencing the results seen. Twelve adults had low level *P. falciparum* parasitaemia (between 125 and 6125 parasites per microlitre of blood), although none of the infected individuals had clinical malaria at the time of sampling. The mean CR1 level of the 12 parasitaemic adults was 394 molecules per cell, which was not significantly different from the non-parasitaemic individuals (mean CR1 level 414 molecules per cell, $P>0.7$, Student's *t*-test). These data do not support the hypothesis that erythrocyte CR1 levels are reduced during *P. falciparum* infection,[15] however it is possible that such an effect might only be seen at higher parasitaemias.

The above data show that erythrocyte CR1 expression level does not correlate with H and L (*Hin*dIII RFLP) or P and A (*Mnl*I RFLP) allele status in an African population, and emphasise the need to assess the relationship between genotype and erythrocyte CR1

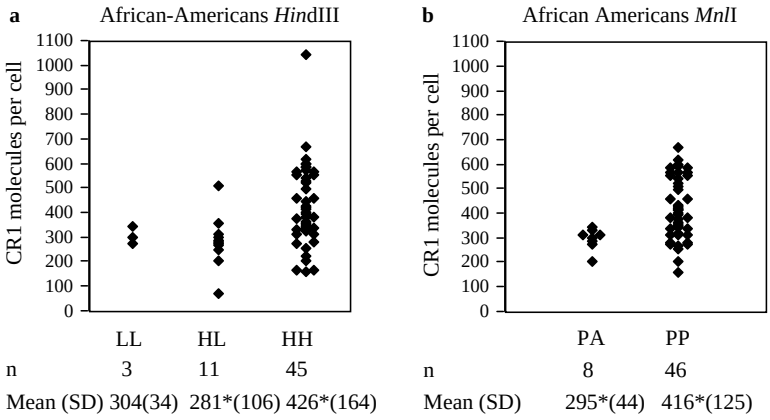

Figure 2 Erythrocyte CR1 expression level and genotype in African-American adults. (**a**) *Hin*dIII RFLP and (**b**) *Mnl*1 RFLP. The mean number of CR1 molecules per cell for each individual was assessed by ELISA, with comparison to known standards[7] and the genotype determined as described in Figure 1. Each point on the graph represents one individual. Five individuals were not assessed for *Mnl*I genotype due to insufficient DNA. Mean CR1 levels for each genotype were compared by Student's *t*-test and statistically significantly differences are indicated (*$P<0.01$).

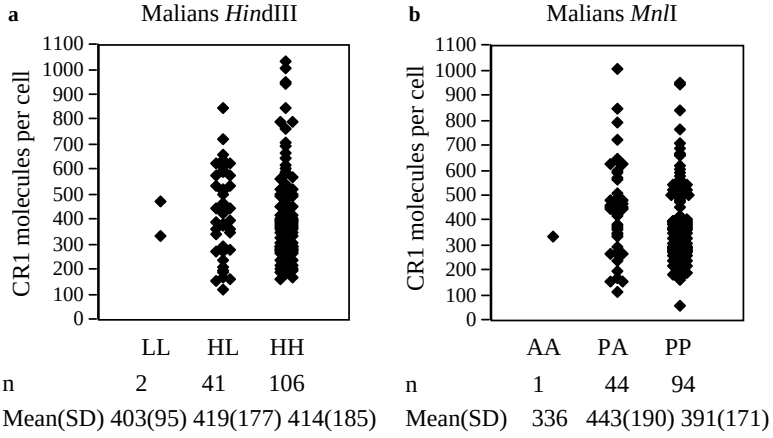

Figure 3 Erythrocyte CR1 expression level and genotype in Malian adults. (**a**) *Hin*dIII RFLP and (**b**) *Mnl*1 RFLP. The mean number of CR1 molecules per cell for each individual was assessed by ELISA, with comparison to known standards[7] and the genotype determined as described in Figure 1. Each point on the graph represents one individual. Ten individuals were not assessed for *Mnl*I genotype due to insufficient DNA. Mean CR1 levels for each genotype were compared by Student's *t*-test and were not statistically significantly different ($P>0.1$).

level in every new population studied. In the light of these results, the conclusions of one previous study that low expression of CR1 does not influence susceptibility to severe malaria in Gambia,[16] may need to be re-evaluated. Bellamy *et al*[16] used the *Hin*dIII RFLP as an indicator of CR1 expression level without determining whether this polymorphism does reflect the quantity of CR1 on erythrocytes in the Gambian population. Future studies on erythrocyte CR1 expression level and malaria susceptibility should either measure the number of CR1 molecules on erythrocytes directly, or require validation that any genetic marker used correlates with CR1 expression level in the population under investigation.

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