Limited antigenic diversity of *Plasmodium falciparum* apical membrane antigen 1 supports the development of effective multi-allele vaccines

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**Abstract**

**Background:** Polymorphism in antigens is a common mechanism for immune evasion used by many important pathogens, and presents major challenges in vaccine development. In malaria, many key immune targets and vaccine candidates show substantial polymorphism. However, knowledge on antigenic diversity of key antigens, the impact of polymorphism on potential vaccine escape, and how sequence polymorphism relates to antigenic differences is very limited, yet crucial for vaccine development. *Plasmodium falciparum* apical membrane antigen 1 (AMA1) is an important target of naturally-acquired antibodies in malaria immunity and a leading vaccine candidate. However, AMA1 has extensive allelic diversity with more than 60 polymorphic amino acid residues and more than 200 haplotypes in a single population. Therefore, AMA1 serves as an excellent model to assess antigenic diversity in malaria vaccine antigens and the feasibility of multi-allele vaccine approaches. While most previous research has focused on sequence diversity and antibody responses in laboratory animals, little has been done on the cross-reactivity of human antibodies.

**Methods:** We aimed to determine the extent of antigenic diversity of AMA1, defined by reactivity with human antibodies, and to aid the identification of specific alleles for potential inclusion in a multi-allele vaccine. We developed an approach using a multiple-antigen-competition enzyme-linked immunosorbent assay (ELISA) to examine cross-reactivity of naturally-acquired antibodies in Papua New Guinea and Kenya, and related this to differences in AMA1 sequence.

**Results:** We found that adults had greater cross-reactivity of antibodies than children, although the patterns of cross-reactivity to alleles were the same. Patterns of antibody cross-reactivity were very similar between populations (Papua New Guinea and Kenya), and over time. Further, our results show that antigenic diversity of AMA1 alleles is surprisingly restricted, despite extensive sequence polymorphism. Our findings suggest that a combination of three different alleles, if selected appropriately, may be sufficient to cover the majority of antigenic diversity in polymorphic AMA1 antigens. Antigenic properties were not strongly related to existing haplotype groupings based on sequence analysis.

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Background
Malaria continues to have a profound impact on the health of children and adults around the world, causing approximately 219 million clinical cases and 660,000 deaths per year [1]. Even though the scale-up of malaria-control interventions has resulted in a notable reduction in morbidity and mortality over the last decade, drug resistance remains a significant concern, and a cost-effective vaccine could play a major role in control and eventual elimination of the disease.

Understanding the mechanisms by which naturally-acquired immunity to malaria protects against death and severe disease is important for informing the rational design and development of effective vaccines. Antibodies constitute a major component of naturally-acquired immunity [2-4]. The merozoite form of the parasite, which invades red blood cells, expresses antigens that are prominent antibody targets [5]. Merozoite antigens are attractive vaccine targets because antibodies to these antigens inhibit red blood cell invasion and promote opsonic phagocytosis and antibody-dependent cellular inhibition that limit blood stage replication and prevent disease [6-9]. Furthermore, antibodies to merozoite antigens are associated with protection from malaria [10], and several merozoite antigen-based vaccines have shown protective efficacy in animal models [6,11]. A major challenge in developing merozoite-based vaccines, and other vaccines based on antigens that are targets of natural immunity, is overcoming potential antigenic diversity. Most major immune targets, and many vaccine candidates, show substantial polymorphism in sequence that have evolved to facilitate immune evasion. Vaccine approaches are needed to account for this polymorphism such that they will cover the majority of strains causing infection and disease. Although sequence polymorphism has been described for many antigens, knowledge is very limited on the extent of antigenic diversity (defined by antigen reactivity to human antibodies) and how polymorphisms relate to antigenic diversity for most leading candidate antigens, yet this is crucial for advancing vaccine development.

There are more than 40 different merozoite antigens on the surface or in the apical organelles of merozoites, few of which have been investigated as immune targets [5,6,10]. One important target, and leading vaccine candidate, is apical membrane antigen 1 (AMA1), which plays an essential role in erythrocyte invasion [12]. Antibodies to AMA1 are highly prevalent in malaria-exposed individuals and their prevalence increases with age as naturally-acquired immunity develops [13]. Antibodies to AMA1 have been associated with reduced risk of clinical malaria in prospective studies [14-16] and in vitro data indicate that AMA1 antibodies can inhibit parasite invasion of erythrocytes [17-19]. AMA1 is a promising blood stage vaccine candidate which is presently being tested in clinical trials. A recent phase II trial of a monovalent AMA1 vaccine in one- to six-year-old children in Mali showed 65% strain specific efficacy [20]. However, AMA1 is a highly polymorphic protein with more than 60 polymorphic sites and more than 200 haplotypes per population [21,22], one of the most polymorphic of all merozoite antigens. Immunization with one allele of AMA1 may not protect against parasites expressing different AMA1 alleles, as highlighted by the Mali trial; there was no overall protection against clinical malaria, but there was evidence of protection against malaria caused by vaccine-like alleles [20]. While sequence analysis has been used to classify AMA1 alleles into related groups that might show cross-reactive immunity, the antigenic diversity of AMA1 and cross-reactivity of antibodies are poorly understood, and it is unclear how sequence polymorphisms and sequence-based groupings relate to antigenic diversity and escape from acquired human antibodies. There are only limited data on antigenic diversity in human studies and limited data to understand how sequence diversity is related to antigenic diversity, which is an impediment to vaccine development. Understanding these issues is essential for advancing AMA1-based vaccines. AMA1 also serves as an ideal model to examine antigenic diversity more broadly, the significance of polymorphism in vaccine development and the feasibility of developing multi-allele vaccines based on polymorphic antigens.

We sought to define antigenic diversity of AMA1 and use this knowledge to understand which AMA1 alleles could be included in a multi-allele vaccine to achieve the broadest coverage of AMA1 diversity, and establish principles that could be applied to other polymorphic vaccine antigens. Antibody reactivity to various geographically-diverse AMA1 alleles was examined among children of
different ages and adults from two geographically diverse malaria endemic regions (Papua New Guinea and Kenya). We examined the relationship between antigenic diversity and sequence diversity, and sought to establish whether overall antigenic diversity of AMA1 is limited and might ultimately be reduced to a small number of major serotypes. To achieve these objectives, we developed a novel approach that we named multiple antigen competition enzyme-linked immunosorbent assay (ELISA) (MACE) that can also be used to define antigenic diversity of other polymorphic antigens.

Methods

Study cohorts and sample collection

Serum samples were collected from a cross-sectional study in Madang Province, Papua New Guinea (PNG) in 2007, and included 118 individuals: 49 adults (median age 28 years) and 69 children (median age 6 years). From these samples we prepared pools of AMA1 antibody positive samples for testing in competition ELISAs; one pool was made from children positive samples for testing in competition ELISAs; one these samples we prepared pools of AMA1 antibody positive samples for testing in competition ELISAs; one pool was made from children’s samples (n = 31; median age 7 years (range 4 to 10)) and one from adult samples (n = 42; median age 28 years (range 16 to 53)). To prepare the pools, all sera were first tested in standard ELISA for immunoglobulin G (IgG) reactivity against five different recombinant AMA1 alleles (3D7, W2mef, FVO, 7G8, and HB3). After screening, we excluded antibody negative samples and samples with low antibody reactivity (defined as below the 25th centile). All samples included in the pools were antibody positive to all five AMA1 alleles, and antibody reactivity to different alleles was highly correlated (>0.9 for all comparisons), as we have found previously in this population [16]. In preparing pools, an equal volume of all individual samples was used, and substantial numbers of samples were included in pools to account for variation in antibody levels and cross-reactivity among individual samples.

Samples were also obtained from an extended longitudinal study in Kilifi, Kenya (Ngerenya cohort). More than 300 children were included in the original Kenyan cohort [23]; in our study we examined serum from a subset of 42 children who were positive for AMA1 antibody responses and were present for screening and sample collection at two different time points, October 2002 and October 2004. From these samples we prepared a pool of AMA1 antibody positive samples for testing in competition ELISAs using samples collected from the same children at the two different times. To select samples for inclusion in the pools, individual samples were first screened for IgG reactivity to threeAMA1 alleles (3D7, W2mef and HB3) in standard ELISA. The levels and proportion of positive samples were lower than for the PNG samples. For preparation of pools we selected the top quartile of responders to AMA1-3D7; however, antibodies to different AMA1 alleles were highly correlated (correlation co-efficient 0.77 to 0.95) and all individuals had antibody reactivity greater than the group median for all AMA1 alleles. The median age (range) of children in the sample pools was 6.5 years (2.1 to 7.6) for October 2002, and 8.3 years (4.2 to 9.8) for October 2004. Sera from unexposed Australian blood donors donated by the Red Cross Blood Bank were used as negative controls in ELISAs and antibody positive samples were defined as those with reactivity greater than the median + three standard deviations (SD) of the Australian controls. Ethical approval was granted by the Kenya National Research Ethics Committee, the Medical Research Advisory Council PNG, the Walter and Eliza Hall Institute, and Alfred Hospital Human Research and Ethics Committees. Written informed consent was obtained from all participants or their guardians.

AMA1 alleles

Eleven alleles of the Plasmodium falciparum AMA1 antigen were used in this study (3D7, D10, W2mef, 7G8, FVO, HB3, XIE, Pf2004, Pf2006, M24, 102-1). The origins of P. falciparum isolates expressing the selected AMA1 alleles are listed in Table 1. Alleles were chosen to represent the broad genetic diversity of AMA1 [24], based on published P. falciparum AMA1 sequences (available from the Protein Data Bank). Sequence alignment of the 11 alleles identified 52 polymorphic amino acid (aa) positions: 7 in the prodomain, 28 in DI, 8 in DII and 9 in DIII [see Additional file 1: Figure S1]. The number of sequence differences between any two alleles ranged from 8 to 27 (see Additional file 1: Figure S2).

Preparation of recombinant AMA1

Recombinant AMA1 proteins used in this study were expressed, purified and refolded using established protocols

| Table 1 | P. falciparum AMA1 alleles selected for this study |
|---------|-----------------------------------------------|
| Isolate | Origin                                      | Reference |
| 3D7     | Amsterdam Airport, origin unknown            | [25]      |
| D10     | Papua New Guinea                             | [26]      |
| W2Mef   | Southeast Asia                               | [27]      |
| 7G8     | Peru                                         | [28]      |
| FVO     | Vietnam                                      | [29]      |
| HB3     | Honduras                                     | [30]      |
| XIE     | PNG                                          | [31]      |
| Pf2004  | Ghana                                        | [32]      |
| Pf2006  | Ghana                                        | [32]      |
| M24     | Kenya                                        | [33,34]   |
| 102-1   | Sudan                                        | [35]      |

AMA1, apical membrane antigen 1.
[19,24,36]. Nucleotide sequences were amplified from genomic DNA using Pfu DNA polymerase and oligonucleotide primers. The amplified products were digested with BamHI and Xho1, and ligated into pProEXHT-B 6xHis, and transformed into *Escherichia coli* strain BL21. (Proteins were solubilized in 6 M guanidine-HCL, which completely denatures the recombinant proteins). After purification on nickel resin, AMA1 protein was refolded with reduced and oxidized glutathione redox pairs. Refolded AMA1 was further purified by anion exchange chromatography, followed by reversed-phase, high performance liquid chromatography (RP-HPLC). Refolded AMA1 was identified by a shift in the monomer peak on RP-HPLC and a migration shift on SDS-PAGE when compared to a reduced sample of the refolded AMA1 preparation. Full details of preparation can be found elsewhere [19].

**Competition ELISAs**

Standard ELISAs to measure IgG to recombinant AMA1 were performed using established methods. Serum samples were tested in single and multiple antigen competition ELISA (MACE) against 11 AMA1 alleles. A novel method, MACE, was developed by modifying the conventional competition ELISA assay to allow cross-reactivity among several alleles of one protein to be examined. In a conventional competition ELISA assay, human antibodies are pre-incubated with a single competing antigen before adding to wells coated with a different allelic variant of the same antigen and proceeding as for a standard ELISA. In MACE, human antibodies are pre-incubated with combinations of up to four competing heterologous recombinant alleles. A total of 75 different combinations were used for competition. The level of inhibition in the presence of one or more competitor alleles indicates the degree of cross-reactivity with the tested allele used to coat the wells [see Additional file 1: Figure S4].

ELISAs were carried out using 96-well polystyrene Maxisorp plates (Nunc, Roskilde, Denmark) coated with one of eleven recombinant AMA-1 proteins at a concentration of 0.5 μg/ml in phosphate-buffered saline (PBS) and were incubated overnight at 4°C. Plates were blocked using 0.1% casein in PBS-tween 0.05%. Plasma was diluted to a concentration of 1:1,000 and one or combinations of several competing recombinant proteins (AMA-1 variants) were added in excess, each at 5 μg/ml. Prior to performing competition ELISAs, we optimized the conditions of our assays to ensure that antibody reactivity with pools was below the saturation point and the serum concentration gave an Optical Density (OD) reading at 405 nm that was in the linear part of a titration curve showing the relationship between antibody concentration and OD (for example, see [37]). Optimization included titration of serum dilutions and antigen coating concentration, and optimization of secondary antibody concentrations and incubation times for the substrate. Additionally, we titrated the concentration of the competitor antigen to determine the concentration required to fully saturate antibody binding and achieve maximal inhibition. A concentration 5 μg/ml of competition antigen was well above the saturation threshold and was used in all competition ELISAs [see Additional file 1: Figure S3]. For each of the 11 AMA-1 variants used as coating antigen, the homologous allele and the heterologous recombinant AMA-1 antigen combinations were added as competitor. The homologous competitor served as internal control for competition [see Additional file 1: Figure S4]. The plasma/competitor mix was allowed to pre-incubate at room temperature for 30 minutes and then added to the plate (following washing) for 2 hours. Incubations were done using 50 μl volume per well in duplicate and washes between each incubation were carried out using PBS-tween 0.05%. All incubations occurred at room temperature. To determine total IgG to plate-bound antibodies, horseradish peroxide (HRP)-conjugated sheep anti-human IgG (Millipore, North Ryde, Australia) at 1 in 5,000 was used and allowed to incubate for one hour. ABTS (2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid)) substrate (Sigma-Aldrich, Castle Hill, Australia) was added to develop color and the reaction was stopped using 1% sodium dodecyl sulfate (SDS) after 20 minutes in the dark. The optical density of each sample was measured at 405 nm (ABTS) using a plate reader (Thermo Fisher Scientific, Scoresby, Victoria, Australia). The mean of duplicate wells was calculated and background (wells coated with antigen and incubated with PBS plus competitor) was subtracted for each sample (using ScanIt MulticanPro software). Results on plates were standardized to adjust for plate-to-plate variance using positive control pools on each plate tested [16], and the mean plus three standard deviations of the OD for Australian negative control sera was used as the cut-off for seropositivity.

**Data analysis**

Cross-reactivity was defined as degree of inhibition of antibody reactivity by one or more competitor. Standardized OD values were used for calculating the proportion of AMA1 specific reactivity to the coating AMA1 allele that was competed by the heterologous competitor(s) ([heterologous competition – homologous competition]/(no competition – homologous competition) × 100) [see Additional file 1: Figure S4]. Clustal-Web 2.1 was used for amino acid sequence alignments. Statistical analysis was performed using GraphPad Prism 5 (Graphpad Software). Correlations between cross-reactivity and sequence polymorphisms between AMA1 alleles were calculated using Spearman’s rank correlation.
Results

Antibody cross-reactivity to different AMA1 alleles

Competition ELISAs with human antibodies were used to examine antigenic diversity of AMA1 and investigate whether strain-specificity of human antibody responses to AMA1 could potentially be overcome with the inclusion of a limited number of alleles in a multi-allele vaccine. Competition ELISAs were required because measuring antibodies to different AMA1 alleles by standard ELISA does not sufficiently discriminate the levels of cross-reactivity and allele-specificity of antibodies. AMA1 alleles of diverse geographical origins were selected (Table 1). Phylogenetic tree analysis has shown previously that these alleles broadly represent global AMA1 diversity [24]. In order to efficiently test the many antigen competition comparisons in this study, we prepared a pool of serum samples from PNG children (n = 31; median age 7 years) and a pool of adult samples (n = 42; median age 28 years) selecting those who were antibody positive after initial screening for reactivity to AMA1 in standard ELISA. We confirmed the use of the pools as an appropriate strategy by demonstrating that competition ELISA results using pools revealed very similar patterns and levels of antibody cross-reactivity as those obtained when testing all samples individually [see Additional file 1: Figure S5]. We have previously reported the use of pools to measure other anti-malarial antibodies [38].

Initially, we investigated the degree to which AMA1 antibodies were strain-specific or could cross-react with other alleles by testing each allele against the 10 other AMA1 alleles in standard single-antigen competition ELISAs using sera from PNG children. Using competition ELISA, we found that all 11 alleles were cross-reactive at least to some extent with all other alleles (Figure 1). There was considerable variation in the extent of human antibody cross-reactivity for different alleles, and the pattern of cross-reactivity appeared distinct for each allele. AMA1-W2mef antibodies, for instance, were highly cross-reactive with the allele 7G8 (76% cross-reactivity), but showed only limited cross-reactivity with other alleles including 3D7, HB3, D10, 2004 and 2006 (range: 25% to 30%, Figure 1A). Antibodies to AMA1-FVO showed a high degree of cross-reactivity overall, with cross-reactivity higher than 50% to W2mef, HB3, D10, XIE and 2006 alleles, greater than 70% to 7G8 and M24, and 82% to 102-1 (Figure 1C). In contrast cross-reactivity of antibodies to the 102-1 allele was rather low, with 50% or less cross-reactivity to most variants, but high cross-reactivity towards W2mef (78%) and 7G8 (88%, Figure 1G). Antibodies to Pf2004 were highly strain-specific, with less than 50% cross-reactivity to all other alleles (Figure 1J). Interestingly, some alleles showed very high cross-reactivity with specific variants, which was as high as 100% (as seen with 3D7 antibodies towards the D10 allele).

Greater cross-reactivity of antibodies among adults compared to children

To assess whether the levels and patterns of antibody cross-reactivity changed over time or with increasing exposure, cross-reactivity of antibodies was compared between pools of serum from adults (n = 42) and children (n = 31) in PNG. For these comparisons we used five representative alleles that had significant differences in sequence: 3D7, HB3, FVO, W2mef, and HB3. Cross-reactivity to all alleles was slightly lower among children compared to adults (P <0.01) (Figure 2). However, it was notable that the pattern of cross-reactivity of antibodies to different alleles was very similar among children and adults. The idea that antibody cross-reactivity increases with increasing age and exposure has been previously suggested, but these are the first data to clearly demonstrate this effect.

Patterns of cross-reactivity are similar over time and between different populations

We compared the patterns of antibody cross-reactivity to AMA1 among PNG children and Kenyan children. The extended longitudinal nature of the Kenyan cohort allowed us to also assess whether the patterns of antibody cross-reactivity change over time. We prepared pools of samples from children (who were positive for AMA1 antibodies) at two collection time points from...
Figure 1 (See legend on next page.)
the Kenyan cohort, October 2002 and October 2004 (n = 42 samples each point; median age 6.5 for 2002 and 8.3 for 2004; same children’s samples in each pool). In the Kenyan cohort cross-reactivity was compared in serum samples collected in October 2002 and October 2004 (Figure 3). The pattern of cross-reactivity of antibodies to different alleles remained the same over time. AMA1 antibody cross-reactivity tended to be higher at the later time-point (P <0.05), but the absolute difference was small (Figure 3A-E). This suggests that significant time and exposure may be required for the development of higher levels of cross-reactivity, such as that seen in adults. Comparing PNG children and adults, or Kenyan children followed longitudinally, the pattern of cross-reactivity remained constant, despite the trend towards an overall increase in cross-reactivity with age. For example, antibodies to W2mef AMA1 showed greatest cross-reactivity to the 7G8 allele, less to FVO and least to 3D7 and HB3, and this pattern was maintained in children and adults (Figure 2A) and when comparing the same children over time (Figure 3A). Patterns of cross-reactivity were similarly maintained for all AMA1 alleles (Figures 1 and 3).

Remarkably, we found that patterns of cross-reactivity among the two geographically distinct populations (PNG and Kenya) were also very similar (Figure 4). Overall, cross-reactivity of antibodies to the five tested alleles was somewhat higher in Kenyan children than in PNG children.
children, but the patterns were comparable. This suggests a similar degree of antigenic diversity of AMA1 in each location, and possibly similar exposure to different alleles, and indicates that sequence or structural differences are the primary determinant of antigenic differences between AMA1 alleles, rather than other population or exposure-specific factors.

### Multiple antigen competition ELISAs

To further define antigenic diversity and relatedness of AMA1 alleles, we developed a novel approach of competition ELISAs with combinations of several competitor AMA1 alleles, which we term MACE. This method allowed us to understand the combined capacity of cross-reactivity of antibodies to two or more AMA1 alleles. Initially we focused on five alleles that differed in sequence (3D7, FVO, HB3, W2mef and 7G8), using combinations of two, three or four alleles as competitors, with PNG children. Combinations were not chosen with regard to their sequence relatedness, instead all possible combinations were tested in order to determine antigenic differences and overlap, and identify which combinations could provide the maximum coverage of AMA1 diversity.

A high degree of cross-reactivity achieved by a certain combination was taken to indicate that those alleles each had a different spectrum of reactivity and so would produce broader cross-reactivity when combined. Results of these studies could, therefore, improve the understanding of antigenic relatedness of different AMA1 alleles and guide selection of AMA1 alleles to be included in a multi-allele vaccine (Figure 5 shows selected examples using combinations of two and three alleles as competitors).

In some assays, we found that a combination of three heterologous alleles could compete almost as successfully as the homologous allele, indicating that most antibodies to the coating AMA1 allele cross-reacted with one or more of the competitors (for example, 3D7, W2mef and HB3 in Figure 5; Additional file 1: Figures S7 and S8). In contrast, for other combinations the addition of the third heterologous allele showed little additional benefit over two competitors, most likely because antibodies reactive with this allele were already binding the other competitor alleles (for example, adding HB3 to 3D7 + FVO, Figure 5B1 and B2). These data were generated using sera from PNG children, but similar results were observed in PNG adults [see Additional file 1: Figure S6]. The complete data set

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**Figure 3** Patterns of cross-reactivity of AMA1 antibodies in children at two different times. Each graph (A-E) shows antibody cross-reactivity between AMA1 alleles in a cohort of Kenyan children (pooled serum) sampled at two time points, in 2002 and 2004. Each allele was tested in competition ELISA against four other AMA1 alleles and cross-reactivity was determined. The pattern of cross-reactivity and allele-specificity of naturally acquired antibodies of children were very similar at the two time-points; antibodies from older children (2004) tended to have slightly higher cross-reactivity than two years earlier (2002) \(P < 0.05\). For each figure (A-E), the competing antigens used in experiments are those listed on the Y-axis. AMA1, apical membrane antigen 1.
of all double and triple combinations in PNG children can be found in Additional file 1: Figure S7. Multiple competition ELISAs with combinations of two or three alleles were also performed with sera from the Kenyan cohort, with similar results [see Additional file 1: Figure S8]. Once again, similar patterns of cross-reactivity were observed in the PNG and Kenyan cohorts, with slightly higher cross-reactivity in Kenya. The combination of three alleles that showed the broadest cross-reactivity in PNG (3D7, W2mef and HB3) also showed the greatest coverage in Kenya. Not only have we found the same patterns of cross-reactivity in both geographical regions, but also that the same combinations may provide almost complete coverage in terms of cross-reactivity in both cohorts (Figure 5 and Additional file 1: Figure S8). These findings provided the rationale for further analysis to identify combinations of alleles that might cover antigenic diversity of AMA1 that could guide the selection of alleles for inclusion in multi-allele vaccine development.

**Figure 4 Patterns of AMA1 antibody cross-reactivity among children from Kenya and PNG.** Each graph (A-E) shows antibody cross-reactivity between AMA1 alleles tested by competition ELISA in pooled serum from children from PNG and Kenya (at 2004 time-point). Each variant (A-E) was tested against four other AMA1 alleles and cross-reactivity was determined. The pattern of cross-reactivity for each allele was similar in both populations, and cross-reactivity was slightly higher in Kenyan children for all alleles (P = 0.017). Data shown here were extracted from the same datasets used in Figures 1 and 3. For each figure (A-E), the competing antigens used in experiments are those listed on the Y-axis. AMA1, apical membrane antigen 1; PNG, Papua New Guinea.

Identifying combinations with broad coverage as possible multi-allele vaccine candidates

To identify allele combinations that would cover most AMA1 diversity, selected combinations of three antigens were tested against eleven available AMA1 alleles using sera from the PNG cohort. Combinations were selected based on: 1) the extent of cross-reactivity observed between alleles in standard competition ELISAs (Figure 1); and 2) cross-reactivity with combinations of two or three antigens against five alleles in the PNG cohort (Figure 5). We found that Combination A consisting of alleles 3D7, W2mef and HB3 gave cross-reactivity of 92% to 100% across all 11 alleles (Figure 6A). Combination C (3D7, W2mef and D10) showed cross-reactivity of 91% to 100%, Combination D (D10, W2mef and HB3) 87% to 100% and Combination E (D10, W2mef and FVO) showed the highest cross-reactivity across all alleles of 97% to 100%. Interestingly, Combination B consisting of 3D7, W2mef and FVO showed a gap of coverage for D10 and HB3. After the principle was established in the PNG cohort, the most promising combinations were selected and tested in the Kenyan cohort (Figure 6B). Combination A (3D7, W2mef and HB3) and combination E (D10, W2mef and FVO) showed broad, high level cross-reactivity in both populations, similar to observations with PNG samples. These results suggest that it may be possible to reduce a multi-allele-vaccine to either of these combinations of three major AMA1 serotypes.
Sequence diversity in AMA1 alleles

To further support our findings, we performed sequence analyses to evaluate how well the 11 AMA1 reference alleles used in our studies represented global diversity of AMA1, and diversity in our study populations. In the 11 AMA1 alleles used in this study, we found 7 polymorphic amino acid positions in the prodomain and 45 in the ectodomain: 28 in domain I, 8 in domain II, and 9 in domain III (Additional file 1: Figures S1 and S2). These make up 81% of known polymorphic positions in AMA1 (64 identified from 355 AMA1 sequences, [39]). This initial analysis suggests that our selection of AMA1 alleles is broadly representative of global AMA1 diversity. To assess this in more detail, we performed phylogenetic network analyses (median-joining networks) to visualize network trees of evolutionary relationships between

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**Figure 5** AMA1 antibody cross-reactivity with multiple competitor alleles in competition ELISAs. Serum pools prepared from PNG children were tested for cross-reactivity of naturally acquired antibodies among five different PfAMA1 variants in competition ELISA. Competition with either two (A1 to D1) or three (A2 to D2) other alleles was tested in multiple antigen competition ELISA against the remaining three AMA1 variants to determine cross-reactivity. Enhancement of cross-reactivity by mixtures of two or three competitor alleles was dependent on the specific combination tested. The combination of 3D7 and 7G8 antibodies shows the highest degree of cross-reactivity towards the three other alleles, w2mef, FVO and HB3 (panel D1). Specific combinations of antibodies, that is, 3D7, w2mef and HB3, result in almost complete cross-reactivity towards the remaining AMA1 variants (panel C2). The degree of cross-reactivity observed with a particular combination of competitor alleles indicates the extent to which immunization with that combination might provide coverage against other alleles. A representative selection of double and triple antigen competition ELISAs is shown; all double and triple combination competition ELISAs are shown in Additional file 1: Figure S7. For each panel (A1-D1, and A2-D2), the coating antigens used in experiments are those listed on the Y-axis, and the antigens used for competition are at the top of the figure (for example, for panel A1, the coating antigens were W2mef, 7G8, FVO, 3D7, and HB3; the competitor antigens were 3D7 and W2mef). AMA1, apical membrane antigen 1; PNG, Papua New Guinea.
different haplotypes. For comparison to our 11 reference alleles, we included 873 AMA1 sequences sourced from all malaria-endemic regions globally, which were found to comprise 269 distinct haplotypes including several high frequency haplotypes (Figure 7A). Our 11 alleles were distributed throughout the network of global sequences, suggesting that they are broadly representative of the global diversity in AMA1 (Figure 7A). The analysis also indicates that there is little evidence of geographic clustering of related sequences, consistent with
previous studies, and that global AMA1 sequences group into a small number of major clusters. We performed a similar analysis restricted to including only sequences from our study populations of Madang Province, PNG, and Kilifi District, Kenya (Figure 7B). Our 11 AMA1 reference alleles were again evenly distributed throughout the network, indicating they are representative of the diversity present in our study populations, which is reflected in our serologic data. Sequences from PNG and Kenya were distributed through the networks indicating substantial overlap in the distribution of alleles and polymorphisms in the two populations.
Relationship between sequence differences in AMA1 alleles and antibody cross-reactivity

To investigate the relationship between antigenic diversity and overall sequence diversity, we related antibody cross-reactivity to the number of sequence differences between each of the AMA1 alleles. For each allele, we correlated the level of antibody cross-reactivity versus the number of polymorphisms in amino acid sequence between alleles across Domain I, II and III of each allele when compared to the other variants (Figure 8 and Additional file 1: Figure S2). Antibody cross-reactivity was generally not strongly related to the number of sequence differences between two alleles, but correlations between sequence differences and antibody cross-reactivity varied for the different alleles. Significant negative correlations were seen between levels of cross-reactivity and number of sequence differences for antibodies to 7G8 (Spearman’s ρ (rho) = -0.822, P = 0.0047) and XIE (ρ = -0.652, P = 0.0438). Negative correlations of borderline statistical significance were observed for antibodies to W2mef (ρ = -0.609, P = 0.0667), FVO (ρ = -0.583, P = 0.0806) and 2006 (ρ = -0.587, P = 0.0806). No significant correlations were observed for the other alleles. At times, cross-reactivity was high despite a high number of sequence differences between alleles (for example, HB3 and D10, Figure 8E). These findings show that although sequence polymorphisms are related to antibody cross-reactivity and allele-specificity, the overall sequence identity between alleles is not strongly predictive of the extent of antigenic relatedness between alleles.

Duan et al. grouped 150 AMA1 haplotypes from diverse locations into six groups using a phylogenetic clustering algorithm, and proposed that these groupings based on sequence might provide an indication of immunological cross-reactivity [35,39,40]. To explore this approach as a predictor of antigenic similarity and differences between alleles, we examined our cross-reactivity data in terms of the six groupings they defined. The 11 alleles used in our study fell into four of the six groups generated by the clustering algorithm (Figure 9A). Cross-reactivity between pairs of alleles within each group was assessed (Figure 9B). Cross-reactivity for pairs of alleles within each cluster group was then compared with cross-reactivity for pairs of alleles from different groups (Figure 9C) (for example, XIE and HB3 are both in cluster three; comparing FVO (cluster five) and 3D7 (cluster one) is an example of comparing heterologous clusters). It was notable that there was a large range in the level of cross-reactivity between alleles within the same cluster and between those in different clusters. Cross-reactivity between alleles in the same sequence cluster was slightly greater than that for alleles from different groups. However, there were many alleles in the same cluster that had limited cross-reactivity, and substantial cross-reactivity was seen between alleles that were in different clusters. These analyses indicate that the sequence clustering approach is not a strong predictor of antigenic differences between alleles defined by human antibodies.

Discussion

Many targets of naturally-acquired immunity to malaria and leading vaccine candidates are polymorphic, which presents challenges to developing these antigens as vaccines that generate broadly protective immunity against different circulating variants or strains in populations. Understanding the antigenic diversity of polymorphic antigens, the level of allele-specific and cross-reactive antibodies, and the relationship between sequence polymorphisms and antigenic escape are essential for advancing vaccine development; however, this knowledge is currently limited for polymorphic malaria antigens. An example of such an antigen is AMA 1, which is a leading malaria vaccine candidate [20,39] and also one of the most polymorphic merozoite antigens. Given the extensive diversity of AMA1 in most populations, single allele immunization is likely to be of limited benefit in endemic areas [20,22]. Here, we investigated antigenic diversity of AMA1 (defined by reactivity to human antibodies) to determine strategies for the development of a potential multi-allele AMA1 vaccine, and more broadly as a model of polymorphic malaria vaccine candidates and the multi-allele vaccine approach. We investigated the antigenic cross-reactivity of a geographically diverse panel of AMA1 alleles with the aim of identifying alleles that might be included in a broadly protective, multi-allele vaccine. Antigenic diversity of AMA1 was found to be surprisingly restricted, despite the large number of haplotypes present in populations. Extensive antibody cross-reactivity against different AMA1 alleles was observed, and results from multiple antigen competition ELISAs indicated that a three-allele vaccine may be sufficient to provide broad coverage against naturally circulating strains, provided the correct alleles are selected. Earlier data from Osier et al. indicated that high levels of antibodies to only three AMA1 alleles measured by ELISA were strongly associated with protection from clinical malaria [41]. These results were suggestive that co-acquisition of different allele specific antibodies may produce cross-protection to a larger number of naturally-circulating strains. Until now, there has been little direct evidence to support this interpretation. Our study on cross-reactivity of human anti-AMA1 antibodies helps resolve this question, defining the extent of cross-reactivity towards different alleles.

Competition ELISA experiments demonstrated that antibodies to AMA1 alleles were extensively cross-reactive. Cross-reactivity of more than 70% was observed for some
Figure 8 (See legend on next page.)
pairs of alleles, suggesting high levels of antigenic relatedness. While other pairs of alleles showed lower cross-reactivity, all combinations displayed at least some cross-reactivity (minimum was 10%). Antibodies that cross-react against different AMA1 alleles probably target shared epitopes (common epitopes that are shared across different alleles) rather than target strictly conserved epitopes (epitopes that are identical on all alleles). Cross-reactive antibodies that protect against different strains of the same pathogen have been well studied in viral diseases, such as influenza or dengue [42-44], and less so in bacteria [45], but there are only limited data on cross-reactive antibodies against individual *Plasmodium falciparum* antigens (for example, [31,46]). This is the first study to comprehensively examine the extent to which naturally-acquired human AMA1 antibodies can react with multiple different AMA1 alleles and the most comprehensive analysis of the antigenic diversity, defined by human antibody reactivity, to any merozoite antigen. Although antigenic diversity to AMA1 has also been assessed through the use of antibodies generated in rabbits by immunization, these responses may not be entirely representative of human responses and may differ in nature and specificity. While studies of responses generated by immunization in animal models are informative, reactivity of human antibodies is the most relevant response to evaluate antigenic diversity of AMA1. In addition, the antigenic diversity of AMA1 has been largely evaluated with growth inhibition assays in published studies [24,35,40], and it is not clear whether this is the primary effector mechanism mediating protection. Therefore, we took an approach to measure human antibodies to all epitopes on AMA1.

Competition ELISA experiments comparing cross-reactivity of AMA1 antibodies among malaria-exposed adults and children showed greater antibody cross-reactivity in adults, reflecting a higher level of protective immunity in adults. This observation is supported by an earlier cross-sectional study of AMA1 antibody responses in PNG [13] that showed that of the few individuals identified as having highly allele-specific responses, most were children younger than 10 years of age; however, they did not directly assess antibody cross-reactivity in children versus adults. If conserved or cross-reactive epitopes are less immunogenic than polymorphic epitopes, antibodies might not be produced by younger children either because their immune systems are functionally immature or they have had insufficient exposure to the antigen. Another explanation is suggested by animal immunization studies, which showed that a higher proportion of cross-reactive antibodies to AMA1 was induced by immunization with multiple alleles compared to immunization with a single allele [47,48]. In humans, acquisition of cross-reactive antibodies may also require exposure to a range of alleles, which will be reflected in an increase in the relative proportion of cross-reactive antibodies with cumulative exposure. Future studies to understand variation in the patterns and levels of antibody cross-reactivity to different AMA1 alleles among individuals, and how that relates to age and exposure, will be important and may further inform the selection of alleles for inclusion in a possible multi-allele vaccine. Further studies using an expanded repertoire of AMA1 alleles in single and multiple competition ELISAs, and in additional populations, may also help refine allele-selection in vaccine design.

Importantly, we found very similar patterns of cross-reactivity and allele specificity when comparing AMA1 antibody responses in geographically distinct populations. This suggests that different populations are exposed to a similar repertoire of AMA1 alleles, and that the antigenic properties of AMA1 alleles are the major determinants of cross-reactivity between alleles rather than differences between populations. Furthermore, it is likely that exposure to multiple different alleles in naturally-acquired infections will also influence the acquisition of cross-reactive antibodies. Consistent with our observations, sequence analyses have indicated that while sequence diversity of AMA1 is high, most of the diversity found globally can be identified within a single geographical location [35,49], although there is some geographic clustering of alleles. Analyses suggested that the 11 alleles included in our studies were broadly representative of global diversity. Encouragingly, our findings suggest that the same multi-allele AMA1 vaccine could be effective in different regions and populations.

Multiple competition ELISAs with mixtures of three competitor alleles suggested that antibodies represented...
in two allele combinations, Combination A (3D7, W2mef and HB3) and Combination E (D10, W2mef and FVO) gave broad, high level cross-reactivity in two geographically distinct human populations (Figure 6), suggesting that a multi-allele vaccine could probably be reduced to either of these combinations of three major AMA1 serotypes. We propose that the novel MACE approach we have used here could be adopted as an efficient and cost-effective means of testing any polymorphic antigen for its antigenic diversity and the potential of multi-allele approaches in vaccine development. Results from competition ELISA experiments could be used to allocate alleles into potential antigenic or serologic groups that could guide allele selection for AMA1 vaccine development. Alleles with high cross-reactivity against each other by single competition ELISA would be considered to belong to the same serogroup. When two competitor alleles from the same serogroup were combined in double-competition ELISAs, cross-reactivity would not be greatly enhanced compared with results when each allele was used alone as a competitor, reflecting antigenic relatedness between the alleles (see Figure 5). In contrast, combining two competitor alleles from different serogroups would increase cross-reactivity against different alleles. This process guides the selection of alleles to use in combinations.

Several studies have described AMA1 sequence diversity and generated allele-clusters based on sequence similarities using the Structure algorithm [22,35,49], with some evidence that these groupings are immunologically relevant. The current study represents the first time AMA1 groupings have been compared to antigenic diversity defined by human antibodies, and we were able to examine how antigenic diversity related to sequence diversity. While there was some relationship between the number of sequence differences between two alleles and the level of antibody cross-reactivity, at times we
observed high antibody cross-reactivity between two AMA1 alleles despite a high number of amino acid sequence differences. Our data indicate that broadly assessing sequence differences by the extent of sequence identity between alleles was not a strong or reliable predictor of antibody cross-reactivity between AMA1 alleles, and antigenic diversity is more restricted than sequence diversity. Similarly, grouping sequences using the Bayesian clustering approach used in Structure [22,35,49] was also not a strong or reliable predictor of antigenic differences. The lack of a strong relationship between overall sequence differences or haplotype clusters and antigenic differences suggests that only a subset of all polymorphisms are important for defining antigenic differences, that groups of polymorphisms are required for significant antigenic differences, or that some polymorphisms play only a minor role in influencing antigenic differences between alleles. For some other polymorphic malaria antigens, results have suggested that antigenic diversity can be relatively restricted despite significant sequence diversity (for example, EBA175, var2csa [31,50,51]). A previous study identified a polymorphic cluster (C1-L) in AMA1 important for escape from vaccine-induced rabbit antibodies; we found that differences in this region, on their own, were not a good predictor of the antigenic differences we measured here (data not shown). Identifying the most important residues that determine antigenic differences and facilitate immune escape is an important focus for future research; this knowledge would enable the development of sequence-based algorithms that better predict antigenic properties of alleles and would be valuable for application in AMA1 vaccine trials. Recent data from growth inhibition assays using rabbit antisera raised against different AMA1 alleles [24] support these conclusions, demonstrating that the extent of sequence identity between alleles was weakly and inconsistently predictive of antibody cross-inhibitory activity in growth inhibition assays. Furthermore, a mixture of antibodies to four AMA1 alleles was sufficient to inhibit growth of a diverse array of P. falciparum isolates [24]. Detailed evaluation of antigenic diversity is crucial for defining which alleles should be included in a multi-allele AMA1 vaccine.

AMA1 antibodies examined here were acquired from natural exposure, and future studies are needed to further evaluate the specificity and cross-reactivity of responses generated by human immunization to support the multi-allele vaccine approach. Immunization of rabbits with a mixture of AMA1 alleles induced a relatively greater proportion of broadly cross-reactive antibodies than single allele immunization [47]; multi-allele immunization appears to shift the antibody responses to epitopes that are conserved or have limited diversity and, therefore, antibodies have greater cross-reactivity than those generated by single-allele immunization [48]. This provides supporting evidence for the multi-allele vaccine approach. However, whether this finding holds true in humans using less potent adjuvants needs to be determined. Supporting these findings, a prior study demonstrated good immunogenicity of a multi-allele AMA1 vaccine in macaques [52]. This vaccine was comprised of AMA1 proteins based on three synthetic sequences (DiCo) that aimed to cover sequence diversity; vaccine-induced antibodies had similar reactivity by ELISA to four different alleles tested (FVO, HB3, 3D7 and CAMP alleles) and gave significant growth inhibition against the three isolates tested (FCR3, HB3 and NF54). Another rabbit immunization study [40] used mixtures of recombinant AMA1 proteins representing each of the cluster groups defined by Duan et al. [35] and showed that a five-allele mixture was sufficient to generate antibodies with broad growth inhibitory activity against different isolates. Our findings are in general agreement with these results and, given the differences between humans and experimental animal models in nature and specificity of antibodies, our study provides valuable additional information to guide selection of allele-combinations for human trials and predict vaccine coverage in malaria-endemic settings rather than relying only on small animal immunization studies; findings from different approaches need to be used together to inform vaccine design.

A bi-allele vaccine consisting of recombinant 3D7 and FVO AMA1 alleles was shown to induce antibodies to both alleles in a clinical trial [53,54]. Vaccine-induced antibodies showed cross-reactivity against a non-vaccine AMA1 allele, although the magnitude of response was lower than that against vaccine alleles [53]. Unfortunately, this vaccine showed no efficacy in a phase II trial in Malian children [55]; subsequent investigation found no evidence of strain-specific efficacy (although the sample size was small), and it was suggested that the lack of efficacy was probably because the vaccine formulation was insufficiently immunogenic [56]. Interestingly, the combination of 3D7 and FVO alleles (as used in this vaccine) showed only limited cross-reactivity against different alleles in our study, highlighting the need to carefully select alleles for inclusion in a multi-allele vaccine, with consideration to be given to antigenic relatedness as well as prevalence of circulating alleles in the target population.

Although antibodies measured by ELISA, as performed in our studies, do not directly assess antibody function, they do appear to be a good correlate of functional activity. Antibody levels by ELISA correlate with growth-inhibitory activity in animal and human studies [19,36,40], and antibodies to functional invasion-inhibitory epitopes of AMA1 correlated with total antibody reactivity to AMA1 by ELISA [36]. Equally, levels of antibody reactivity to circumsporozoite proteins measured by ELISA appear to be a good correlate of protection with the RTS,S vaccine [57,58]. Correlations of protection for AMA1 vaccines are
not yet available. Further studies that are able to demonstrate comparable levels of cross-reactivity between functional human antibodies to AMA1 alleles (for example, using growth inhibition assays) would provide additional support for the development of a multi-allele AMA1 vaccine, and further research to define the functional activity of AMA1 antibodies is needed. The extent to which human immunization with a multi-allele AMA1 vaccine could protect against naturally circulating *P. falciparum* strains needs to be formally assessed in clinical trials; however, our approach represents a useful tool to predict this coverage.

**Conclusions**

In conclusion, AMA1 has been a strong vaccine candidate for many years, but its extensive sequence polymorphism and the limited efficacy of mono- or bi-valent formulations in clinical trials has presented challenges for developing it as an effective vaccine. It has not been clear whether protection would need to be provided by a large repertoire of allele-specific antibodies or antibodies that are able to cross-react with multiple alleles. Here, we have shown extensive cross-reactivity of naturally-acquired antibodies between alleles of AMA1. Whereas sequence polymorphism in AMA1 is high, antigenic diversity is surprisingly restricted, suggesting the feasibility of a multi-allele AMA1 vaccine. Selected combinations of three AMA1 alleles showed high-level, broad cross-reactivity against a range of AMA1 alleles. While further studies with additional alleles are needed to consolidate our findings and further define the alleles for vaccine inclusion, and subsequent testing of allele combinations in clinical trials is needed, our results support the development of AMA1 as a multi-allele vaccine, possibly in combination with other antigens to maximize protective efficacy against malaria. These findings are also broadly relevant to numerous malaria vaccine candidates that are polymorphic, supporting the concept of multi-allele vaccines as a feasible approach and indicating that antigenic diversity may be much lower than predicted by sequence analyses. Our novel MACE method to define antigenic differences and serogroups could be valuable for examining other polymorphic antigens, to characterize antigenic diversity and assess the potential of antigens as future vaccine candidates.

**Additional file**

Additional file 1: Figure S1. Amino acid sequence alignment of the 11 AMA1 reference alleles. Figure S2. Differences in amino acid sequence between 11 AMA1 alleles. Figure S3. Optimising competing AMA1 antigen concentration. Figure S4. Example of results from competition ELISA presented as degree of cross-reactivity. Figure S5. Antibody cross-reactivity to different AMA1 alleles using individual samples versus a pool of samples. Figure S6. AMA1 antibody cross-reactivity with multiple competitor alleles in competition ELISAs with PNG adults. Figure S7. AMA1 antibody cross-reactivity with multiple competitor alleles in competition ELISAs with PNG children. Figure S8. AMA1 antibody cross-reactivity with multiple competitor alleles in competition ELISAs using samples from the Kenyan cohort.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

UT, JGB designed the study; UT, NJC, DRD, CKM, performed experiments; UT, JSR, AEB, JGB performed analysis and interpretation of data; DRD, ANH, AEB, FHHQ, KM, CKM, RFA, SD, IM, PMS, DIS, NJC, SRE, and JGB contributed reagents, clinical samples and data, and AMA1 sequences; UT, JGB, and JSR wrote the manuscript with contributions from DRD, ANH, AEB, RFA, SD, KM, DIS, NS, and SRE. All authors read and approved the final manuscript.

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**References**

1. World Health Organization: *World Malaria Report 2012*. Geneva: 2012.
2. Cohen S, McGregor IA, Carrington S: Gamma-globulin and acquired immunity to human malaria. *Nature* 1961, 192:733–737.
3. Beeson JG, Osier FH, Engwerda CR: Recent insights into humoral and cellular immune responses against malaria. *Trends Parasitol* 2008, 24:578–584.
4. Folley SD, Conway DJ, Cavanagh DR, McBride JS, Lowe BS, Williams TN, Mwangi TW, Marsh K: High levels of serum antibodies to merozoite surface protein 2 of Plasmodium falciparum are associated with reduced risk of clinical malaria in coastal Kenya. *Vaccine* 2006, 24:4233–4246.
5. Richards JS, Arumugam TU, Reiling L, Healer J, Hodder AN, Fowkes FJ, Cross N, Langer C, Takeo S, Ubeda AD, Thompson JH, Gilson PR, Coppell RL, Siba PIA, King CL, Torii M, Olitina CE, Narum DL, Mueller I, Crabbe BS, Cowman AF, Tsukib T, Beeson JG: Identification and prioritization of merozoite antigens as targets of protective human immunity to Plasmodium falciparum malaria for vaccine and biomarker development. *J Immunol* 2013, 191:795–809.
6. Richards JS, Beeson JG: The future for blood-stage vaccines against malaria. *Immunol Cell Biol* 2009, 87:377–390.
7. Osię F, Feng G, Boyle MJ, Langer C, Zhou J, Richards JS, McCallum FJ, Reiling L, Jaworowski A, Anders RF, Marsh K, Beeson JG: Opsonic phagocytosis of Plasmodium falciparum merozoites: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

8. Guevara-Patino JA, Holder AA, McBride JS, Blackman MJ: Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. J Exp Med 1997, 186:1689–1699.

9. Jos C, Marrama L, Polson HE, Corre S, Diatta AM, Dufou B, Trape JF, Tall A, Longacre S, Perrat R: Clinical protection from falciparum malaria correlates with neutrophil respiratory bursts induced by merozoites opsonized with human serum antibodies. PLoS One 2010, 5:e10871.

10. Fowkes FJ, Richards JS, Simpson JA, Beeson JG: Extreme polymorphism in a vaccine antigen and risk of clinical malaria: implications for vaccine development. Sci Transl Med 2009, 1:21ra5.

11. Olotu A, Fegan G, Williams TN, Sasi P, Ogada E, Bauni E, Wambua J, Marsh K, Bormann S, Bejon P: Defining clinical malaria: the specificity and incidence of endpoints from active and passive surveillance of children in rural Kenya. PLoS One 2010, 5:e15369.

12. Drew DR, Hodder AN, Wilson DW, Foley M, Mueller I, Siba PM, Dent AE, Cowman AF, Beeson JG: Defining the antigenic diversity of Plasmodium falciparum apical membrane antigen 1 and the requirements for a multi-allele vaccine against malaria. PLoS One 2012, 7:e31023.

13. Miller LH, Roberts T, Shahabuddin M, McCutchan TF: Analysis of sequence diversity in the Plasmodium falciparum merozoite surface protein-1 (MSP1). Mol Biochem Parasitol 1993, 59:1–14.

14. Triglia T, Duraisingh MT, Good RT, Cowman AF: Reticulocyte-binding protein homologue 1 is required for sialic acid-dependent invasion into human erythrocytes by Plasmodium falciparum. Mol Microbiol 2005, 55:162–174.

15. Fowler EV, Peters JM, Gattoufi M, Chen N, Cheng G: Genetic diversity of the DBLalpha region in Plasmodium falciparum var genes among Asia-Pacific isolates. Mol Biochem Parasitol 2002, 117:1–12.

16. Burke TR, Williams JL, Schneider I: Identification of Plasmodium falciparum-falciparum infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. Am J Trop Med Hyg 1984, 33:783–788.

17. Rayner JC, Tran TM, Corredor V, Huber CS, Barnwell JW, Galinski MR: Dramatic difference in diversity between Plasmodium falciparum and Plasmodium vivax reticulocyte binding-like genes. Am J Trop Med Hyg 2007, 77:666–674.

18. Bhaskar NK, Trager W: Gametocyte-forming and non-gametocyte-forming clones of Plasmodium falciparum. Am J Trop Med Hyg 1984, 33:534–537.

19. Hommel M, Elliott SR, Soma V, Kelly G, Fowkes FJ, Chesson JM, Duffy MF, Bockhorst J, Avil M, Mueller I, Raiko A, Stanisic DI, Rogerson SJ, Smith JD, Beeson JG: Evaluating the antigenic diversity of placental binding Plasmodium falciparum variants and the antibody repertoire among pregnant women. Infect Med 2010, 78:1963–1978.

20. Elliott SR, Payne PD, Duffy MF, Byrne TJ, Tham WH, Rogerson SJ, Brown GV, Eisen DP: Antibody recognition of heterologous variant surface antigens after a single Plasmodium falciparum infection in previously naive adults. Am J Trop Med Hyg 2007, 76:860–864.

21. Hume JC, Tunnicliff C, Runford-Cartwright LC, Day KP: Susceptibility of Anopheles gambiae and Anopheles stephensi to tropical isolates of Plasmodium falciparum. Mol Biochem Parasitol 2007, 6:139.

22. Barry AE, Leliwa-Sytek A, Tavul L, Imrie H, Mogot-Nabias F, Brown SM, McVean GA, Day KP: Structural basis of antigenic escape of a malaria vaccine candidate. Proc Natl Acad Sci U S A 2008, 115:534–537.

23. Mwii NY, Tabor E, Kariuki SW, Ngugi WN, Wangari M, Mwangi T, Bull PC, Thomas AW, Cavanagh DR, McBride JS, Lanar DE, Eisen DP, Coomaraswamy A, Kerke M, Rocklov J, Walliker D, Molyneux ME, Brown GV, Eisen DP: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

24. Bhasin VK, Trager W: Gametocyte-forming and non-gametocyte-forming clones of Plasmodium falciparum. Am J Trop Med Hyg 1984, 33:534–537.

25. Morlon-Guyot J, Dubremetz JF, Fauquenoy S, Tomavo S, Faber BW, Kocken CH, Thomas AW, Boulanger MJ, Bentley GA, Lebrun M: The RON2-AMA1 interaction is a critical step in moving junction-dependent invasion of apicomplexan parasites. PLoS Pathog 2011, 7:e1001276.

26. Duan J, Mu J, Thera MA, Joy D, Kosakovsky Pond SL, Diemert D, Long CA, Zhou H, Miura K, Dolo A, Doumbo O, Su XZ, Miller L: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

27. Tongren JE, Drakeley CJ, McDonald SL, Reyburn HG, Manjurano A, Nkya WM, Asensio-Ortega B, Chidumi C, Embolu B, Ketana-Bwambale W, Stothard J, Kaufmann SH, Roper TD, Marsh K, Beeson JG: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

28. Bhasin VK, Trager W: Gametocyte-forming and non-gametocyte-forming clones of Plasmodium falciparum. Am J Trop Med Hyg 1984, 33:534–537.

29. Hommel M, Elliott SR, Soma V, Kelly G, Fowkes FJ, Chesson JM, Duffy MF, Bockhorst J, Avil M, Mueller I, Raiko A, Stanisic DI, Rogerson SJ, Smith JD, Beeson JG: Evaluating the antigenic diversity of placental binding Plasmodium falciparum variants and the antibody repertoire among pregnant women. Infect Med 2010, 78:1963–1978.

30. Elliott SR, Payne PD, Duffy MF, Byrne TJ, Tham WH, Rogerson SJ, Brown GV, Eisen DP: Antibody recognition of heterologous variant surface antigens after a single Plasmodium falciparum infection in previously naive adults. Am J Trop Med Hyg 2007, 76:860–864.

31. Hume JC, Tunnicliff C, Runford-Cartwright LC, Day KP: Susceptibility of Anopheles gambiae and Anopheles stephensi to tropical isolates of Plasmodium falciparum. Mol Biochem Parasitol 2007, 6:139.

32. Barry AE, Leliwa-Sytek A, Tavul L, Imrie H, Mogot-Nabias F, Brown SM, McVean GA, Day KP: Structural basis of antigenic escape of a malaria vaccine candidate. Proc Natl Acad Sci U S A 2008, 105:7857–7862.

33. Mwii NY, Tabor E, Kariuki SW, Ngugi WN, Wangari M, Mwangi T, Bull PC, Thomas AW, Cavanagh DR, McBride JS, Lanar DE, Eisen DP, Coomaraswamy A, Kerke M, Rocklov J, Walliker D, Molyneux ME, Brown GV, Eisen DP: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

34. McVean GA, Day KP: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

35. McVean GA, Day KP: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

36. McVean GA, Day KP: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

37. McVean GA, Day KP: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.

38. McVean GA, Day KP: Antibodies to polymorphic invasion-inhibitory and non-inhibitory merozoites of Plasmodium falciparum: mechanism in human immunity and a correlate of protection against malaria. BMC Med 2014, 12:108.
43. Wu CY, Yeh YC, Chan JT, Yang YC, Yang JR, Liu MT, Wu HS, Hsiao PW: A VLP vaccine induces broad-spectrum cross-protective antibody immunity against H5N1 and H1N1 subtypes of influenza A virus. PLoS One 2012, 7(1):e2363.

44. Warter L, Appanna R, Fink K: Human poly- and cross-reactive anti-viral antibodies and their impact on protection and pathology. Immunol Res 2012, 53:148-161.

45. Tyler JW, Culbtr JS, Spier SJ, Smith BP: Immunity targeting common core antigens of gram-negative bacteria. J Vet Intern Med 1990, 4:17-25.

46. Conway DJ, Cavanagh DR, Tanabe K, Roper C, Miles ZS, Sakihana N, Bojang KA, Oduwo AM, Kremsner PG, Amor DE, Greenwood BM, McBride JS: A principal target of human immunity to malaria identified by molecular population genetic and immunological analyses. Nat Med 2000, 6:689-692.

47. Kusi KA, Faber BW, Thomas AW, Remarque EJ: Humoral immune response to mixed PfAMA1 alleles; multivalent PfAMA1 vaccines induce broad specificity. PLoS One 2009, 4(8):110.

48. Dutta S, Dlugosz LS, Drew DR, Ge X, Ababacar D, Rovira YI, Moch J, Shi M, Long CA, Foley M, Beeson JG, Andersen RF, Miura K, Haynes JD, Batchelor AH: Overcoming antigenic diversity by enhancing the immunogenicity of conserved epitopes on the malaria vaccine candidate apical membrane antigen-1. PLoS Pathog. 2013, 9(10):103840.

49. Barry AE, Schultz L, Buckee CO, Reeder JC: Contrasting population structures of the genes encoding ten leading vaccine-candidate antigens of the human malaria parasite, Plasmodium falciparum. PLoS One 2009, 4(9):e6977.

50. Mambilapalli A, Pattnaik P, Sharma M, Sharma SK, Tyagi PK, Joshi H, Chinis CE: Sequence polymorphisms in the receptor-binding domain of Plasmodium falciparum EBA-175: implications for malaria vaccine development. Mol Biochem Parasitol 2006, 146:120–123.

51. Dicko A, Diemert DJ, Sagara I, Sagouba M, Niamebe MB, Assadou MH, Guindo O, Kamate B, Baby M, Sissoko M, Diallo DA, Saul A, Doumbo O, Miller LH: Impact of a Plasmodium falciparum AMA1 vaccine on antibody responses in adult Malians. PLoS One 2007, 155:1039–112.

52. Kusi KA, Remarque EJ, Rasat V, Walraven V, Thomas AW, Faber BW, Kocken CH: Safety and immunogenicity of multi-antigen AMA1-based vaccines formulated with CoVaccine HT and Montanide ISA 51 in chesu macaques. Malar J 2011, 10:182.

53. Dicko A, Diemert DJ, Sagara I, Sagouba M, Niamebe MB, Assadou MH, Guindo O, Kamate B, Baby M, Sissoko M, Malik EM, Pay MP, Thera MA, Miura K, Dolo A, Diallo DA, Muller GE, Long CA, Saul A, Doumbo O, Miller LH: Structural polymorphism and diversifying selection on the pregnancy malaria vaccine candidate VAR2CSA. Mol Biochem Parasitol 2007, 155:103–112.

54. Dicko A, Sagara I, Ellis RD, Miura K, Guindo O, Kamate B, Sogoba M, Niamebe MB, Sissoko M, Baby M, Dolo A, Muller GE, Pay MP, Pierce M, Diallo DA, Saul A, Miller LH, Doumbo OK: Phase 1 study of a combination AMA1 blood stage malaria vaccine in Malian children. PLoS One 2008, 3(1):e1563.

55. Sagara I, Dicko A, Ellis RD, Pay MP, Diawara S, Lamine B, Oduwo AM, Guindo O, Kamate B, Sogoba M, Niamebe MB, Sissoko M, Buye P, Long CA, Kante O, Diallo DA, Mullen GE, Pay MP, Hanshaw JD, Miller LH, Saul A, Doumbo OK: A randomized controlled phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. Vaccine 2009, 27:3090–3098.

56. Ouattara A, Mou J, Takala-Harrison S, Saye R, Sagara I, Dicko A, Niangaly A, Duan J, Ellis RD, Miller LH, Sun XZ, Plowe CV, Doumbo OK: Lack of allele-specific efficacy of a bivalent AMA1 malaria vaccine. Malar J 2010, 9:175.

57. White MT, Bejon P, Olotu A, Griffin JT, Bojang K, Lusingu J, Salim N, Abdullah S, Oyamo N, Aparajita ST, Lelli B, Asante K, Owusu-emyi S, Malama E, Agbenyega T, Ansong D, Sagaral J, Aponte JI, Ghani AC: A combined analysis of immunogenicity, antibody kinetics and vaccine efficacy from phase 2 trials of the RTS, S malaria vaccine. BMC Med 2014, 12:117.

58. Beeson J, Fowkes FJ, Reiling L, Osis F, Drew D, Brown G: Correlates of protection for Plasmodium falciparum malaria vaccine development. In Malaria Vaccine Development: Over 40 Years of Trials and Tribulations. Edited by Corradin G, Engers H. London: Future Medicine; 2014.
Author/s:
Terheggen, U; Drew, DR; Hodder, AN; Cross, NJ; Muyengyi, CK; Barry, AE; Anders, RF; Dutta, S; Osier, FHA; Elliott, SR; Senn, N; Stanisic, DI; Marsh, K; Sib, PM; Mueller, I; Richards, JS; Beeson, JG

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