Supplementary Material for:

Title: Longitudinal analysis of naturally acquired PfEMP1 CIDR domain variant antibodies identifies associations with malaria protection

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Table S1. Antigens tested in multiplex immunoassay.

Original Antigen Labels	Figure Labels	Group	Binding Phenotype	Genome/ Isolate	Domain Class	Name used in figures of Rambathla et al PMID. 30365003
HB3var03	CIDRα1.4 (a)	A	EPCR	HB3	CIDRα1	α1.4 (a)
IT4var7	CIDRα1.4 (b)	A	EPCR	IT4	CIDRα1	α1.4 (b)
X1965_2	CIDRα1.5a (a)	A	EPCR	1965	CIDRα1	α1.5a (a)
ERS010323	CIDRα1.5a (b)	A	EPCR	GA013	CIDRα1	α1.5a (b)
ERS01002	CIDRα1.5a (c)	A	EPCR	GA014	CIDRα1	α1.5a (c)
X198_5	CIDRα1.5b (a)	A	unknown	1918	CIDRα1	α1.5b (a)
X198313	CIDRα1.5b (b)	A	unknown	1983	CIDRα1	α1.5b (b)
HB3var02	CIDRα1.6a	A	EPCR	HB3	CIDRα1	α1.6a
ERS01057	CIDRα1.6b (a)	A	EPCR	GA018	CIDRα1	α1.6b (a)
ERS01003	CIDRα1.6b (b)	A	EPCR	GA019	CIDRα1	α1.6b (b)
X1965_8	CIDRα1.7 (a)	A	EPCR	1965	CIDRα1	α1.7 (a)
X1918_3	CIDRα1.7 (b)	A	EPCR	1918	CIDRα1	α1.7 (b)
ERS01043	CIDRα1.7 (c)	A	EPCR	GA024	CIDRα1	α1.7 (c)
IT4var08	CIDRγ3	A	unknown	IT4	CIDRγ	γ
HB3var05	CIDRδ (a)	A	unknown	HB3	CIDRδ	δ (a)
HB3var35	CIDRδ (b)	A	unknown	HB3	CIDRδ	δ (b)
IT4var02	CIDRδ (c)	A	unknown	IT4	CIDRδ	δ (c)
IT4var30	CIDRα2.10	B	CD36	IT4	CIDRα2-6	α2.10
IT4var24	CIDRα2.2	B	CD36	IT4	CIDRα2-6	α2.2
IT4var33	CIDRα2.4	B	CD36	IT4	CIDRα2-6	α2.4
IT4var61	CIDRα2.7	B	CD36	IT4	CIDRα2-6	α2.7
IT4var45	CIDRα2.9	B	CD36	IT4	CIDRα2-6	α2.9
DD2var01	CIDRα3.1 (a)	B	CD36	DD2	CIDRα2-6	α3.1 (a)
HB3var27	CIDRα3.1 (b)	B	CD36	HB3	CIDRα2-6	α3.1 (b)
IT4var21	CIDRα3.1 (c)	B	CD36	IT4	CIDRα2-6	α3.1 (c)
IT4var26	CIDRα3.3	B	CD36	IT4	CIDRα2-6	α3.3
IT4var15	CIDRα3.5	B	CD36	IT4	CIDRα2-6	α3.5
IT4var14	CIDRα5	B	CD36	IT4	CIDRα2-6	α5
IT4var12	CIDRα6	B	CD36	IT4	CIDRα2-6	α6
IT4var20	CIDRα1.1 (a)	B/A	EPCR	IT4	CIDRα1	α1.1 (a)
igh_var19	CIDRα1.1 (b)	B/A	EPCR	IGH	CIDRα1	α1.1 (b)
raj116_var	CIDRα1.1 (c)	B/A	EPCR	raj116	CIDRα1	α1.1 (c)
ERS010178_NODE_17	CIDRα1.8a	B/A	EPCR	GA026	CIDRα1	α1.8a
X2053_3	CIDRα1.8b (a)	B/A	EPCR	GA027	CIDRα1	α1.8b (a)
ERS010532_NODE_326	CIDRα1.8b (c)	B/A	EPCR	GA029	CIDRα1	α1.8b (c)
AMA1	AMA1	non-var	N/A	N/A	AMA1	N/A
BSA	BSA	non-var	N/A	N/A	BSA	N/A
CSP	CSP	non-var	N/A	N/A	CSP	N/A
MSP1	MSP1	non-var	N/A	N/A	MSP1	N/A
tetanus toxoid	tetanus toxoid	non-var	N/A	N/A	tetanus	N/A

Listed antigens were used in a multiplex bead-based immunoassay to determine antigen-specific IgG reactivity of plasma from participants in the Kalifabougou chort. Bovine serum albumin (BSA) and tetanus toxoid were used as controls for non-specific cross-reactivity and the predictable response to tetanus vaccination, respectively.

Table S2. Differential acquisition of CIDR domain class-specific IgG antibodies with age and/or malaria exposure.

Domain Class	n	PfEMP1 group	Binding Phenotype	Slope for domain class	Coefficient (age:domain class interaction term)	Standard error	t	P value	BH-adjusted P value
CIDRγ	340	A	unknown	0.224	0.0485	0.0134	3.63	0.000283	0.00141
CIDRδ	340	A	unknown	0.209	0.0357	0.00796	4.49	<0.0001	<0.0001
CIDRα1	340	A	EPCR	0.208	0.0682	0.00435	15.7	<0.0001	<0.0001
CIDRα2-6	340	B	CD36	0.115	-0.0935	0.00446	-21.0	<0.0001	<0.0001

Refers to Figure 3B. To determine CIDR domain classes for which specific IgG was acquired more rapidly than the other variants, the change in variant-specific IgG reactivity with age was compared between all variants within each CIDR domain class and all other variants. Specifically, for each CIDR domain class, a linear regression model was performed for children <8 years of age, which represents the linear portion of the plot. The dependent variable was log-transformed antigen-specific IgG reactivity; the independent variables were presence of *P. falciparum* parasitemia (determined by PCR), age, and PfEMP-1 variant type dichotomized as the PfEMP-1 domain class of interest or variants in all other domain classes with the latter being the reference level. An interaction between age and CIDR class was included in the model. Tabulated coefficient and statistics are for the age:domain class interaction term. P values were adjusted for multiple testing (5 coefficients per model times 4 domain classes) using the Benjamini-Hochberg (BH) method. Non-PfEMP-1 antigens were not included in the analysis.

Table S3. Pairwise comparison of slopes between CIDR classes.

Domain class 1	Domain class 2	F statistic	P value
CIDRα1	CIDRγ	1.3852	0.2393
CIDRα2-6	CIDRγ	88.663	< 2.2e-16
CIDRδ	CIDRγ	0.816	0.3665
CIDRα1	CIDRδ	0.0333	0.8552
CIDRα2-6	CIDRδ	164.38	< 2.2e-16
CIDRα2-6	CIDRα1	415.19	< 2.2e-16

Refers to Figure 3B. To determine if the slopes for IgG reactivity between each CIDR domain classes were significantly different from each other in a pairwise manner, we compared a simple linear model in which the slopes of any two CIDR domain classes were parallel to a more complex linear model that included an interaction between age and CIDR domain class using analysis of variance tables. For these models, the predictor variable was IgG reactivity (as $\log_{10}$ arbitrary units) and independent variables were age, *P. falciparum* PCR status at baseline, and domain class as two-level factor variable. Analysis was limited to children <8 years of age (n = 340). A P value < 0.05 indicates that the slopes for the two domain classes are significantly different.

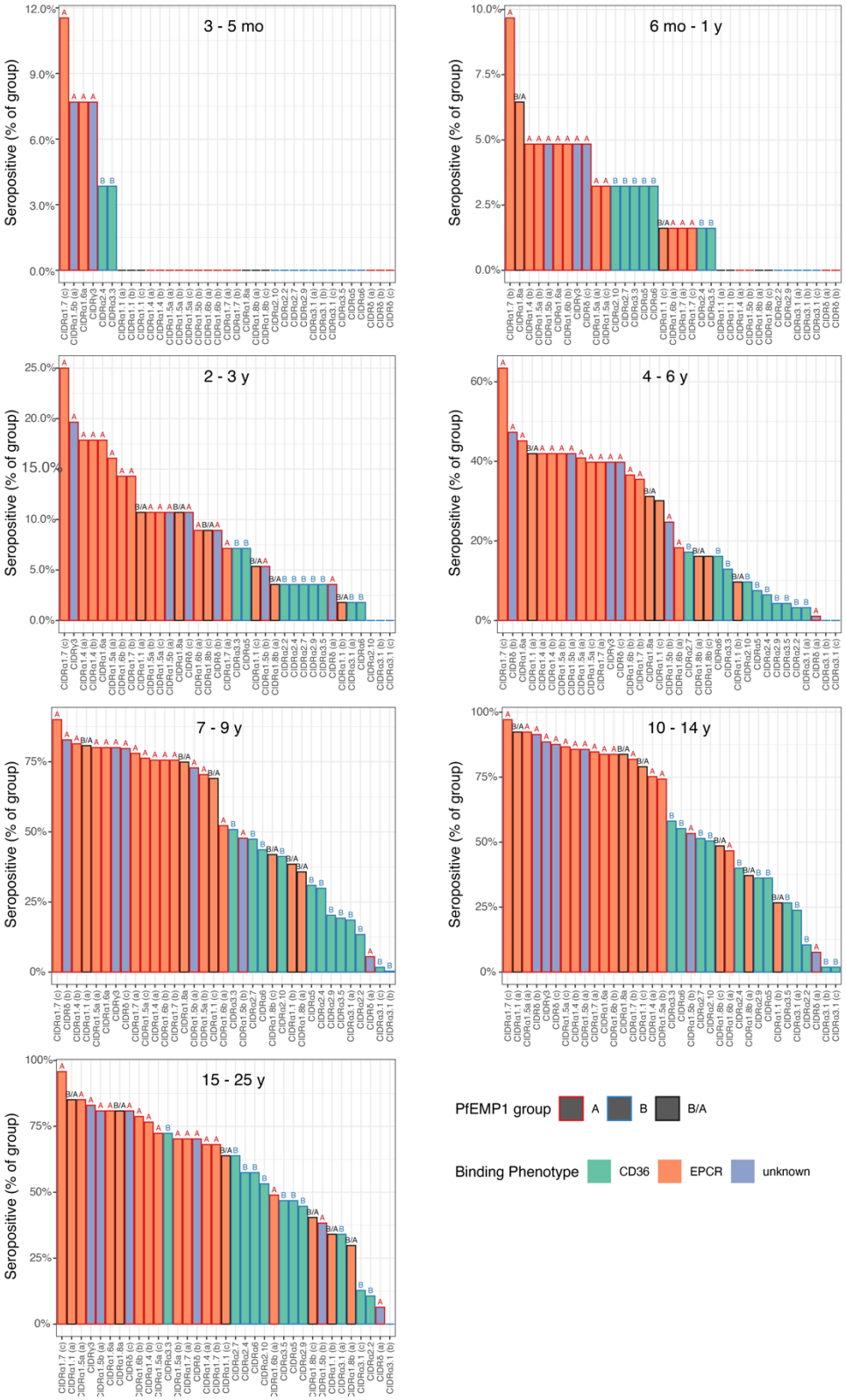

Fig. S1. Antibodies specific for group A and EPCR-binding phenotypes are acquired earlier in life.
Antigens were ranked by seroprevalence to determine the dominant antibody responses for each age group. A response was considered seropositive if the antigen-specific IgG reactivity was greater than the mean reactivity of 20 malaria-naive US donors plus 3 standard deviations.

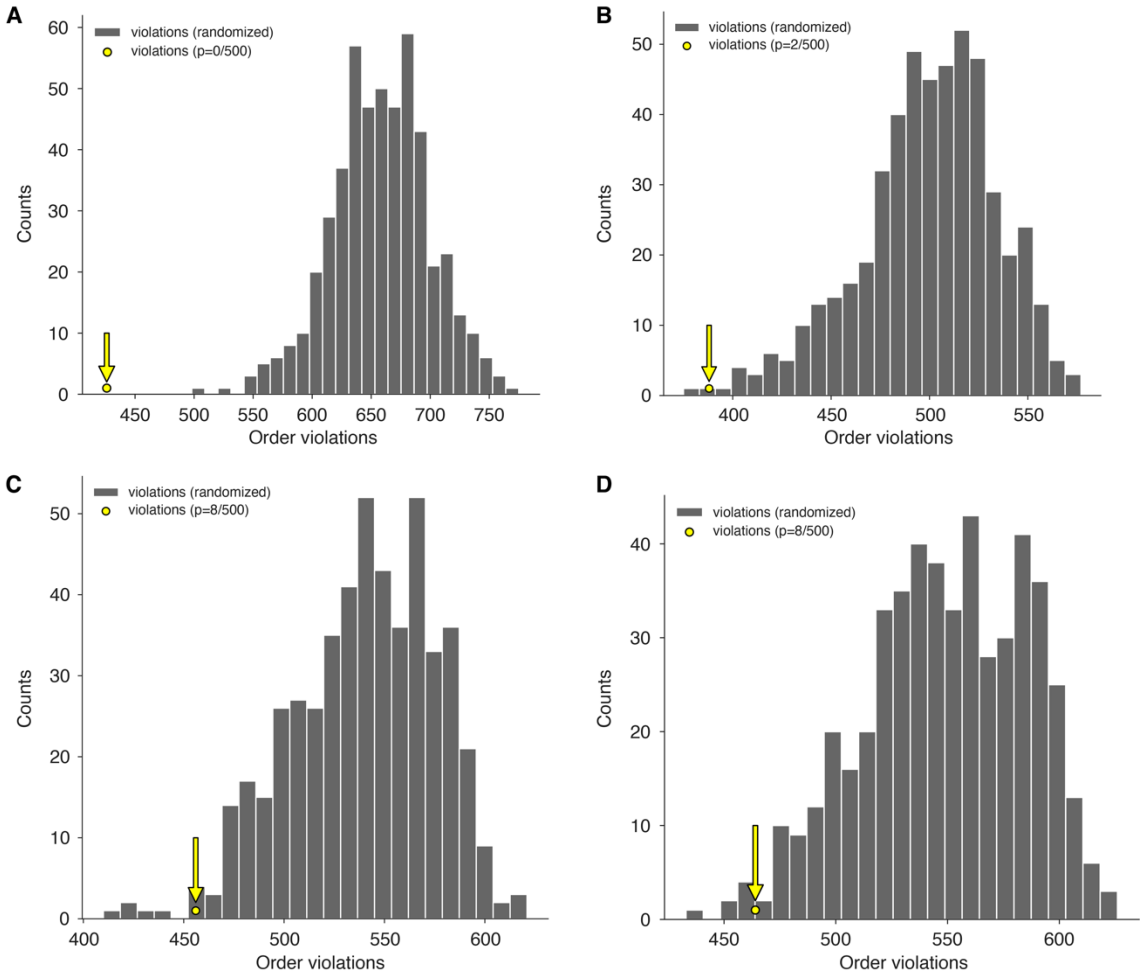

Fig. S2. Significance testing for consensus ordering.

Related to Fig. 5. Observed consensus ordering was compared against 200 independent procedures in which the seroconversion orders for each subject was randomized and consensus ordering was carried out in the same manner, using the total count of consensus-violating seroconversions as a test-statistic (right panel). Fewer consensus violations in real data than in randomized data implies the reported seroconversion ordering is statistically significant. Analysis was performed at the level of **A** individual variants, **B** CIDR domain class, **C** upstream sequence group, and **D** binding phenotype.

Table S4. Relationship between CIDRγ3 seropositivity and protection from febrile malaria with inclusion of blood group O as a co-variate.

	without group O covariate				with group O covariate			
Covariate	**HR**	**LCI**	**UCI**	**P value**	**HR**	**LCI**	**UCI**	**P value**
Age	1.07	0.998	1.14	0.0582	1.07	0.999	1.14	0.055
CIDRγ3	0.411	0.276	0.613	1.26E-05	0.407	0.274	0.605	8.73E-06
group O blood type					0.963	0.678	1.37	0.831
Male	0.858	0.605	1.22	0.39	0.859	0.606	1.22	0.395
presence of HbS allele	0.53	0.311	0.903	0.0196	0.529	0.311	0.902	0.0194

Results of Cox regression models assessing CIDRγ3-specific IgG on the risk of febrile malaria after incident *P. falciparum* infection in which covariates were age, gender, presence of the HbS allele, and AMA1-specific IgG reactivity without or with group O blood type. Analysis was restricted to children within the cohort who were at least 6 months of age, began the study negative for *P. falciparum* infection by PCR, and had ABO blood typing performed (218 subjects; 140 malaria events). Malaria risk was determined based on time to clinical malaria, defined as axillary temperature >37.5° C and any parasitemia, once parasitemia was detected by PCR. Results are ordered by increasing significance values.