Emergence of *FY*A*^{null} in a *Plasmodium vivax*-endemic region of Papua New Guinea

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In Papua New Guinea (PNG), numerous blood group polymorphisms and hemoglobinopathies characterize the human population. Human genetic polymorphisms of this nature are common in malarious regions, and all four human malaria parasites are holoendemic below 1500 meters in PNG. At this elevation, a prominent condition characterizing Melanesians is α^+-thalassemia. Interestingly, recent epidemiological surveys have demonstrated that α^+-thalassemia is associated with increased susceptibility to uncomplicated malaria among young children. It is further proposed that α^+-thalassemia may facilitate so-called "benign" *Plasmodium vivax* infection to act later in life as a "natural vaccine" against severe *Plasmodium falciparum* malaria. Here, in a *P. vivax*-endemic region of PNG where the resident Abelam-speaking population is characterized by a frequency of α^+-thalassemia ≥0.98, we have discovered the mutation responsible for erythrocyte Duffy antigen-negativity (Fy[a−b−]) on the *FY*A* allele. In this study population there were 23 heterozygous and no homozygous individuals bearing this new allele (allele frequency, 23/1062 = 0.022). Flow cytometric analysis illustrated a 2-fold difference in erythroid-specific Fy-antigen expression between heterozygous (*FY*A*/*FY*A*^{null}) and homozygous (*FY*A*/*FY*A*) individuals, suggesting a gene-dosage effect. In further comparisons, we observed a higher prevalence of *P. vivax* infection in *FY*A*/*FY*A* (83/508 = 0.163) compared with *FY*A*/*FY*A*^{null} (2/23 = 0.087) individuals (odds ratio = 2.05, 95% confidence interval = 0.47–8.91). Emergence of *FY*A*^{null} in this population suggests that *P. vivax* is involved in selection of this erythroid polymorphism. This mutation would ultimately compromise α^+-thalassemia/*P. vivax*-mediated protection against severe *P. falciparum* malaria.

Substantial overlap between malaria endemicity and erythroid polymorphisms suggests that these genetic conditions protect against malaria (1, 2). Whereas mechanisms governing "balanced polymorphism" presumably constrain the increase in frequency of mutations conferring homozygous lethal phenotypes [HbS, sickle cell anemia (3)], other mutations have increased to fixation, including erythroid Duffy blood group-negativity (Fy[a−b−]) in Africans (4) and α^+-thalassemia in Melanesians (5).

Molecular genetic studies on the Duffy (*FY*) blood group antigen receptor for chemokines (alternative gene name *DARC*) have identified mutations underlying the major serologically defined antigens of the Fy blood group system, Fy^a, Fy^b (6), Fy^{bweak} (7), and the Fy-negative phenotype (Fy[a−b−], GATA-1 promoter mutation; ref. 8). Individuals homozygous for this GATA-1 mutation fail to express Fy specifically on erythroid cells, providing the molecular basis for Fy(a−b−). In Africans the GATA-1 mutation is haplotypically associated with 625A, encoding Fy^b (*FY*B*^{null}). Because Fy(a−b−) prevents *Plasmodium vivax* from invading host erythrocytes (9–11) and completing its complex life cycle, it is not surprising that vast regions of Africa inhabited by Fy(a−b−) human populations are devoid of this malaria parasite (12). Although the specificity of the parasite–receptor interaction suggests that selective pressure by the pathogen drove the allele to fixation, the reported lack of significant mortality from *P. vivax* infection counters this hypothesis. Alternatively, debate suggests that preexisting high frequency Fy(a−b−) prevented *P. vivax* from becoming established in Africa (13).

The primary mutations responsible for α^+-thalassemia in Melanesians are 3.7- and 4.2-kilobase deletions that eliminate one of the two tandemly repeated α-globin genes on human chromosome 16 (5). In contrast to Fy(a−b−), the mechanism by which α^+-thalassemia protects against malaria is unknown (14). Recent studies in Melanesian populations have demonstrated an increased susceptibility to uncomplicated malaria among young α^+-thalassemic children. It is further proposed that this genetic condition may facilitate *P. vivax* infection to act later in life as a natural vaccine against severe *P. falciparum* malaria (14, 15).

As in other areas in the South Pacific, *P. vivax* causes significant morbidity and coexists with the other three human malaria parasites in Papua New Guinea (PNG). *P. vivax* is responsible for 25% of malaria cases in the East Sepik Province in PNG; *P. falciparum* (55%), *P. malariae* (20%), and *P. ovale* (0.04%) are also holoendemic in this area (16). Resident human populations have been phenotypically characterized to be 100% Fy antigen-positive (more than 85% are Fy[a+b−]) (4, 17), and as high as 90% α^+-thalassemic (18). If, as previously suggested (14, 15), α^+-thalassemia predisposes children to *P. vivax* infection, examination of similar host populations would present a unique opportunity to observe the influence of this malaria species on *FY* sequence polymorphism. To this end, we have studied individuals from two different bioclimatic regions of *P. vivax*-endemic East Sepik Province, PNG. The inclusion of these two populations enabled the study of communities where environmental factors are likely to vary the exposure to malaria-infected mosquitoes, and was predicted to increase the genetic diversity of the study because of the relative isolation of the resident Abelam and Urat linguistic groups (16, 19).

Methods

Study Area and Population. The study was conducted in villages in East Sepik, PNG. Details describing South Wosera ($n \approx 6000$) and villages near the government administrative unit in Dreikikir ($n \approx 4000$–5000) have been provided earlier (16, 19). Study subjects from these areas are members of the Abelam ($n = 531$) and Urat ($n = 381$) linguistic groups, respectively. Sample collection was organized through the Papua New Guinea Institute of Medical Research, Goroka. Blood samples from West African individuals ($n = 200$) from 17 villages distributed

Abbreviation: PNG, Papua New Guinea; RFLP, restriction fragment length polymorphism; MFI, median frequency intensity.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF100634).

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between Senegal and Ghana were collected as part of the studies conducted in conjunction with the Onchocerciasis Control Programme, Ouagadougou, Burkina Faso (20). North American random blood donors, whose race or ethnicity was self-identified at the time of sample collection ($n = 400$), were obtained from the National Histocompatibility Laboratory, American Red Cross/University of Maryland Medical System, Baltimore, MD. Blood samples from all individuals participating in this study were collected under clinical protocols approved by the corresponding institutional review boards.

Malaria Diagnosis. Blood films were prepared and examined as described previously (16). Briefly, thick and thin films were stained with 4% Giemsa and examined under oil immersion objective (100×) for 100 fields. Parasite species were identified by using both thick and thin film preparations. Parasite densities were recorded as the number of parasites per 200 white blood cells (WBC). Previous studies have determined the average WBC count for individuals living in this area to be approximately 8000 WBC/μl.

DNA Extraction. Whole blood was collected in K+EDTA vacutainers and stored at −20°C until DNA extraction could be performed. DNA extraction was performed by using the QIAamp blood extraction protocol (Qiagen, Valencia, CA) or by protocols described previously (21).

PCR-Restriction Fragment Length Polymorphism (RFLP) Genotyping. PCR amplification was performed in a solution (25 μl) containing 2.5 pmol of the appropriate positive-strand and negative-strand primer; 67 mM Tris·HCl (pH 8.8); 6.7 mM $MgSO_4$; 16.6 mM $(NH_4)_2SO_4$; 10 mM 2-mercaptoethanol; 100 μM dATP, dGTP, dCTP, and dTTP; 2.5 units of thermostable DNA polymerase; and 10–50 ng of purified human genomic DNA. Strategies used to characterize *FY* DNA sequence polymorphisms (Fig 1*A*; refs. 6–8) include fragment A (primers −*FY*Pup 5′-GTAAAATCTCTACTTGCTGGAAG-3′ and *FY*Pdn 5′-CCATGGCACCGTTTGGTTCAGG-3′), fragment B (primers *FY*up 5′-GACTCTTCCGGTGTAACTCTGATG-3′ and *FY*851[−] 5′GGCCAAGACGGGCACCACAATG-3′), fragment C (primers *FY*2014[+] 5′-ACTCAGTTCAGGGAACATATC-3′ and *FY*2224[−] 5′-TGCTGACAGAGATGCAAGCT-3′), and fragment D (primers *FY*Pup and *FY*851[−]). The thermocycling program used for each of the above amplifications was 30 seconds at 94°C, 30 seconds at 65°C, and 30–60 seconds at 72°C for 40 cycles. RFLP genotyping was performed following supplier-recommended protocols (New England Biolabs) as follows, fragment B/*Sty*I, fragment C separate assays by using *Ban*I, *Aci*I, and *Mwo*I. Restriction fragments were visualized after electrophoresis on either 4% (5:1, GTG:NuSieve:LE; fragment A/*Sty*I fragments) or 2% (fragment B/*Ban*I, *Aci*I and *Mwo*I fragments) agarose gels (FMC) stained with a 1:10,000 dilution of SYBR Gold (Molecular Probes) and detected with a Storm 860 (Molecular Dynamics). Genotyping of α-globin was directed by a combination of primers described previously (22).

Cloning and DNA Sequence Analysis. After amplification of fragments C and D (Fig. 1*A*) from human genomic DNA templates, PCR products were cloned into pCR2.1TOPO (Invitrogen). Multiple clones from subjects of interest were sequenced by using fluorescence-based sequencing protocols on an ABI377 automated sequencer (Perkin–Elmer).

Microsatellite Genotyping. Microsatellite primer pairs identified in Fig. 3 were selected from the Version 9 Marker Screening Set listed by the Marshfield Center for Medical Genetics (http://www.marshmed.org/genetics) and GDB6.3 (http://www.gdb.org/gdb/gdbtop.html). One primer of each microsatellite primer pair was 5′ end-labeled with Cy5 at the time of synthesis (Research Genetics, Huntsville, AL). Microsatellite amplification was performed by using the same buffer conditions as described above. The thermocycling program used for amplification of all microsatellites was 2 minutes at 94°C; 5 cycles of 30 seconds at 94°C, 30 seconds at 65°C, and 60 seconds at 72°C; 25 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 60 seconds at 72°C; 5 cycles of 30 seconds at 94°C, 60 seconds at 55°C, and 2 minutes at 72°C; and a final extension of 7 minutes at 72°C. After PCR amplification of human genomic DNA samples, Cy 5-labeled microsatellite amplicons were visualized after electrophoresis on 6% denaturing polyacrylamide gels (7.7 M urea/32% formamide). For each microsatellite, allelic determination was normalized by inclusion of reference samples on each gel.

Flow Cytometry. Five-microliter blood samples were suspended in PBS (50 μl) and then pelleted by microcentrifugation ($4.5–6 \times 10^3 \times g$). This washing procedure was repeated twice. After the second washing, the red blood cell pellet was resuspended in 50 μl of PBS/Fy6 mouse monoclonal antibody (23) (Fy6 was kindly provided by J. Barnwell, Centers for Disease Control and Prevention, Atlanta, GA) at a 1:50 ratio, then incubated for 15 minutes at 37°C. The samples were then washed twice in PBS, resuspended in 15 μl of PBS/goat anti-mouse Ig labeled with phycoerythrin at a 1:3 ratio, and incubated for 15 minutes at 37°C. Red blood cells were then washed twice in PBS and resuspended in 1 ml of PBS. Flow cytometry was performed with a Coulter Elite ESP flow cytometer; forward light scatter, side light scatter, and fluorescence data were analyzed.

Statistical Analysis. Odds ratios and confidence intervals were calculated by using standard methodologies and computed with the programs available through Simple Interactive Statistical Analysis (SISA, http://home.clara.net/sisa/two2hlp.htm). The association between microsatellites and Fy locus-specific markers was analyzed by using procedures described by Weir (24).

Results and Discussion

Genotyping Studies. PCR-based genotyping of α-globin polymorphism used strategies developed by Oron-Karni *et al.* (22) to amplify a 298-bp fragment downstream from the α2 gene and a 446-bp fragment downstream from the α1 gene. Consistent with other studies, the frequency of α^+-thalassemia ($-\alpha/-\alpha$ or $-\alpha/\alpha\alpha$) was ≥98% in both linguistic groups.

PCR-RFLP genotyping (Fig. 1*A*) of *FY* promoter (Fig. 1*B*) and open reading frame (ORF) (Fig. 1*C*) polymorphisms was performed for a West African (lane 1), an African-American (lane 2), and three Abelam speakers (lanes 3–5). The West African was homozygous for the inactive-GATA-1 site (65-bp fragment) and *FY*B* (363-bp fragment) RFLPs (*FY*B*^{null}/*FY*B*^{null}); the African-American was heterozygous for the GATA-1 site (65- and 77-bp fragments) and ORF (151-, 212-, and 363-bp fragments) RFLPs (*FY*A*/*FY*B*^{null}); the three Abelam speakers were heterozygous for the GATA-1 (65- and 77-bp fragments) and homozygous for *FY*A* (151- and 212-bp fragments) RFLPs (*FY*A*/*FY*A*^{null}). PCR-RFLP and DNA sequence analyses of cloned allelic amplicons confirmed that the 65-bp restriction fragment (lane 7) was due to the same T-46C transition observed in Fy(a−b−) Africans, and that this mutation was on the ORF allele encoding the Fy^a antigen (625 G). Within the PNG study population, 23 Abelam speakers were heterozygous, *FY*A*/*FY*A*^{null}, for this new allele (Table 1). This report of the *FY* GATA-1 mutation in PNG and of the association between the inactive GATA-1 promoter site and *FY*A* suggests the identification of an *FY*A*^{null} allele. The latter contrasts with the association between the GATA-1 mutation with *FY*B* observed in Africans (8) and African-Americans (*FY*B*^{null}; Tables 1 and 2), suggesting that this new allele is

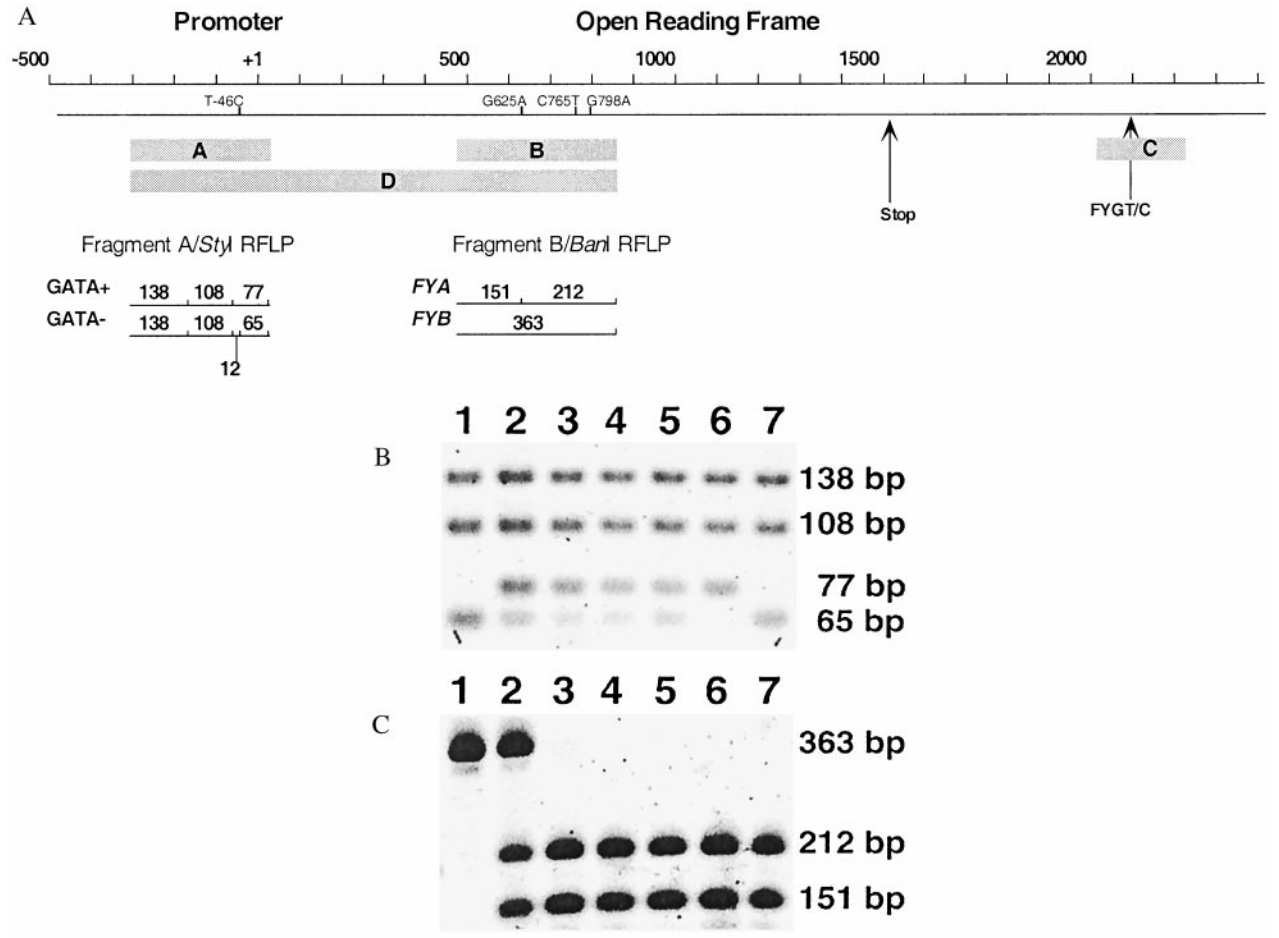

Fig. 1. (*A*) *FY* gene locus (human chromosome 1q22-23) and PCR-RFLP genotyping strategies for *FY* promoter and ORF (6–8). The scale at the top of the figure provides orientation to the *FY* gene sequence (−/+ coordinates relative to the transcription start site). Positions of previously characterized single nucleotide polymorphisms identified along the linear representation of the *FY* gene [by using nucleotide (amino acid) nomenclature consistent with erythroid gene product (8)] include T-46C, G625A (G44D), C765T (R91C), G798A (A102T). Promoter-specific (−304 to +25, 328 bp; fragment A) and ORF-specific (+474 to +851, 387 bp; fragment B) amplicons were subject to *Sty*I and *Ban*I restriction endonuclease digestion, respectively. *Sty*I RFLP analysis reveals polymorphism where the 77-bp fragment corresponds with −46T (active GATA-1 site) and the 65-bp fragment corresponds with −46C (inactive GATA-1 site). *Ban*I RFLP analysis reveals polymorphism where the 151- and 212-bp fragments correspond to the Fy^a-specific sequence 625G (encoding G) and the 363-bp fragment corresponds with the Fy^b-specific sequence 625A (encoding D). Additional polymorphisms include C765T (R91C), eliminating an *Aci*I restriction endonuclease cleavage specific for the $FY*B^{weak}$ allele (14), and G798A (A102T) eliminating an *Mwo*I restriction endonuclease cleavage site in the wild-type *FY* allele. PCR-based cloning and DNA sequence analysis were applied to the 3′ untranslated region microsatellite (fragment C, arrow) and contiguous promoter-ORF (fragment D) sequences. Allele-specific analysis of fragments C and D enabled precise determination of the linear order of DNA sequence polymorphism potentially obscured by heterozygosity in direct DNA sequencing approaches. (*B* and *C*) PCR-RFLP analysis of individual study subjects, lane 1; West African, lane 2; African-American, lanes 3–5; Papua New Guineans, lanes 6 and 7; cloned amplicons (fragment D) derived from the individual in lane 5. (*B*) Results from *Sty*I digestion of the promoter-specific amplicon-producing restriction fragments of 138, 108, 77, and/or 65 bp. The 12-bp product was not visualized after staining of agarose gels. (*C*) Results from *Ban*I digestion of the ORF-specific amplicon-producing restriction fragments of 363 and/or 212 and 151 bp. The figure shows the reverse SYBR Gold-stained image scanned with the Storm 860.

independent of African origin. Results on 400 North Americans (Table 2) illustrate that $FY*A^{null}$ is not observed in a more ethnically diverse study population where recombination between *FY*A* and $FY*B^{null}$ would be possible. Identification of $FY*B^{null}$ alleles in non-African populations (Table 2) is likely to be the result of admixture with the African-American genetic background. Earlier studies have observed African-based genetic polymorphisms in the coding region of *ICAM-1* (25) and

Table 1. Summary of *FY* alleles in PNG and African study groups

	South Wosera†	Dreikikir‡	West Africa
*FY*A*	1039	762	0
$FY*A^{null}$	23	0	0
*FY*B*	0	0	0
$FY*B^{null}$	0	0	400

†Abelam speakers.
‡Urat speakers.

Table 2. Summary of *FY* alleles in North American study groups†

	Caucasian-American	African-American	Asian-American	Hispanic-American
*FY*A*	91	24	159	103
$FY*A^{null}$	0	0	0	0
*FY*B*	109	31	33	90
$FY*B^{null}$	0	145	8	7

†Self-identified ethnicity.

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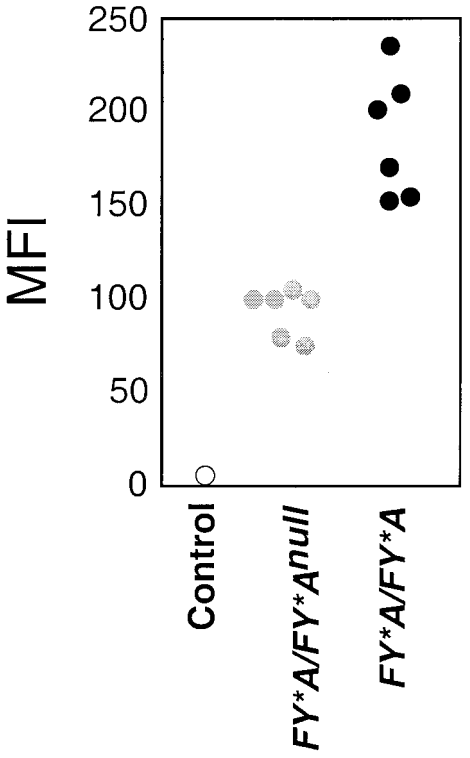

Fig. 2. Flow cytometric analysis of phycoerythrin-labeled Fy6-specific fluorescence intensity comparing erythrocytes from 12 PNG study subjects. Mean fluorescence intensity (MFI) is indicated on the *y* axis, and *FY* genotype is indicated along the *x* axis. MFI readings were compared for six PNG *FY*A*/*FY*A*^{null} individuals (shaded circles), 93.33 (SEM = 12.81), and six PNG *FY*A*/*FY*A* individuals (solid circles), 187.36 (SEM = 33.29), (Mann–Whitney $U = 36$, $P = 0.004$). The open circle represents the average MFI for experiments omitting primary (Fy6) and secondary phycoerythrin-labeled goat anti-mouse IgG incubation with erythrocytes from an individual with an *FY*A*/*FY*A* genotype (MFI = 5.98). In a separate experiment, the average MFI for individuals with the *FY*B*/*FY*B* genotype ($n = 2$) was 2.72 times greater than that for individuals with the *FY*B*/*FY*B*^{null} genotype ($n = 2$).

the *TNF-α* promoter (*TNFAp-4*; ref. 26) in Caucasian-, Hispanic-, and Asian-American populations studied here.

Flow Cytometric Analysis of Fy Expression. Evidence correlating *FY*A*^{null} with the Fy antigen-negative phenotype was developed by flow cytometry, comparing Fy6-specific (23) phycoerythrin labeling of erythrocytes from six PNG *FY*A*/*FY*A* and six PNG *FY*A*/*FY*A*^{null} individuals (Fig. 2). Results show a significant difference in Fy6-specific mean fluorescence intensity for the homozygous wild-type [187.36 (±33.29)] vs. the heterozygous [93.33 (±12.81)] genotypes (Mann–Whitney U, $P = 0.004$). These findings indicate a gene-dosage effect, where individuals with two erythroid-functional alleles express approximately twice the amount of Fy on erythrocytes compared with individuals with one erythroid-functional allele. Furthermore, these results illustrate that T-46C affects Fy expression on the *FY*A* and *FY*B* genetic backgrounds in a similar manner (Fig. 2 legend).

Association Between *FY* Genotype and *P. vivax* Infection. Given this reduction in Fy expression on erythrocytes of heterozygous individuals, it was next determined whether there was an association between *FY*A*/*FY*A*^{null} and reduced prevalence of *P. vivax* blood-stage infection. Although the prevalence of *P. vivax* infection was approximately 2-fold greater in *FY*A*/*FY*A* (83/508 = 0.163) compared with *FY*A*/*FY*A*^{null} (2/23 = 0.087)

*FY*A*^{null} - MS allele associations

Chr. 1map units (cM)	Chr. 1 MS loci	Allele	P-value	Adjusted P-value
	GATA13C08	4	ns	ns
160	*D1S1595*	3	ns	ns
	D1S1600	3	ns	ns
165	***D1S2635***	4	0.0003	0.0012
	ATA73A08	2	0.005	0.025
170				
175	*D1S1677*	4	ns	ns

Fig. 3. Test for association between microsatellite alleles and *FY*A*^{null}. Association between microsatellite and *FY*A* and *FY*A*^{null} alleles was performed on 47 *FY*A*/*FY*A* and 15 *FY*A*/*FY*A*^{null} unrelated individuals from South Wosera following the approach described by Weir (24). *P* values were adjusted (Bonferroni correction) for the number of alleles observed at each individual locus. Linear organization of microsatellites in relation to the *FY* locus was performed by using the "Build your own map" function, supplied by the Marshfield Center for Medical Genetics (http://www.marshmed.org/genetics). Genetic distances between *FY*-specific amplicons and microsatellite (MS) markers were determined following radiation hybrid mapping by using the G3 RH panel (Research Genetics) and the Stanford Human Genome Center Radiation Hybrid Mapping E-mail Server (http://www-shgc.stanford.edu/RH/rhserverformnew.html). *D1S2635* maps to within 0.3–0.6 cM of *FY*. ns, not significant.

individuals, this difference was not statistically significant (odds ratio = 2.05, 95% confidence interval = 0.47–8.91). Moreover, consistent with *in vitro* parasite invasion studies (10), these results show that heterozygous individuals are susceptible to infection by *P. vivax*. For other malaria species, prevalence of *P. falciparum* (*FY*A*/*FY*A*, 158/508 = 0.311; *FY*A*/*FY*A*^{null}, 5/23 = 0.217; odds ratio = 1.63, 95% confidence interval = 0.59–4.45) and *P. malariae* (*FY*A*/*FY*A*, 65/508 = 0.128; *FY*A*/*FY*A*^{null}, 3/23 = 0.130; odds ratio = 0.98, 95% confidence interval = 0.28–3.38) infection was similar based on *FY* promoter genotype. It is important to acknowledge that these findings provide only a preliminary assessment of differential susceptibility to *P. vivax* infection based on this new *FY* promoter genotype. More definitive examination of this issue requires a longitudinal, age-matched, case-control study in which individuals are treated to clear existing *Plasmodium* infection and then followed prospectively to compare not only incidence, but also time to infection in *FY*A*/*FY*A* and *FY*A*/*FY*A*^{null} individuals.

In contrast to *FY*B*^{null} fixation in the absence of *P. vivax* in Africa, which precludes further study of how this parasite acts as a selective agent for the T-46C promoter mutation, the apparent recent emergence of *FY*A*^{null} in PNG provides the opportunity to study the significance of this mutation for susceptibility to malaria infection and clinical morbidity. Support for a recent emergence of *FY*A*^{null}, relative to *FY*A*, is presented in Fig. 3. These findings identify specific microsatellite alleles associated with *FY*A*^{null} at *D1S2635* (adjusted $P = 0.0012$) and *ATA73A08* (adjusted $P = 0.025$) that map to within 3 cM of the *FY* gene locus on human chromosome 1. No significant associations were observed between microsatellite alleles and *FY*A*, indicating that longer time, enabling more genetic recombination, has obscured haplotype associations with the wild-type allele. Beyond this comparison of haplotype associations with the *FY*A* and *FY*A*^{null} alleles in the Abelam-speaking population, it would be premature to estimate when *FY*A*^{null} arose in this setting. To

assess properly the origin of this new allele would require broader sampling and assessment of population structure within Abelam-speakers (estimated population size, 40,000; ref. 27) and among neighboring communities.

Conclusion

The emergence of *FY*A*^{null} raises important questions regarding the complex relationships between malaria parasites and the human host. Whereas Duffy-negativity clearly explains resistance to *P. vivax* merozoites in individuals and host populations, little is known regarding the factors that contributed to fixation of the *FY*B*^{null} allele throughout ethnically diverse African populations. To complicate this discussion, although early studies have reported that *P. vivax* was responsible for significant disease and death in Britain (28), it has become consistently regarded as a "benign" or "non-lethal" malaria parasite. Therefore, the mechanisms through which it might act as a selective agent are not clear. The evolution of the *FY*A*^{null} allele and its emergence in a *P. vivax*-endemic region associate this very specific mutation with vivax malaria for a second time. This association may suggest that infection by this parasite cannot be benign and that it is influencing polymorphism in the human genome through mechanisms not previously considered.

Additionally, identification of *FY*A*^{null} in PNG must also be considered as an important variable with the potential to influence severe pathogenesis resulting from *P. falciparum* infection. Recent studies have proposed that α^+-thalassemia increases susceptibility to *P. vivax* infection to serve later as a natural vaccine against severe falciparum malaria. As the frequency of α^+-thalassemia was found to be $\geq$98% in the East Sepik Province groups studied here, this hypothesized heterologous immune phenomenon should be at work within these host populations. If this complex hypothesis is correct, an increase in the frequency of *FY*A*^{null} leading to Duffy-negativity and resistance to *P. vivax* blood-stage infection may be suppressed to maintain the proposed protective effect of *P. vivax* infection, because severe falciparum malaria kills millions of children each year. Alternatively, it may be necessary to reevaluate the proposed heterologous immunological relationship if the frequency of *FY*A*^{null} rises in this *P. vivax*-endemic region of PNG.

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1. Haldane, J. B. S. (1949) *Proc. Int. Congr. Genet.*, **8,** 267–273.
2. Weatherall, D. J. (1987) *Ann. Trop. Med. Parasitol.* **81,** 539–548.
3. Allison, A. C. (1964) *Cold Spring Harbor Symp. Quant. Biol.* **29,** 137–149.
4. Roychoudhury, A. K. & Nei, M. (1988) *Human Polymorphic Genes: World Distribution* (Oxford Univ. Press, New York).
5. Yenchitsomanus, P.-T., Summers, K. M., Bhatia, K. K., Cattani, J. & Board, P. G. (1985) *Am. J. Hum. Genet.* **37,** 778–784.
6. Tournamille, C., Le Van Kim, C., Gane, P., Cartron, J. P. & Colin, Y. (1995) *Hum. Genet.* **95,** 407–410.
7. Tournamille, C., Le Van Kim, C., Gane, P., Le Pennec, P. Y., Roubinet, F., Babinet, J., Cartron, J. P. & Colin, Y. (1998) *Blood* **92,** 2147–2156.
8. Tournamille, C., Colin, Y., Cartron, J. P. & Le Van Kim, C. (1995) *Nat. Genet.* **10**, 224–228.
9. Miller, L. H., Mason, S. J., Clyde, D. F. & McGinniss, M. H. (1976) *N. Engl. J. Med.* **295,** 302–304.
10. Mason, S. J., Miller, L. H., Shiroishi, T., Dvorak, J. A. & McGinniss, M. H. (1977) *Br. J. Haematol.* **36,** 327–335.
11. Chitnis, C. E. & Miller, L. H. (1994) *J. Exp. Med.* **180,** 497–506.
12. Barnwell, J. W. & Galinski, M. R. (1995) *Ann. Trop. Med. Parasitol.* **89,** 113–120.
13. Livingstone, F. B. (1984) *Hum. Biol.* **56**, 413–425.
14. Williams, T. N., Maitland, K., Bennett, S., Ganczakowski, M., Peto, T. E., Newbold C. I., Bowden, D. K., Weatherall, D. J. & Clegg, J. B. (1996) *Nature (London)* **383,** 522–525.
15. Allen, S. J., O'Donnell, A., Alexander, N. D., Alpers, M. P., Peto, T. E. A., Clegg, J. B. & Weatherall, D. J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 14736–14741.
16. Genton, B., Al-Yaman, F., Beck, H.-P., Hii, J., Mellor, S., Narara, A., Gibson, N., Smith, T. & Alpers, M. P. (1995) *Ann. Trop. Med. Parasitol.* **89,** 359–376.
17. Simmons, R. T., Gajdusek, D. C., Gorman, J. G., Kidson, C. & Hornabrook, R. W. (1967) *Nature (London)* **213,** 1148–1149.
18. Serjeantson, S. W., Board, P. G. & Bhatia, K. K. (1992) in *Human Biology in Papua New Guinea: The Small Cosmos*, eds. Attenborough, R. D. & Alpers, M. P. (Clarendon, Oxford), pp. 198–233.
19. Alexander, N. J. (1998) Ph.D. thesis (Univ. of Cambridge, Cambridge, U.K.).
20. Zimmerman, P. A., Dadzie, K. Y., De Sole, G., Remme, J., Alley, E. S. & Unnasch, T. R. (1992) *J. Infect. Dis.* **165**, 964–968.
21. Zimmerman, P. A., Phadke, P. M., Lee, A., Elson, L. H., Aruajo, E., Guderian R. & Nutman, T. B. (1995) *Hum. Immunol.* **42,** 233–240.
22. Oron-Karni, V., Filon, D., Oppenheim, A. & Rund, D. (1998) *Am. J. Hematol.* **58,** 306–310.
23. Nichols, M. E., Rubinstein, P., Barnwell, J., Rodriguez de Cordoba, S. & Rosenfield, R. E. (1987) *J. Exp. Med.* **166,** 776–785.
24. Weir, B. S. (1996) *Genetic Data Analysis* (Sinauer, Sunderland, MA).
25. Zimmerman, P. A., Wieseman, M., Spalding, T., Boatin, B. A. & Nutman, T. B. (1997) *Tissue Antigens* **50,** 645–656.
26. Zimmerman, P. A., Guderian, R. H. & Nutman, T. B. (1996) *Immunogenetics* **44,** 485–486.
27. Foley, W. A. (1992) in *Human Biology in Papua New Guinea: The Small Cosmos*, eds. Attenborough, R. D. & Alpers, M. P. (Clarendon, Oxford), pp. 136–149.
28. James, S. P. (1929) *Proc. R. Soc. Med.* **23,** 71–87.