Temporal changes in *Plasmodium falciparum* reticulocyte binding protein homolog 2b (*PfRh2b*) in Senegal and The Gambia

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**Abstract**

**Background:** The *Plasmodium falciparum* reticulocyte binding protein homolog 2b (*PfRh2b*) is an important *P. falciparum* merozoite ligand that mediates invasion of erythrocytes by interacting with a chymotrypsin-sensitive "receptor Z". A large deletion polymorphism is found in the c-terminal ectodomain of this protein in many countries around the world, resulting in a truncated, but expressed protein. The varying frequencies by region suggest that there could be region specific immune selection at this locus. Therefore, this study was designed to determine temporal changes in the *PfRh2b* deletion polymorphism in infected individuals from Thiès (Senegal) and Western Gambia (The Gambia). It was also sought to determine the selective pressures acting at this locus and whether prevalence of the deletion in isolates genotyped by a 24-SNP molecular barcode is linked to background genotype or whether there might be independent selection acting at this locus.

**Methods:** Infected blood samples were sourced from archives of previous studies conducted between 2007 and 2013 at SLAP clinic in Thiès and from 1984 to 2013 in Western Gambia by MRC Unit at LSHTM, The Gambia. A total of 1380 samples were screened for the dimorphic alleles of the *PfRh2b* using semi-nested Polymerase Chain Reaction PCR. Samples from Thiès were previously barcoded.

**Results:** In Thiès, a consistent trend of decreasing prevalence of the *PfRh2b* deletion over time was observed: from 66.54% in 2007 and to 38.1% in 2013. In contrast, in Western Gambia, the frequency of the deletion fluctuated over time; it increased between 1984 and 2005 from (58.04%) to (69.33%) and decreased to 47.47% in 2007. Between 2007 and 2012, the prevalence of this deletion increased significantly from 47.47 to 83.02% and finally declined significantly to 57.94% in 2013. Association between the presence of this deletion and age was found in Thiès, however, not in Western Gambia. For the majority of isolates, the *PfRh2b* alleles could be tracked with specific 24-SNP barcoded genotype, indicating a lack of independent selection at this locus.

**Conclusion:** *PfRh2b* deletion was found in the two countries with varying prevalence during the study period. However, these temporal and spatial variations could be an obstacle to the implementation of this protein as a potential vaccine candidate.

**Keywords:** *Plasmodium falciparum*, *PfRh2b*, SNPs, Senegal, Gambia

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Background

Despite significant efforts and progress directed towards malaria prevention and control, malaria from Plasmodium falciparum parasite infection remains a major global health challenge. This is due to multiple factors, including insecticide resistance in anopheline vectors, the emergence and rapid spread of drug-resistant parasite strains and especially the lack of an effective vaccine.

Fig. 1 Evolution of PRRh2b deletion in Thiès (a) and Western Gambia (b). Scheme showing the variation in the prevalence of PRRh2b deletion over the time, including mixed infections (PRRh2 full-length and PRRh2b deletion) in both areas using formula described previously by Ahouidi et al. [10]. Alleles frequencies of the dimorphic PRRh2b gene were determined using semi-nested Polymerase Chain Reaction (PCR). a A decrease of PRRh2b deletion from 2007 to 2013 in Thiès was noted. b A fluctuation of this deletion from 1984 to 2013 in The Gambia was observed. N, number of isolates; Del, deletion.
Therefore, the development of an effective malaria vaccine remains critical for malaria eradication. However, efforts at developing a malaria vaccine have been hampered by the extensive genetic diversity in malaria parasite populations and allele specific immunity in endemic human populations [1, 2].

Characterization of genetic polymorphisms in key vaccine antigens of P. falciparum will enable a better understanding of the molecular evolution of parasite populations that could affect efficacy of future vaccines [3]. Plasmodium falciparum Reticulocyte binding protein homologues (PfRhs) are expressed at the apical surface of invasive merozoite [4–7] and are believed to play a role in the recognition of the erythrocyte and in tight junction formation [8]. Towards design of a vaccine, several studies are looking at the immunity and diversity of the PfRh family of proteins [6, 8–13]. There are five functional PfRh genes: P. falciparum Reticulocyte-binding protein homolog 1 (PfRh1) [14, 15], PfRh2a, PfRh2b [16–18], PfRh4 [19, 20], and PfRh5 [21–23]. Two members of the P. falciparum Rh ligands, PfRh2a and PfRh2b, are important mediators of parasite invasion. PfRh2b mediates invasion by interacting with a chymotrypsin-sensitive erythrocyte receptor Z [24]. These proteins are currently being assessed as invasion-blocking vaccine candidates [11, 25]. However, they differ structurally at the c-terminal.

The c-terminal region of PfRh2b gene presents a large structural polymorphism (0.58 kb) and this was found at high frequencies in field isolates from different areas of Africa [10, 26, 27]. The prevalence of this deletion was characterized for the first time in 2006 in parasite populations from different areas [10]. However, the prevalence and evolution of the PfRh2b protein as malaria declines remains unknown. Understanding the changes over time in the frequencies of allelic variants in potential vaccine candidate will be important, as these changes could affect the efficacy of a candidate vaccine. Therefore, it is important to assess temporal changes in the PfRh2b gene and prevalence of the dimorphic alleles PfRh2b deletion (PfRh2bdel) over time in endemic populations.

As shown for Thiès, in Senegal, neutral single nucleotide polymorphism (SNP) markers can sensitively determine temporal changes in P. falciparum genotypic diversity [28]. There are extensive hotspots of diversity across the genome, mostly within genes exposed on the surface of the parasite and erythrocyte, including invasion ligands. These ligands are a target of immune responses and remain a major focus for development of a blood stage vaccine [29, 30].

As the frequency of genotypes changes as well as the PfRh2b alleles, the prevalence of the deletion was determined using molecular barcode to examine whether allelic frequencies are strictly related to barcode haplotype clusters or independent selection at the PfRh2b locus could be driving frequencies in the population. This provides data relevant for further consideration of PfRh2b and other structurally variant proteins as targets for vaccine development.

Methods

Study sites and Plasmodium falciparum field isolates

Archived P. falciparum DNA samples from infected blood samples of consenting individuals were sourced from previous studies conducted between 2007 and 2013 at the Service de Lutte Anti-Parasitaire (SLAP) clinic in...
Thiès (70 km from Dakar, the capital city of Senegal) and from 1984 to 2013 in Western Gambia. Overall, malaria prevalence is moderate in The Gambia with high seasonal transmission [31, 32]. In contrast, Thiès is characterized by a perennial hypo-endemic transmission. These studies had received ethical approval from the Institutional Review Boards of the Harvard School of Public Health, the Ethics Committee of the Ministry of Health in Senegal and the Joint Gambian Government/MRCG Ethics Committee. A total of 1380 (849 from Thiès and 531 from Western Gambia) *P. falciparum* malaria infected blood samples were analysed. Among the Thiès samples, 580 that has been previously genotyped using a molecular barcode of 24 SNPs by Daniels et al. [33] were used for determining the association between alleles of *PfRh2b* and the specific barcode of parasite.

**PfRh2b genotyping**

Semi-nested PCR method, as described previously [10], was used to amplify an *PfRh2b* gene fragment including the deletion in all extracted DNA samples. A positive control (*P. falciparum* laboratory cloned line 3D7) and negative control (reagent grade water) were included in all PCR amplifications. The size of the PCR products was estimated using Gene Ruler 100 bp DNA ladder marker (Quick Load®, 100pb DNA Ladder).

**Statistical analysis**

For continuous variables median and interquartile range were calculated, while for categorical variables, the proportion or prevalence of the outcome with 95% CI was calculated.

For each year, to take into account the prevalence in mixed genotype isolates, the prevalence of *PfRh2b* full-length and *PfRh2b* deletion was calculated as follows:

\[
\text{prevalence} = \frac{\text{number of } PfRh2b \text{ allele isolates} + \text{0.5*number of mixed isolates}}{\text{total number of isolates}}
\]

The Chi square linear trends was used to determine if the differences in frequency over the years was statistically significant. Differences between groups were assessed using Mann–Whitney U-test. Wright’s fixation index (*Fst*) was also calculated to assess the extent of genetic differentiation of *PfRh2b* polymorphism in Thiès and Western Gambia over time.

**Results**

**Evolution of the prevalence of *PfRh2b* deletion in fields isolates from Thiès and Western Gambia**

In Thiès, the prevalence of the deletion decreased significantly from 66.54% in 2007 to 38.1% in 2013 (P < 0.0001). This decline was not homogeneous with the presence of a peak in 2012, where *PfRh2bdel* form was present in 43.62% of infections (Fig. 1a).

In Western Gambia, temporal variation in prevalence in *PfRh2b* deletion was observed between 1984 and 2013. The prevalence of *PfRh2b* deletion increased between 1984 and 2005 from (58.04%) to (69.33%) (P = 0.03). From 2005 to 2007 there was a decline of the deletion to (47.47%) (P = 0.004). Between 2007 and 2012, the prevalence of this deletion increased significantly from 47.47 to 83.02% (P = 0.00005) and finally declined significantly to 57.94% in 2013 (P = 0.001) (Fig. 1b). Since mixed

**Table 1** Allele frequencies used to calculate *Fst* according to years

| Years | THIES | | | WESTERN GAMBIA | | |
|-------|-------|-------|-------|----------------|-------|-------|
|       | PfRh2bdel | PfRh2bfull | Gene diversity (h) | PfRh2bdel | PfRh2bfull | Gene diversity (h) |
| 1984  | –      | –      | 0.58   | 0.42   | 0.487 |
| 2005  | –      | –      | 0.69   | 0.31   | 0.428 |
| 2007  | 0.67   | 0.33   | 0.442  | 0.47   | 0.53   | 0.498 |
| 2008  | 0.65   | 0.35   | 0.455  | 0.66   | 0.34   | 0.449 |
| 2009  | 0.58   | 0.42   | 0.487  | –      | –      | –      |
| 2010  | 0.39   | 0.61   | 0.372  | 0.73   | 0.27   | 0.394 |
| 2011  | 0.37   | 0.63   | 0.466  | –      | –      | –      |
| 2012  | 0.44   | 0.56   | 0.493  | 0.83   | 0.17   | 0.282 |
| 2013  | 0.38   | 0.62   | 0.471  | 0.58   | 0.42   | 0.487 |
| Average | 0.497 | 0.503 | 0.455 | 0.648 | 0.351 | 0.432 |

*Fst* = 0.09

*Fst* = 0.057

Temporal differentiation of *PfRh2b* polymorphism in Thiès and Western Gambia populations. *Fst* value was calculated to assess the extent of temporal variation in the frequencies of *PfRh2b* alleles in both areas. The overall estimated value of *Fst* from 2007 to 2013 in Thiès was 0.09. In Western Gambia the *Fst* value from 1984 to 2013 was 0.057

*Fst*, wright’s fixation index; h, gene diversity
Table 2  Nucleotide sequence of haplotype clusters in Thiès

| Haplotype cluster       | Molecular barcodes   | N (%) | N (isolats) | PfRh2bdel (%) | PfRh2bfull (%) |
|-------------------------|----------------------|-------|-------------|---------------|---------------|
| Haplotype cluster 36    | CACTCGAGACCCGACCCCAAGCCTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 38    | CACTCGAGATCGTACACACGCATTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 45    | TATCCGGATCGTCCCTCCCTGCTTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 51    | TACTCCGGTTCCGACACACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 49    | TACTTGAATATGCACCTGAGTTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 48    | TACTCGAGATCCCTACACACGATTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 46    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 57    | CACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 54    | CGCTCCGAGACTACACCTAAGCTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 53    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 59    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 61    | TACCCCGAGATTTGACCTAAGCTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 15    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 63    | TACTCCGAGACCCGACCCCAAGCCTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 9     | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 62    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 13    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 68    | TACCCCGAGACCCCAATAAGCTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 69    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 67    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 70    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 22    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 74    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 71    | CACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 80    | CACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 72    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 77    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 97    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 87    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 89    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 92    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 84    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 96    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 50            | 50            |
| Haplotype cluster 93    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 44    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 43    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 58    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 33            | 67            |
| Haplotype cluster 7     | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 33            | 67            |
| Haplotype cluster 19    | TACTCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 21    | TACCCCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 23    | TACCCCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 95    | TACCCCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 0             | 100           |
| Haplotype cluster 99    | TACCCCGAGATGTTGACCCAAACACTG | 0.345 | 2           | 100           | 0             |
| Haplotype cluster 55    | TACCCCGAGATGTTGACCCAAACACTG | 0.69  | 4           | 100           | 0             |
| Haplotype cluster 64    | TACCCCGAGATGTTGACCCAAACACTG | 0.69  | 4           | 0             | 100           |
| Haplotype cluster 10    | TACCCCGAGATGTTGACCCAAACACTG | 0.69  | 4           | 100           | 0             |
| Haplotype cluster 24    | TACCCCGAGATGTTGACCCAAACACTG | 0.69  | 4           | 25            | 75            |
| Haplotype cluster 81    | TACCCCGAGATGTTGACCCAAACACTG | 0.69  | 4           | 75            | 25            |
| Haplotype cluster 98    | TACCCCGAGATGTTGACCCAAACACTG | 0.69  | 4           | 25            | 75            |
infections are uncommon in endemic populations, the frequency of infections with both deleted and full-length parasites (mixed) was determined. The result was highest in 2007 (0.10) and lowest in 2009 (0.02) in Thiès. In Western Gambia, mixed infections were most common in the earliest population from 1984 (0.23) and lowest in 2008 with (0.04) (Additional file 1: Table S1).

Overall, a significant decrease of the prevalence of \( PfRh2b \) deletion from 2007 to 2013 in Thiès (\( P < 0.0001 \)) and fluctuating prevalence from 1984 to 2013 in Western Gambia were observed.

**Prevalence of \( PfRh2b \) deletion according to age in Thiès and western Gambia**

The presence of the deletion at high frequency in the general population and the acquisition of antibodies in an age-dependent manner against the c-terminal region of \( PfRh2b \) [10], have raised the interest to determine the prevalence of this deletion by age-group to see if the deletion would be more frequent in adults since they are long exposed. Thus, among the 1380 \( Plasmodium \) isolates assayed, 934 samples were available for age data and were analysed (651 from Thiès and 283 from Western Gambia). A median age was used to divide each population into two numerically equal groups.

In Thiès, since the study population mainly consists of adults (62%), the median age of the 651 patients was 18 years. Thus, the \( PfRh2b \) deletion form was less common in children compared to older patients (Fig. 2a). Using the Mann–Whitney U-test, a significant difference in the presence of \( PfRh2b \) deletion was found (\( P = 0.037 \)), suggesting that there is an association between age and the presence of this deletion in Thiès.

In contrast, in Western Gambia, the population study is consisting of children (under 16 years of age); the median age of the 283 patients was 5 years. The \( PfRh2b \) deletion was more common in younger children compared to older children (Fig. 2b), but the difference is not significant by the Mann–Whitney U-test (\( P = 0.316 \)).

**Temporal differentiation of \( PfRh2b \) polymorphisms in Senegal and Gambia populations**

Genetic diversity of the genes in natural parasites populations is a real obstacle for the validation of potential vaccine candidate. In this study, the degree of divergence to which \( PfRh2b \) gene is subject to selection was estimated by calculating \( Fst \) from the allelic frequencies of this locus according to years.

In Senegal the \( Fst \) value obtained from 2007 to 2013 was high (0.09), suggesting high degree of allelic divergence in this region over the time. In contrast, the \( Fst \) value obtained in The Gambia from 1984 to 2013 was low (0.057), suggesting less genetic differentiation of these alleles in this area over the time (Table 1).

**Prevalence of \( PfRh2b \) in isolates grouped by molecular barcode**

It has been observed by barcoding in Thiès that the parasite, having undergone various interventions and pressures, has adapted by evolving towards to a type of parasite [28]. It is also assumed that parasite, initially having the \( PfRh2b \) full-length, has adapted by presenting the deletion following the different pressures. To test the hypothesis that the parasite with \( PfRh2b \) deletion may be linked by specific cluster, the prevalence of \( PfRh2b \) deletion according to molecular barcode was determined.
The number of samples is differently distributed in each of the 20 haplotype clusters with the deletion, and this distribution varies also over time (Table 3). Evolution over time of the 20 haplotype clusters containing only the PfRh2b deletion shows a decrease of the prevalence between 2008 and 2010 as well as a slight increase between 2010 and 2013 (Fig. 3). Since the numbers in the majority of the cluster group with the PfRh2b deletion are limited, haplotypes with n > 5 were examined further (haplotype 4 and 16) (Fig. 4). Haplotype cluster 4 with the deletion was found in 2008 (n = 12) and 2009 (n = 3), but not in the other years. Meanwhile, haplotype cluster 16 with the deletion was present in 2011 (n = 1), and 2012 (n = 6), but not in the other years (Fig. 5). The analysis of the results over time suggests that the frequency of PfRh2b deletion is related to the presence of some haplotype clusters in this population.

Among the samples that were analysed for PfRh2b polymorphism, 580 from Thiès had been previously genotyped using the 24-SNP barcode [33] and the results were classified into two groups: a group named “cluster” which includes the samples having similarities with at least one other isolate in their nucleotide sequences and a group called “unique” grouping parasites with unique SNP barcode. 41.73% (242/580) of samples belonged to the cluster-group and 58.27% (338/580) were in the unique group (Table 2).

No significant difference in the frequency of PfRh2b deletion between the cluster-group and the unique group was found (P = 0.5014). Among the 338 isolates having unique barcodes, 166 (49.11%) had the deletion and 172 (50.89%) had the full-length fragment of PfRh2b gene. Therefore, both alleles PfRh2b deletion and PfRh2b full-length, had a similar distribution within the unique genotype group. Furthermore, all the 242 isolates belonging to the cluster-group were also tested to assess the frequency of PfRh2b alleles. In this group, 62 distinct subgroups were identified. The results showed that 20 haplotype clusters had only the deletion variant of PfRh2b gene, 24 subgroups had only the full-length fragment and the remaining 18 subgroups were parasites having the deletion and full-length (Table 2).

Table 3 Frequency of the deletion over time within each cluster that contain only the deletion

| Cluster | Samples | Molecular barcode | Sample ID | Del (%) |
|---------|---------|-------------------|----------|---------|
| Cluster 63 | Th 107.2010 | TACGCACTGTGACTATGATGATG | Th 107.2010 | 1 0 |
| Cluster 64 | Th 108.2010 | TACGCACTGTGACTATGATGATG | Th 108.2010 | 1 0 |
| Cluster 65 | Th 109.2010 | TACGCACTGTGACTATGATGATG | Th 109.2010 | 1 0 |
| Cluster 66 | Th 110.2010 | TACGCACTGTGACTATGATGATG | Th 110.2010 | 1 0 |
| Cluster 67 | Th 111.2010 | TACGCACTGTGACTATGATGATG | Th 111.2010 | 1 0 |
| Cluster 68 | Th 112.2010 | TACGCACTGTGACTATGATGATG | Th 112.2010 | 1 0 |
| Cluster 69 | Th 113.2010 | TACGCACTGTGACTATGATGATG | Th 113.2010 | 1 0 |
| Cluster 70 | Th 114.2010 | TACGCACTGTGACTATGATGATG | Th 114.2010 | 1 0 |
| Cluster 71 | Th 115.2010 | TACGCACTGTGACTATGATGATG | Th 115.2010 | 1 0 |
| Cluster 72 | Th 116.2010 | TACGCACTGTGACTATGATGATG | Th 116.2010 | 1 0 |
| Cluster 73 | Th 117.2010 | TACGCACTGTGACTATGATGATG | Th 117.2010 | 1 0 |
| Cluster 74 | Th 118.2010 | TACGCACTGTGACTATGATGATG | Th 118.2010 | 1 0 |
| Cluster 75 | Th 119.2010 | TACGCACTGTGACTATGATGATG | Th 119.2010 | 1 0 |
| Cluster 76 | Th 120.2010 | TACGCACTGTGACTATGATGATG | Th 120.2010 | 1 0 |
| Cluster 77 | Th 121.2010 | TACGCACTGTGACTATGATGATG | Th 121.2010 | 1 0 |
| Cluster 78 | Th 122.2010 | TACGCACTGTGACTATGATGATG | Th 122.2010 | 1 0 |
| Cluster 79 | Th 123.2010 | TACGCACTGTGACTATGATGATG | Th 123.2010 | 1 0 |
| Cluster 80 | Th 124.2010 | TACGCACTGTGACTATGATGATG | Th 124.2010 | 1 0 |
| Cluster 81 | Th 125.2010 | TACGCACTGTGACTATGATGATG | Th 125.2010 | 1 0 |
| Cluster 82 | Th 126.2010 | TACGCACTGTGACTATGATGATG | Th 126.2010 | 1 0 |
| Cluster 83 | Th 127.2010 | TACGCACTGTGACTATGATGATG | Th 127.2010 | 1 0 |
| Cluster 84 | Th 128.2010 | TACGCACTGTGACTATGATGATG | Th 128.2010 | 1 0 |
| Cluster 85 | Th 129.2010 | TACGCACTGTGACTATGATGATG | Th 129.2010 | 1 0 |
| Cluster 86 | Th 130.2010 | TACGCACTGTGACTATGATGATG | Th 130.2010 | 1 0 |
| Cluster 87 | Th 131.2010 | TACGCACTGTGACTATGATGATG | Th 131.2010 | 1 0 |

Fig. 3 Evolution over time of PfRh2b deletion in haplotype clusters having only the deletion in Thiès
Discussion

*Plasmodium falciparum* reticulocyte binding protein homolog 2b (*PfRh2b*), has been reported to present a large structural polymorphism (0.58 kb deletion) in the c-terminal region. It is an important target of immunity [10] and could be considered as a potential candidate for blood stage vaccine development. Previous works showed that *PfRh2b* deletion was highly prevalent in isolates from Senegal and Africa [10, 26, 27] and present in different populations of the world [10]. However, malaria prevalence has been declining and interventions are taking place across sub-Saharan Africa. As these may be selecting for parasite subtypes, the changes imposed on this PfRh2b protein remains unknown. Therefore, the goal of this study was to follow the temporal evolution of the prevalence of *PfRh2b* deletion using samples from 2007 to 2013 in Thiès and from 1984 to 2013 in Western Gambia.

For the two populations, the prevalence of the deletion variant of *PfRh2b* ranged from 30 to 80%. However, while the prevalence of this deletion declined in Senegal, there was a steady increase in the Gambian populations studied until 2012. The differences in the trends between the two countries and within each country can be the result in random shifts in parasite allele frequencies in different geographic region due to genetic drift in isolated populations. Other explanations could be the genetic background of the host, the environmental modification, immune pressure, drug pressure and or drug resistance [28, 34].

Furthermore, the prevalence of *PfRh2b* deletion in different age groups in Thiès showed that the deletion was
significantly less present in children compared to older patients. In Western Gambia, the prevalence of PfRh2b deletion was more common in younger children compared to older children, but the difference is not significant. With those results observed in these two sites, the association between age and deletion is not clear. However, a previous study did not find a relation between age and deletion [10].

Moreover, the evaluation of temporal variation of PfRh2b polymorphism in Senegal and Gambia populations was performed by using Fst to estimate the degree by which this locus is subject to a selection. Indeed, selection intensity can lead to differences in diversity and generate divergence among natural populations.

Thus, the Fst value (0.09) in Thiès, from 2007 to 2013 is higher than those observed for PfRh2b, Msp2, EBA 175 and Pf48/45 within Senegal and between African countries [10]. However, this Fst value was lower than those observed for Msp2 and EBA 175 dimorphisms, which has been reported to exhibit less genetic differentiation and possible balancing selection in the global populations (African, South East Asian and Latin American populations) [10]. In Western Gambia, the Fst value was low and similar to that observed for the EBA 175 dimorphism within Senegal, which has been shown to have also minimal genetic differentiation globally [10]. Overall, the Fst values obtained in this study indicate less genetic differentiation suggesting that PfRh2b polymorphism is under balancing selection over time in Thiès and Western Gambia.

Additionally, the prevalence of PfRh2b deletion according to the molecular barcode of isolates from Thiès was analysed to determine whether the deletion is associated with a specific barcode in this region. Analysis of the distribution of PfRh2b polymorphism in shared barcode clusters suggests that deletion is associated at some haplotype clusters in the population. However, the removal of large clusters from the population do not effect the prevalence of the deletion over time. The results of the study provide information on the genetic diversity of the PfRh2b gene that could be useful in the validation of this antigen as a vaccine candidate.

**Conclusion**

Temporal trends in the frequency of the deleted PfRh2b variant differed in Senegal and The Gambia. This may suggest an effect of local factors on the prevalence of PfRh2b deletion between the years. Changes in the frequency of PfRh2b polymorphism over the time could be an obstacle to the implementation of this protein as a potential vaccine candidate as allele specific immunity may affect its efficacy. It will be important to investigate the natural antibodies responses against PfRh2b over the time in different malaria endemic countries to evaluate this antigen as a vaccine candidate.

**Additional file**

**Additional file 1: Table S1.** Number of samples by year of PfRh2b polymorphism in Thiès and Western Gambia. Column N shows the number of samples analysed. n = the number of samples of each allele. PfRh2b-del = deletion present; PfRh2b-full = full-length sequence; Mix = PfRh2bDel/PfRh2bfull.

**Abbreviations**
PfRh2b: Plasmodium falciparum reticulocyte binding protein homolog 2b; PfRh2b-del: PfRh2b deletion; PfRh2b-full: PfRh2b full-length; DBL: Duffy binding-like family; SNPs: single nucleotide polymorphisms; SLAP: Service de Lutte Anti-parasitaire; MRC: Medical Research Council; DNA: deoxyribonucleic acid; PCR: polymerase chain reaction.

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**Authors’ contributions**

CKD performed experiments, analysed the data and wrote the manuscript. RAM performed experiments and analysed data. ADA designed and supervised the study, edited and reviewed the manuscript. AKB designed the study, contributed to the analyses of data, and reviewed the manuscript. AAN, RFD and NPM contributed to the analyses of data and reviewed the manuscript. DFW, DN, NF and SM reviewed final version of the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets generated and analysed during the current study are included within the article.

**Ethics approval and consent to participate**

These studies were approved by the Institutional Review Boards of the Harvard School of Public Health, the Ethics Committee of the Ministry of Health in Senegal and the Joint Gambian Government/MRC Ethics Committee. Written informed consent was obtained directly from adult subjects and from parents or other legal guardians of all participating children.

**Consent for publication**

Written informed consent was obtained from all enrolled patients for publication of this study.

**Competing interests**

The authors declare that they have no competing interests.

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