G6PD and HBB polymorphisms in Senegalese population: prevalence and correlations with clinical malaria

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Research

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Running title

G6PD and *HBB* polymorphisms in Senegalese population: prevalence and correlations with clinical malaria.

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Abstract

Background

Several genetic polymorphisms were reported to be prevalent among populations living in tropical endemic regions and induce protection against malaria. In this study, we investigated the prevalence of key malaria-protective polymorphisms in G6PD and HBB genes in a Senegalese population.

Methods

We performed a retrospective study in 323 samples from patients recruited in hospitals located in three different endemic areas where malaria episodes were confirmed. Patients enrolled were classified in two groups: severe (153 patients) and uncomplicated malaria (170 patients). PCR and DNA sequencing assessed host genetic polymorphisms in HBB and G6PD. Using a multivariate regression and additive model, estimates the impact of human HBB and G6PD polymorphisms on malaria incidence were performed.

Results

We identified 12 SNPs in HBB gene. Among them, 6 SNPs (rs7946748, rs7480526, rs10768683, rs35209591, HbS (rs334) and rs713040) were detected with high frequencies in global population. The MAF of the sickle cell HbS polymorphism was estimated to 0.026, 0.069 and 0.035; and HbC polymorphism was estimated to be 0, 0.009, 0.029, in SM, UM and CTR group respectively. The MAF of G6PD deficiency polymorphisms such as G6PD-202 G>A were 0.022, 0.032 and 0.018 in SM, UM and CTR, respectively. Analysis of selected HbS polymorphism showed significant association with protection against severe malaria with a significant *p-value* = 0.033 (OR 0.38, 95%CI: 0.16–0.91). Surprisingly, HbC polymorphism is not a protective variant in our population. Finally, we found that the selected SNPs were associated to biological parameters such as PNE, PNB and lymphocytes.

Conclusion

Our data report at the first time the prevalence of HBB and G6PD mutations in senegalese population. These deficiencies are very common in West Africa endemic regions such as Gambia, Mali and Burkina Faso. Our findings show the important role of genetic factors in malaria outcome and these genetic markers could be good tools for malaria diagnosis and prognosis.

Keywords: severe malaria, HBB, G6PD, Polymorphisms, Senegal, severe malaria.

1. Background

Malaria caused by *Plasmodium* species affects hundreds of millions of people per year and forms the major cause of death in the world. In 2017, an estimated 219 million cases of malaria occurred worldwide. Moreover, most of them (92%) affect children living in sub-Sahara Africa region followed by South-East Asia region (5% of the cases) and the Eastern Mediterranean region with 2%. Per World Health Organization, there were an estimated 20 million malaria cases in 2017, and data for the period 2015–2017 highlighted a non-significant progress in reducing global malaria cases **[1]**. The mortality of malaria remains higher was estimated to 435000, and the comprehension of these mechanisms are necessary for global malaria eradication **[2-4]**.

Host genetic factors contribute to the variability of malaria phenotypes **[3]** and can allow better understanding of mechanisms involved in susceptibility and/or resistance to *P. falciparum* infection outcome [5]. Several genetic polymorphisms were reported to be prevalent among populations living in tropical malaria endemic regions and induce protection against malaria **[5, 6]**. Mutations in Glucose-6-phosphate dehydrogenase (*G6PD*) and β-chain of hemoglobin (*HBB*) genes are responsible for sickle cell trait and several globinopathies such as G6PD enzyme deficiency and thalassemia. As known, G6PD and HBB deficiencies are more prevalent in malaria-endemic countries and are two of the most important loci conferring resistance to severe malaria in humans **[2]**.

In endemic areas, the protective effect of *HBB* variants (HbS and HbC) on *P. falciparum* malaria clinical phenotypes were reported **[7]**. HbC is prevalent only in West Africa **[8, 9]** and HbS is encountered at frequencies up to 18% across sub-Saharan Africa, the Middle East and south Asia. HbS homozygoty is responsible for disease and is associated to mortality rates while HbC homozygosity is clinically benign, as HbAS and HbAC heterozygous traits **[9]**. The *HBB* mutations are known to confer protection against *P. falciparum* malaria **[10-15]**. HbAS has been associated with 50% and 80% reduced risks of developing uncomplicated and severe malaria **[15]**, and HbAC and HbC have been associated with 30% and 93% reduced risks of developing mild and severe malaria, respectively **[11]**. Additional studies have confirmed the protective effects of HbC **[12, 16, and 17]**.

G6PD deficiency affects over 400 million people living in tropical and subtropical countries, 15-30% of whom were founded in sub-Saharan Africa **[18-20]**. A high diversity of G6PD variants has been reported, including the most common form G6PD B (wild type enzyme), which is characterized by alleles G and A at the 202 and 376 nucleotides of the gene (202G-376A), respectively. In sub-Saharan Africa, up to 40% of the population carries the G6PD A form (non-deficient type) characterized by 202G-376G allele with 15% reduction in enzyme activity **[21]**. The presence of an added mutation at the 202 nucleotide (G > A), the double mutant (202A-376G) is named G6PD A with 12% of enzyme activity compared to the B allele **[22]**. Although, the 202A- allele has been associated with protection against severe malaria in African populations, such as in Mali, in Gambia and Uganda **[23, 24]**, associations for this polymorphism have been less consistent than for sickle hemoglobin and α-thalassemia **[25]**.

High prevalence of malaria protective polymorphisms is clearly associated with endemicity, but prevalence varies among populations living in endemic areas. Some of this difference can be explained by local malaria risk, as suggested by decreased prevalence of protective polymorphisms with increasing altitude in endemic countries **[26]**. The prevalence of some protective polymorphisms has also been shown to vary between ethnic groups **[26]**.
In Senegal, no data have been obtained concerning prevalence of G6PD and HBB deficiencies. In Gambia, a neighbor country of Senegal, it was reported that the minor allele frequency of G6pD-202A in control sample was 0.03, considerably lower than reports from eastern African countries such as Kenya (0.18) and Malawi (0.19) **[20]**. In addition, the prevalence of HBB and G6PD deficiencies polymorphisms, among West African populations, present a major challenge in a context of decreasing and/or malaria pre-elimination.

Our present study aimed to investigate the prevalence of key malaria-protective polymorphisms in G6PD and HBB genes in a Senegalese population. Senegal is West African country, where malaria risk varies between regions and ethnic diversity is great. We characterized polymorphisms on *HBB* and G6PD genes in residents from three ecological zones where the malaria endemicities were different. Having more data on G6PD and HBB polymorphisms and their incidence on malaria outcome and haematological parameters is important for different strategies of malaria eradication in endemic western African countries.

2. Material and Methods

2.1. *Ethics Statement*

Objectives of the study were explained clearly using local dialect before performing inclusion of patients in hospitals centers. Protocol has been review according to the rules issued by the National Committee for Ethics for Health Research (CNERS) of Senegal, and in accordance with the procedures established by the University Cheikh Anta Diop of Dakar (UCAD) for the ethical approval of any research involving human participants. Written informed consent was obtained from adult participants and from parents, or legal representatives of children. In addition, based on the information provided, UCAD's Committee on Research and Ethics (CER) considers that the research proposed, respects the appropriate ethical standard and, as a result, approves its execution under "Protocole 0344/2018/CER-UCAD". All patients enrolled in the cohort/ or legal representative gave informed consent after signed and informal written consent to provide a blood sample for furthers studies.

2.2. *Study sites*

The cohort included black senegalese-born individuals recruited between 2003 and 2015, whose parents and grandparents were born in Senegal, a malaria-endemic country in the Sahelian zone of West Africa. Malaria patients were enrolled from five regions in Senegal: St Louis, Louga, Dakar, Tambacounda and Kolda **(Figure 1)**. For commodity, these 5 regions were divided into 3 ecological areas according to the climatic gradient, which is translated by a north-south gradient according the prevalence of malaria. The northern part that presents sahelian climates with an unstable or seasonal malaria included St Louis and Louga. This part is a rural zone where malaria is hypoendemic and where annual incidence in 2017 was less than five cases per 1000 inhabitants, and in some sites under 1/1000 with very low anopheles vector density. The center part is Dakar, which represents an urban pool where the prevalence rate remains low, however health conditions are different with better disease coverage. The southern part includes Kolda, Tambacounda, and corresponding to Sudano-Guinean zone, with annual rainfall exceeding 800 mm **[27]**. In southern regions, permanently transmitting malaria is hyperendemic with and immunity is acquired in the first

five years of life. The high transmission season in Senegal occurs mainly between July and October **[28, 29]** .

2.3. *Sampling, malaria cohort and controls subjects*

Malaria patients corresponded to subjects with Plasmodium-positive QBC, a test that is more sensitive than the Giemsa thick stain **[30]**. The malaria patients were assigned in two groups: uncomplicated malaria (UM) and severe malaria (SM); according to the criteria defined by Saissy et al. **[31]**. Severe anemia and cerebral form are the two most 'severe form' currently explored, but other complications such as hypoglycemia, thrombocytopenia, renal insufficiency, hepatic or even pulmonary edema may appear alone or in combination. To ensure homogeneity of data, inclusion criteria were: (1) Only black's Senegalese individuals with *P. falciparum* infection confirmed in diagnostic; (2) persons borned in Senegal; who parents and "grandparents" borned in Senegal. Exclusion criteria were: (1) others racial or ethnicity group living in Senegal; (2) subjects with clinical signs of severity or any state may interfere with the study, such as a recent pregnancy and childbirth. The healthy subjects corresponded to control group (CTR) with age, sex and ethnics-matched individuals residing in the same areas. For each patient, a blood sample (10 mL) was taken on venous blood in heparin-containing vacutainer tubes. A total of 114 CTR, 170 UM and 153 SM subjects were included in this study **(Table 1)**.

2.4. *DNA extraction, PCR and Sequencing*

Genomic DNA was extracted from peripheral blood using a standard Qiagen Kit following the manufacturer's recommendations (QIAmp kit Cat. No 51306). Oligonucleotide primers were design to amplify interest region in HBB and G6PD by PCR. The PCR reactions were performed using a Gotaq®Green Master Mix (Promega, Germany) in a total volume of 25 µl containing 25 ng of genomic DNA (5 ng/µl) and 2.5 µl of each primer (10 µM). The PCR conditions were initial denaturation at 95 °C for 5 min, 35 cycles at 95 °C for 30 s, 62 °C for 45 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR amplification of DNA fragments was performed with forward (5'-GTCTTCTGGGTCAGGGAT-3') and reverse (5'-GGAGAAAGCTCTCTCTCC-3') primers for G6PD deficiencies. For Hemoglobin S (rs334) and hemoglobin C (rs33930165), the forward primer was (5'-ACTCCTAAGCCAGTGCCAGA-3'), and the reverse one was (5'-CGATCCTGAGACTTCCACAC-3'). The PCR products were checked by

1.5% agarose gel electrophoresis to verify the product size and the amplicons were purified using BioGel P100 gels (Bio-Rad). Sequencing reactions (2 µl of PCR product) were performed using the Dye Terminator v3.1 method in an ABI PRISMs 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing conditions were: 96 °C for 5 min, 25 cycles of 96 °C for 10 s; 60 °C for 4 min and 15 °C forever, and PCR products were purified with Sephadex G50 superfine columns (GE Healthcare). Alignment of acquired sequences and SNP discovery were performed using NC_000014.9 as a reference. Analysis was performed with Genalys version 2.0 software **[32]**.

2.5. *Statistical analysis*

Statistical analysis was performed to evaluate the association between malaria status and *G6PD* and *HBB* polymorphisms. Hardy-Weinberg equilibrium was tested and allelic frequencies were calculated, as described **[33]**. Differences in allele frequencies among the three groups (SM, UM, CTR) were tested using logistic regression analysis. Linkage disequilibrium (LD) was computed for each pair of polymorphisms within the HBB and G6PD genes using Haploview software **[34]**. Haplotype estimates were obtained using Shapeit4 **[35]**. Nominal p values were corrected under cofounder's association effects HbS and HbC polymorphisms. Associations with p values < 0.05 were considered statistically significant.

3. Results

3.1. *Study sites and populations.*

We performed a retrospective study in 437 subjects recruited between 2003 and 2015 in three ecological areas distinguished by their malaria endemicity (**Figure 1, Table 1**). Of those, 27 (6%) were from St Louis-Louga 258 (59%) from Dakar, and 147 (33%) from Tambacounda-Kolda. In total, 153 subjects had fulfilled WHO criteria for SM **[36]**, 170 had UM, and 117 CTR. Population controls were recruited mainly in Dakar and represent 26.7% of study population. The median age was 25.8 years in individuals with SM individuals, and 18.9 years in individuals with UM. Eighteen percent (18%) were children under 5 years of age, 15% were 5-15 years, and 55% were above 15 years. In this cohort, no mortality was observed in UM and CTR groups, however in severe malaria patients, 13.7% of mortality was observe. In our population, 207 cases (47.36 %) were male and 194 (44.39 %) were female.

3.2. *Haemotological and parasitaemia parameters of the study population.*

The mean values of selected haematological parameters were determined for three SM, UM and CTR groups and the association were calculated using *ANOVA* statistical tests. The haematological parameters of the three groups were compared each other as shown in **Table 2**. In our study, we found a difference between the levels of parasitaemia in both groups, which were statistically significant (P < 0.01). Several blood cell indices (RBC (red blood cells) count, Hematocrit, MCV (mean corpuscular volume) and MCHC (mean corpuscular volume concentration) differed significantly between malaria groups **(Table 2)**. We noted anemia in SM group with hemoglobin level <10 g/dl red blood cells and platelet counts were significantly lower (p < 0.0012) while the leucocytes count was significantly increased (p = 0.001) in SM compared to UM patients.

3.3. *G6PD and HBB polymorphisms and structure in Senegalese population*

To explore the prevalence of G6PD and HBB deficiencies in our cohort, the polymorphism of G6PD (Chr.X) and HBB (Chr.11) genes and their flanking sequences were analyzed in 437 Senegalese population, including 153 SM, 170 UM and 117 CTR subjects. **Tables 3 and 4** summarizes the frequency of each polymorphism in the SM, UM and CTR groups living in three ecological area. Allelic frequencies obtained in this study are like the data provided by the NCBI dbSNP database about African population.

For ***G6PD*** gene, a set of 6 SNP were identified (**Table 3**). Among them, 3 SNPs (+10707 G>A, +10776 G>C and +10983 G>C were a newly identified genetic variants, with low frequencies (MAF<3%). G6PD deficiency polymorphisms such as G6PD-202 G>A, was 0.022, 0.032 and 0.018, in SM, UM and CTR groups respectively. Other G6PD deficiency allele such as +10588A/G (rs762515) was observed at high frequency > 3% in global population. At specific clinical phenotype population, the MAF were 0.28, 0.37 and 0.32 in SM, UM and control groups respectively. There was no evidence of genotypic deviation from HWE at all, except +10588_A>G (rs762515).

Rather than *G6PD, HBB* gene showed more polymorphisms in Senegalese population with 12 characterized SNP. Among them, 6 SNP (rs7946748, rs7480526, rs10768683, rs35209591, rs334 (HbS) and rs713040) were detected with high frequencies (with MAF> 3%), unlike 6

other SNPs (rs33945777, rs111851677, rs33930165 (HbC), rs34598529, rs72561473, rs33944208) with MAF < 3% were observed **(Table 4)**. The MAF of the sickle cell HbS polymorphism was estimated to 0.026, 0.069 and 0.035; and HbC polymorphism was estimated to 0, 0.009, 0.029, in SM, UM and CTR group respectively.

The structure of *G6PD* and *HBB* genes in Senegalese population were investigated, and the presence of an haploblock structure gene was analyzed by measuring the pairwise linkage disequilibrium (LD) in each pair of polymorphisms. The **Figure 2** shows the values of D' between SNP. For G6PD rs762515 A>G, rs1050828 G>A, and for HBB rs10768683 G>C, rs7480526 T>G, rs7946748 C>T polymorphisms, the values D'> 0.97, showing a high linkage disequilibrium, and defining a block structure in HBB gene. In fact, a genetic segment appeared to be part of a haploblock structure in HBB locus, composed of 3 'successive' SNPs, for which the confidence interval D' was 0.9–1 (rs10768683 G>C, rs7480526 T>G, and rs7946748 C>T).

3.4. *G6PD, HBB polymorphisms and clinical malaria.*

To test whether G6PD and HBB polymorphisms were associated to malaria protective effect in our global population, statistical association analysis was performed using logistic regression by comparing the three phenotypes groups, SM, UM and CTR. For G6PD, the SNP +10588 A>G (rs762515) yielded a significant association with protection to severe malaria in Senegalese populations. For SM vs UM, the SNP +10588 A>G (rs762515) showed a borderline p-value = 0.047 (OR 0.65, 95% CI 0.43-0.99). For G6PD-202 G>A, no association with protection against severe malaria was found in our global population (**Table 3**).

Analysis of HbS and HbC polymorphisms showed significant association with protection against severe malaria. For SM vs.UM, the sickle cell trait HbS polymorphism (HBB +20 A>T, rs334) yielded a significant *p-value* = 0.033 (OR 0.38, 95%CI: 0.16–0.91). The HbC polymorphism (HBB +19 G>A, rs33930165) showed a significant p value when comparing SM *vs.* CTR (*p* value = 0.0084, OR 1.67; 95% IC 0.99–3.42) (**Table 4**). The comparative analysis UM *vs.* CTR of the others Hb polymorphisms showed significant associations with rs7946748 C>T and rs713040 C>T, respectively (*p* value = 0.033, OR 2.67 95% IC: 2.01–3.50) (*p*-value = 0.008, OR 1.67; 95% IC 0.99 - 3.42) (**Table 4**).

3.5. *G6PD, HBB polymorphisms and ecological malaria areas.*

We then performed comparisons of frequencies among the known malaria protective SNPs in 3 ecological areas and tried to distinguish relations with endemicity. The SNPs HbS (rs334), HbC (rs33930165) and G6PD +10588A>G (rs762515) were associated with protection against severe malaria in the 3 regions, by comparison of SM *vs* UM (**Data not shown).** However, HBB +9 C>T (rs713040) has variable protective effects depending on endemicity. In Dakar and St-Louis, the SNP rs713040 was associated with the protection of the severe form, unlike Tambacounda/Kolda, which are more endemic areas. The polymorphism rs7946748 was found in the 3 regions and was not associated to protection against severe malaria. For G6PD-202 G>A, we found differences per the locality. Indeed, the SNP is associated with the protection against SM form in Dakar, but not in the other regions (**Data not shown**).

3.6. *G6PD, HBB polymorphisms and biological parameters*

Analyses were conducted to test whether *G6PD* and *HBB* polymorphisms were associated with biological parameters of severity, performing comparisons *SM vs. UM*. Then correlation of HBB polymorphisms HBB +226 C>T (rs7946748), HbS (rs334), HbC (rs33930165), HBB +9 C>T (rs713040), and G6PD 202 G>A with parasitaemia, biological parameters (hemoglobin levels, blood platelets, lymphocytes, monocytes, basophils, neutrophils and eosinophils) and survival/death outcome, were performed using the Mann–Whitney test. Results showed significant association of rs7946748 with PNB levels ($p = 0.015$) and lymphocytes ($p = 0.04$) ***(Table 5)*;** the rs10768683 and rs713040 were associated to PNB with $p = 0.023$ and $p = 0.020$ respectively. HbS (rs334) triggered a significant association with PNE ($p = 0.024$). Platelets were associated with G6PD-SNP+10707 G>A, G6PD-SNP+10983 G>C and HBB-rs72561473, with p = 0.009, 0.048, 0.006 respectively. Finally, G6PD-SNP+11000_C>T (rs782500951) was associated with parasitaemia with a p value = 0.016 (***Table 5)***.

4. Discussion

In present study, we characterized the polymorphisms of *G6PD* and *HBB* genes in Senegalese population, two loci previously associated with protection against severe malaria. To identify polymorphisms associated with clinical malaria and their prevalence, genetic analysis was performed, using three specific phenotypes of severity, including SM, UM and CTR groups living, from North to South, in three ecological malaria transmission areas.

We identified 6 polymorphisms on G6PD gene and found that the minor allele frequency of G6PD 202 G>A was 0.022, 0.032, 0.018, in SM, MM, and CTR group respectively. The frequency of these polymorphisms was 0.026 in global population. In Gambia, a neighbor country of Senegal, it was reported that the minor allele frequency of this polymorphism in CTR sample was 0.03, considerably lower than reports from other east African countries such as Kenya (0.18) and Malawi (0.19) **[20].** Then our results confirm the weak prevalence of allele deficiency form G6PD 202 G>A in Senegambia area, compared to Kenya and Malawi population. These results could confirm that G6PD polymorphisms prevalence is highly population specific, and thus, attention should be paid to the study population from which future estimates may be withdrawn **[37]**.

In our Senegalese population cohort, we found that G6PD-202 G>A polymorphism was not associated with protection against severe malaria at global level. However, differences were founded splitting in ecological locality. The polymorphism was associated with protection against SM in Dakar Capital center, but not in the other regions (St Louis-Louga and Tambacounda-Kolda). Our results could be in contradiction with published data, indeed the A-allele of G6PD-202 G>A polymorphism has been associated with protection against severe malaria in African populations, such as in Mali, Gambia and Uganda **[23, 24]**. However, this protective effect seems to depend on others parameters such as sex and locality. In fact, this protective effect against severe malaria have been inconsistent across large studies, observing in females **[38, 39]**, in males **[23]**, in both **[24]**, or no protection **[40, 41]**. This disparity could be explained in part by variation in phenotype definition, choice of controls (village surveys *vs.* hospital based studies), age or immune status of subjects, and study designs (case control *vs.* cohort) **[40].** G6PD deficiency can potentially protect against uncomplicated malaria in African countries, but not severe malaria. Interestingly, this protection was mainly in heterozygous, being x-linked thus related to gender **[23, 42]**. No such protection was evident from the mosaic state of G6PD deficiency in heterozygous females. Previous study confirmed highly significant protection against severe malaria in hemizygous males but not in heterozygous females **[23]**. A case-control study showed significant association between G6PD A- and risk of severe malaria, with protection against cerebral malaria, but increased risk of severe anemia **[20]**. However, compared to HbAS or

α–thalassemia and associations between 'G6PD A- deficiency' and risk of severe malaria have been less straightforward, with studies yielding inconsistent results **[39, 42].** In fact, it has been showed that the G6PD-202 polymorphism may not be a good marker of A- deficiency, and/or other polymorphisms are required to confirm the protective effect **[40]**. In our study, we found the G6PD SNP +10588_A>G (rs762515) yielded a significant association with protection to severe malaria in Senegalese population.

In the *HBB* gene, located on chromosome 11, we identified 12 polymorphisms and characterized their frequencies. The SNP HbS yielded in the substitution of a glutamate residue to a valine or lysine residue in the ß-globin chain at position 6. The Minor Allele Frequency of HbS and HbC polymorphisms were estimated to be 0.05 and 0.01 respectively in global population. Several studies have shown a low frequency of the HbS allele in the control group (~3.8%). The frequency is in keeping with other west (Burkina Faso 5.2%, Cameroon 6.5%, Gambia 7.6%, and Ghana 6.5%) and east African populations (Kenya 6.4%, Malawi 2.7%, and Tanzania 7.8%) (http://www.map.ox.ac.uk/). A higher frequency of the HbC allele has been observed in other West African populations **[16, 41].**

Our analysis confirmed the known protective effects of HbS and HbC. We found that HbS and HbC polymorphisms showed significant association with protection against severe malaria in the three ecological regions. For SM *vs.*UM, the sickle cell trait HbS polymorphism (HBB +20_A>T, rs334) yielded a significant p-value = 0.033. The HbC polymorphism (HBB +19_G>A, rs33930165) showed a significant p-value = 0.008 when comparing SM *vs*. CTR. Previous studies showed that HbAS has been associated with 50% and 80% reduced risks of developing uncomplicated and severe malaria **[15]**, HbAC and HbCC have been associated with 30% and 93% reduced risks of developing mild and severe malaria, respectively **[11].** A recent study conducted in Mali, a neighboring country of Senegal indicated that HbC is associated with protection against the SM form of *P falciparum* malaria in the Dogon of Bandiagara population **[20]**. In this ethnic group, the prevalence of HbC was significantly lower among cases of severe malaria than among cases of uncomplicated malaria. The protective effect indicated 80% reduction in the risk of severe malaria. The data also suggest that the protective effect associated with HbC may be greater than that of HbS in this population **[16]** .

It was suggested a “balanced polymorphism” where the HbS homozygote disadvantage is recompensed through the resistance of the heterozygote HbAS in regions where malaria is endemic **[43]**.

Our results suggest differences in protective effect hemoglobin among the three ecological areas. The possibility that protective effects associated with different hemoglobin mutations vary among different human populations. This is consistent with report findings. In fact in West Africa, the Fulani ethnic group has decreased susceptibility to malaria compared to Dogon populations **[44]**, and the Fulani also have a decreased prevalence of sickle hemoglobin **[45]**, α-thalassemia **[11]**, and G6PD A- **[11, 46]** compared to other groups.

Results of this study showed significant associations between several polymorphisms on *HBB* and *G6PD* genes and immunological parameters such as PNB, PNE and lymphocytes. An early and vigorous development of malaria immunity has been associated with a protection effect of sickle cell trait and thalassemia. Indeed, ethnic variations in the immunological response to *P falciparum* infection and rates of the clinical episodes of malaria are described from clinical studies in West Africa [**11, 16**].

5. Conclusions

This study gives an overview of importance of the sickle cell polymorphisms on severe malaria susceptibility. It provides the prevalence of polymorphisms in HBB and G6PD genes associated with clinical malaria and their prevalence in Senegalese population. Our findings show the important role of genetic factors in malaria outcome and these genetic markers could be good tools for malaria diagnosis and prognosis.

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Authors’ contributions

FT and GD conceived the study, the methodology and drafted the manuscript.

JFZ and CC conducted the data analysis, revised the manuscript and approved the final version.

CD and EC conducted the methodology, performing Sanger Sequencing, molecular biology experiments and approved the final version.

BM conducted the recruitment of malaria cohort, revising the manuscript from hospital center and approved the final version.

RND contributed to the correction and revision of the manuscript, and approved the final version.

CMN contributed to the correction and revision of the manuscript, and approved the final version.

YD contributed to the correction, revising the manuscript and approved the final version.

JFD & AD coordinated this study, revised the manuscript, and approved the final version.

All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Objectives and benefits were explained to patients using local dialect before inclusion. Protocols were reviewed according to the rules issued by the National Committee for Ethics for Health Research (CNERS) of Senegal, and in accordance with the procedures established by the University Cheikh Anta Diop of Dakar (UCAD) for the ethical approval of any research involving human participants. Written informed consent was obtained from participants. Based on the information provided, UCAD's Committee on Research and Ethic (CER) considers that the research proposed, respects the appropriate ethical standard and, as a result, approves its execution under “Protocole 0344/2018/CER-UCAD”. Patients and/or legal representative give consent, to provide a blood sample for studies.

Consent for publication

Patients and/or legal representative give consent for furthers studies and gave written informed consent for publication under “Protocole 0344/2018/CER-UCAD”.

Competing interests

The authors declare that they have no competing interests.

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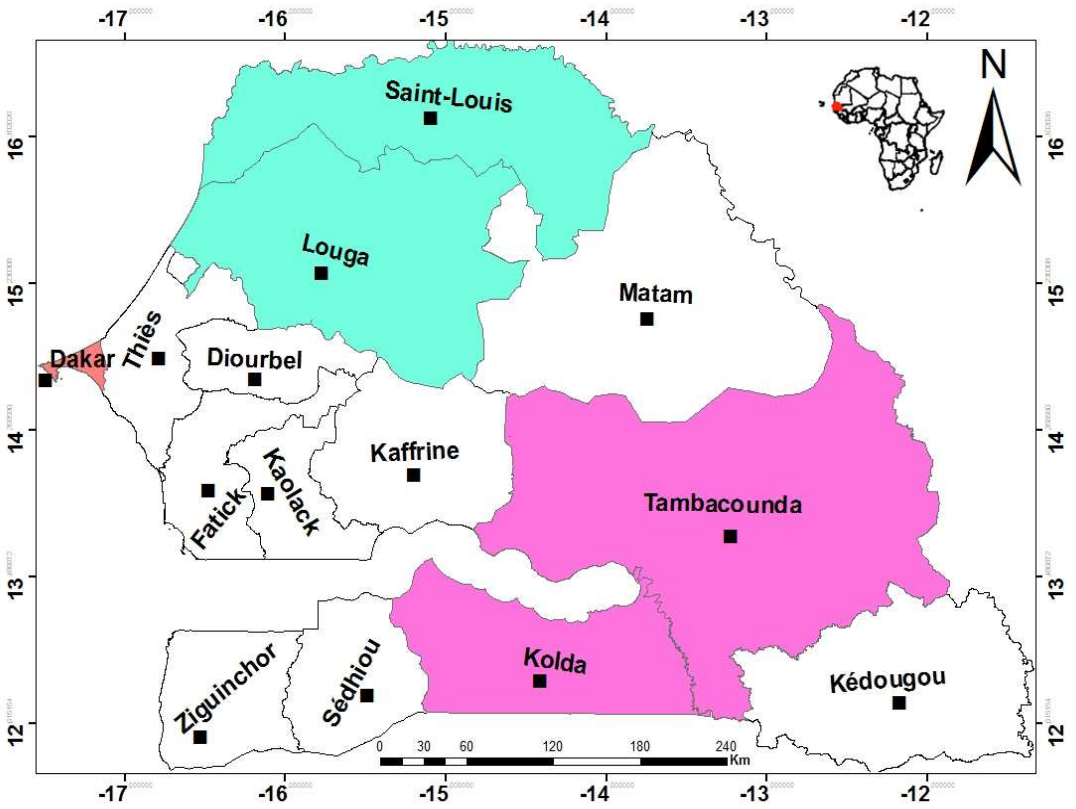
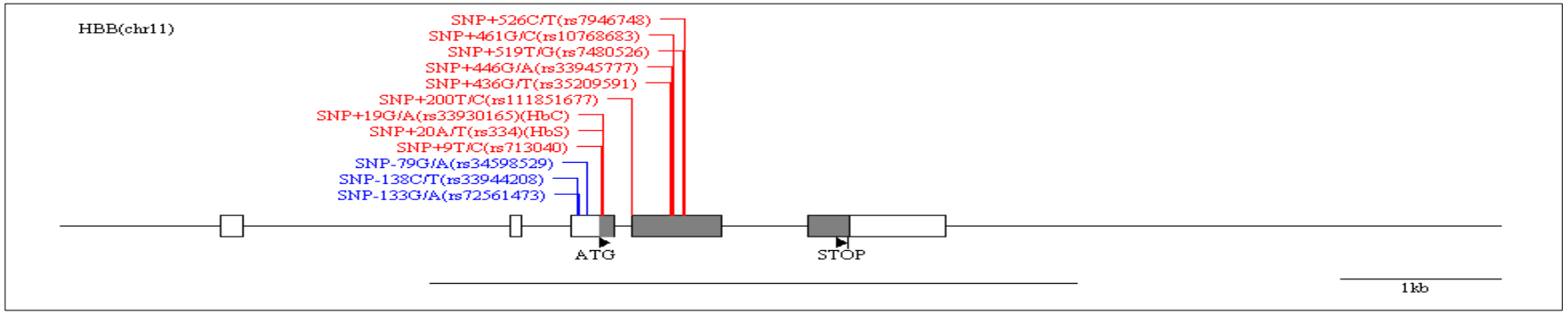


Fig 1: Study region map. Black point mark the towns surveyed of Senegal and showing the study sites. The colors zones marked the three different ecological area of endemicity, with green color (preelimination area: St Louis-Louga regions); the plink color zone (the capital Dakar-Diamniadio center) and the red zone (south endemic region Tambacounda-Kolda).

Fig 2: Structure and organization of *HBB (chr.11) (A)* and G6PD *(chrX) (B)* genes in Senegalese population.

A)

1)



2)

B)

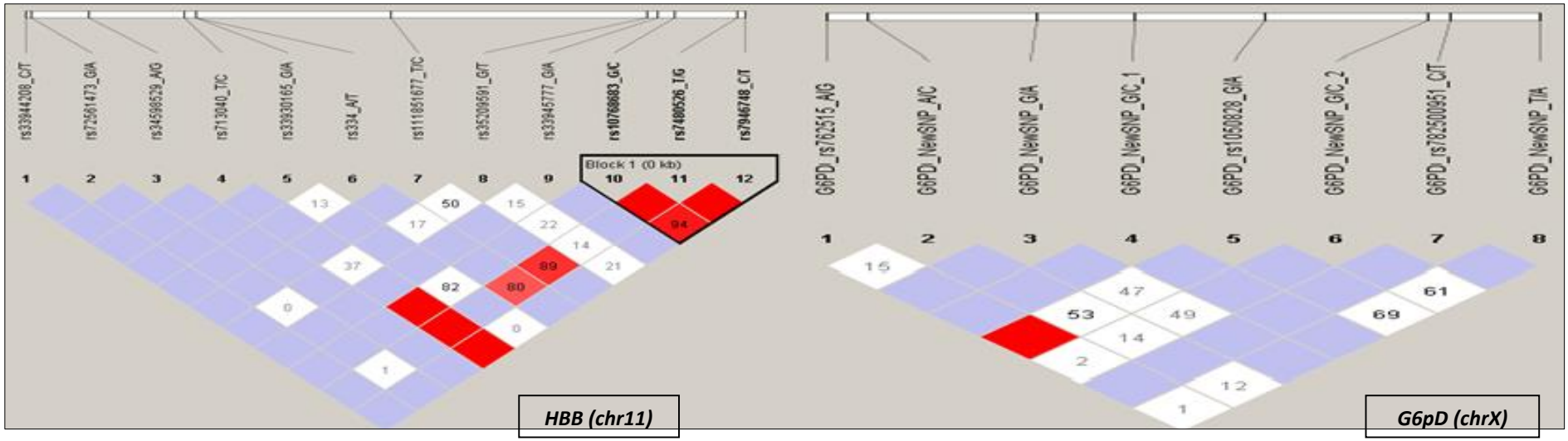


Fig2: shows *HBB* and G6PD polymorphisms located on chromosome chr11 and chr X respectively. The linkage disequilibrium map of *HBB* and *G6PD* genes were provided by the Haploview software. **A)** The coding and UTR regions were indicated by black and white rectangles respectively, for each locus. SNP Positions are numbered per the initiation codon ATG considered as + 1 (indicated by a black triangle). The genomic sequence used for alignment is GenBank sequence was NG_009015.2 and NG_059281 for *G6PD and HBB* respectively**. B)** The linkage disequilibrium map of *G6PD* and *HBB* genes. The LD plot shows pairwise *D*' values given in the squares for each statistical comparison between the SNPs. The different shade of color represents *D*' values (between 0 and 1). An empty red square indicates that D' > 0.97. The Lewontin's D' coefficient correlate to level of recombination, useful for the finding of an haploblock.

Table 1: General Characteristics of the study participants

The number of patients in each group SM, UM and CTR, was showned. Age is given with median values (the number of subject and corresponding percentage was given in parenthesis). *ND* the number for witch, data were undetermined and/or unknowned.

		Severe Malaria, SM	**Uncomplicated Malaria, UM**	**Control, CTR**	**Total**
		(N = 153)	**(N = 170)**	**(N = 117)**	**(N = 437)**
Age (years)	< 5, n (%)	29 (18.9)	45 (26.4)	6(5.1)	79 (18.1)
	5 à 25, n (%)	66 (43.1)	43 (25.3)	26 (22.2)	66 (15.1)
	> 25, n (%)	52 (33.9)	48 (28.2)	76 (64.9)	241 (55.1)
	ND, n (%)	6 (3.9)	34 (20)	9 (7.6)	49 (11.2)
	Mean, [interval]	25.8 (25-89)	18.9 (13-77)	35.4 (32-87)	24.9 (23-89)
Survivals	Survivors, n (%)	117 (76.5)	169 (99.4)	117 (100)	403 (92.2)
	Deaths, n (%)	21 (13.7)	0 (0)	0 (0)	21 (4.8)
	ND, n (%)	15 (9.8)	1 (0.6)	0 (0)	16 (3.6)
Gender	Male, n (%)	86 (56.2)	71 (41.7)	50 (42.7)	207 (47.4)
	Female, n (%)	55 (36)	81 (47.6)	58 (49.6)	194 (44.4)
	ND, n (%)	12 (7.8)	18 (10.7)	9 (7.7)	39 (8.9)
Locality	Dakar, n (%)	82 (53.6)	67 (39.4)	114 (97.4)	263 (60.2)
	Tambacounda/Kolda, n (%)	62 (40.4)	85 (50)	0 (0)	147 (33.6)
	Saint-Louis/Louga, n (%)	9 (6)	18 (10.6)	0 (0)	27 (6.2)

Table 2: Haematological and parasitaemia parameters of the study participants.
M severe malaria, *UM* Uncomplicated malaria, *CTR* Control group, *M* male; *F* female. The number of patients showed in each group SM, UM and CTR. Age is given with median values (minimum and maximum values are in parenthesis). Hb levels and parasite density distribution are given with the mean and SD values (standard deviation). *Hb** values statistically significant when compared SM to UM with $p < 0.001$. *Parasite density** $p < 0.001$ when comparing SM to UM.

Parameter	**Severe Malaria (SM)**	**Uncomplicated Malaria (UM)**	**Control (CTR)**	**P-value (SM/MM)**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Parasitaemia (P/µL)	27220 ± 5458,3	3993 ± 1327,3	0,00 ± 0,0	< 0,001
Leukocyte ($^{x}10^{3}$/µL)	12,9 ± 1,0	8,8 ± 0,5	6,1 ± 0,2	< 0,001
Neutrophil (%)	52,02 ± 3,7	60,29 ± 2,5	45,77 ± 1,3	< 0,001
Lymphocyte (%)	22,32 ± 2,4	26,21 ± 2,1	40,09 ± 1,3	< 0,001
Monocyte (%)	6,27 ± 0,9	7,56 ± 0,9	8,65 ± 0,3	0,009
Eosinophil (%)	0,49 ± 0,1	2,77 ± 0,48	4,33 ± 0,45	0,0098
Basophil (%)	0,44 ± 0,1	0,72 ± 0,1	1,14 ± 0,08	< 0,001
RBCs ($^{x}10^{6}$/µL)	3,71 ± 0,29	4,11 ± 0,07	4,66 ± 0,06	0,0012
Hb[a] (g/dL)	9,49 ± 0,3	12,11 ± 0,2	12,96 ± 0,2	< 0,001
Hematocrit (%)	27,88 ± 0,9	36,68 ± 0,7	38,44 ± 0,5	< 0,001
MCV[b] (fL)	79,96 ± 0,64	87,70 ± 1,37	82,98 ± 0,64	< 0,001
MCHC[c] (pg/cell)	26,48 ± 0,47	29,26 ± 0,47	28,04 ± 0,23	< 0,001
Platelet ($^{x}10^{3}$/µL)	124.1 ± 7,6	241.8 ± 5,2	286.8 ± 11,2	< 0,001

Table 3: Frequencies and Single Nucleotide Polymorphism (SNP) of G6PD gene and association analysis with susceptibility to severe malaria (SM)

G6PD-SNP	**NCBI dbSNP Number**	**Phénotype**	**MAF**				***HWE***			
			SM	**UM**	**CTR**	**Global Population**	**SM *vs* UM**		**UM *vs* CTR**	**SM *vs* CTR**
							P-value	**OR (95%IC)**	***P-value***	***P-value***
+10588_A>G	rs762515		0.280	0.373	0.325	0.329	***0.047***	***0,65 (0.43-0.99)***	0.344	0.415
+10707_G>A	newSNP		0	0.004	0	0.001	1		1	1
+10776_G>C	newSNP		0	0.008	0	0.003	0.509		0.517	1
+10869_G>A	rs1050828	G6PD202A	0.022	0.032	0.018	0.026	0.568		0.536	1
+10983_G>C	newSNP		0.005	0.008	0.012	0.008	1		1	0.607
+11000_C>T	rs782500951		0,005	0.004	0	0.003	1		1	1

Table 4: Frequencies of Single Nucleotide Polymorphism (SNP) of HBB gene and association analysis with susceptibility to severe malaria (SM).

HBB-SNP	NCBI dbSNP Number	Phénotype (NCBI)	MAF				HWE				
			SM	UM	CTR	Global Population	*SM vs UM*		*UM vs CTR*		*SM vs CTR*
							P-value	OR (95% IC)	*P-value*	OR (95% IC)	*P-value*
+526_C>T	rs7946748	β-thalassemia	0.064	0.029	0.060	0.052	0.062		*0.048*	*0.41 (0.17-0.97)*	1
+519_T>G	rs7480526	Benign	0.340	0.404	0.331	0.365	0.136		0.138		1
+461_C>G	rs10768683	Benign	0.091	0.064	0.094	0.086	0.264		0.139		0.753
+446_G>A	rs33945777	E6 familial, Fetal HQTL-1, Hb SS disease, H-B-A, MTH β type, α-β-thalassemia.	0.008	0	0	0.002	0.196		1		0.503
+436_G>T	rs35209591	E6 familial	0.393	0.414	0.419	0.408	0.607		0.927		0.563
+200_T>C	rs111851677	β-thalassemia	0.024	0.028	0.025	0.025	0.797		1		1
+20_A>T	rs334	HbS	0.026	0.069	**0.035**	0.050	***0.033***	*0,38 (0.16-0.91)*	0.257		0.446
+19_G>A	rs33930165	HbC	0	0.009	0.029	0.011	0.258		0.1661		*0.008*
+9_C>T	rs713040	Hemoglobin OKAYAMA	0.107	0.068	0.119	0.099	0.097		*0.029*	*0.51 (0.27-0.93)*	0.660
- 79_A>G	rs34598529	E6 familial , Fetal HQTL-1, Hb SS disease, H-B-A, MTH β type, α-β-thalassemia.	0.004	0	0	0.001	0.443		1		1
-133_G>A	rs72561473	B-thalassemia	0.012	0.019	0.010	0.014	0.737		0.486		1
-138_C>T	rs33944208	B-thalassemia, Hemoglobinopathy	0	0	0.005	0.002	1		0.159		0.206

Table 3 and 4: For each polymorphism, the *p* values for statistical tests were indicated. Statistical tests used were logistic regression analysis. (A) Association analysis for *G6PD* polymorphism and (B) Association analysis for HBB polymorphisms. By comparisons of SM *vs* UM, UM *vs* CTR and SM *vs* CTR. The calculation mode used for logistic regression test was an additive model. Borderline ($0.05 \leq p \leq 0.1$) and significant ($0 \leq p \leq 0.05$) *p* values are in bold. The OR (odds ratio) and CI (Confidence intervals) were showed when *p* values are significant. The phenotype attributed by from each polymorphism was mentioned (from NCBI) as: Erythrocytosis 6 familial (E6 familial), Beta thalassemia (β-thalassemia), Fetal hemoglobin quantitative trait locus 1 (Fetal HQTL-1), Heinz body anemia (H-B-A), METHEMOGLOBINEMIA (MTH).

Table 5: Associations between HBB and G6PD polymorphisms and biological parameters. Statistical analysis of correlations, between an assigned ***HBB* and *G6PD* polymorphisms** and cohort parameters. Statistical analysis was performed using Man Whitney test. Significant association was showed with a p value $p < 0.01$ (under additive model).

Biological parameters	**SNP**	**CHR**	**BP**	**A1**	**P-values (under *Additive model*)**
PNB	**rs7946748**	11	5226496	T	0.015
	rs10768683	11	5226561	G	0.023
	rs713040	11	5227013	T	0.020
Lymphocyte	rs7948748	11	5226496	T	0.041
PNE	rs7480526	11	5226503	G	0.002
	rs334	11	5227002	T	0.024
Platelet	rs72561473	11	5227154	A	0.009
	SNP_10707_GA	23	10707	A	0.048
	SNP_10983_GC	23	10983	C	0.006
Parasitaemia	rs782500951	23	11000	T	0.016
Age	rs111851677	11	5226822	C	0.032

Figures

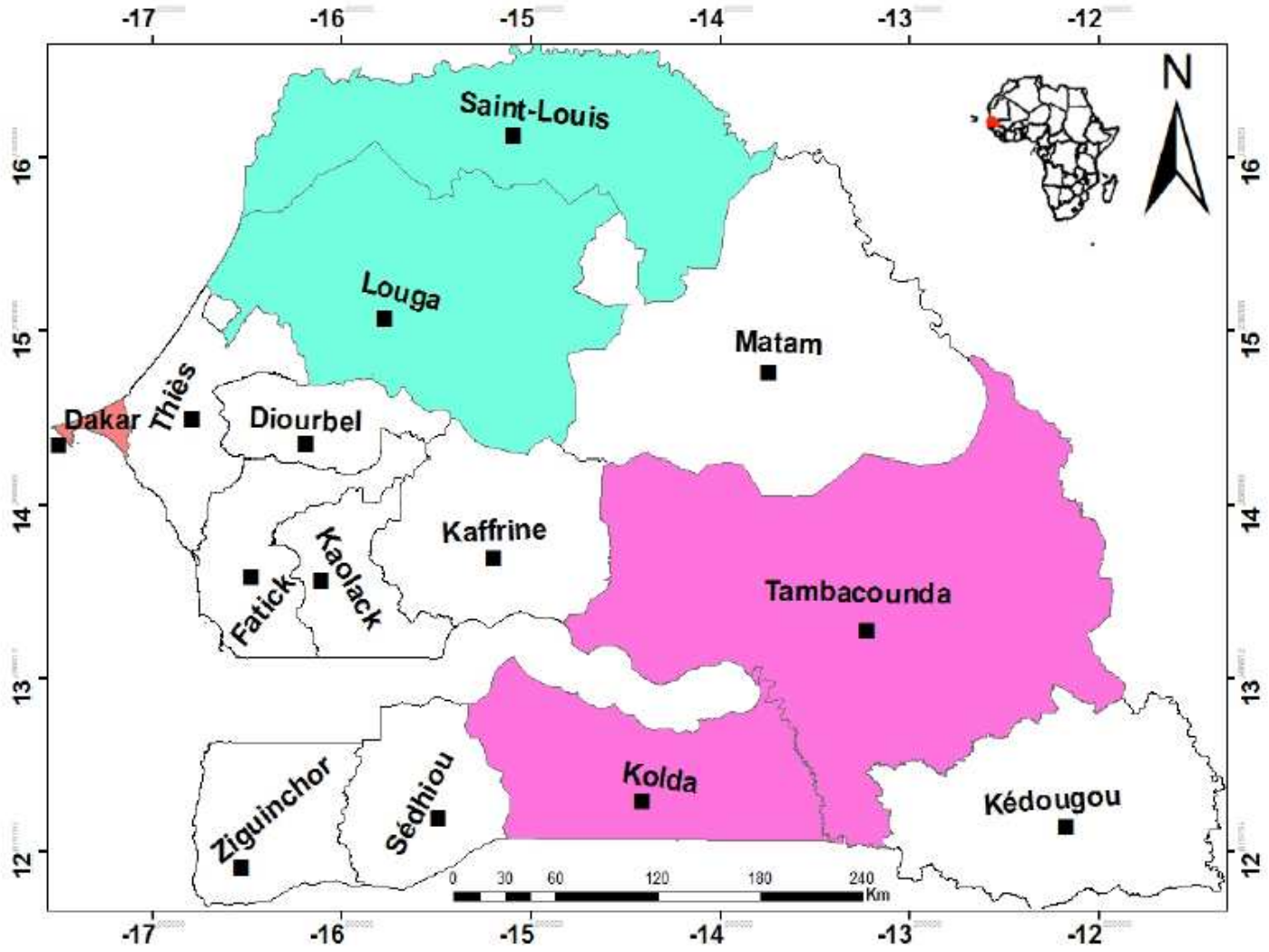


Figure 1

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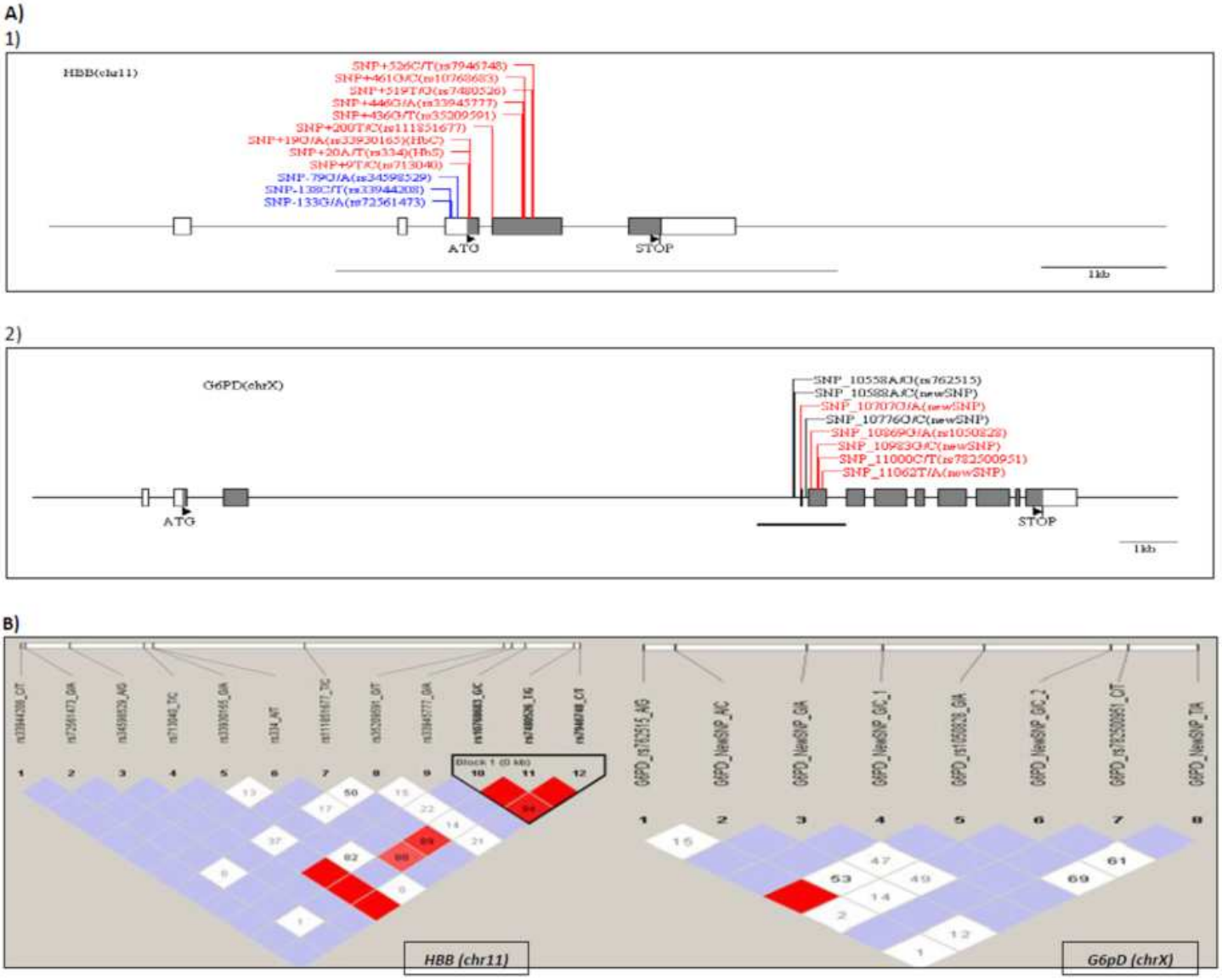

Figure 2

Structure and organization of HBB (chr.11) (A) and G6PD (chrX) (B) genes in Senegalese population. Shows HBB and G6PD polymorphisms located on chromosome chr11 and chr X respectively. The linkage disequilibrium map of HBB and G6PD genes were provided by the Haploview software. A) The coding and UTR regions were indicated by black and white rectangles respectively, for each locus. SNP Positions are numbered per the initiation codon ATG considered as + 1 (indicated by a black triangle). The genomic sequence used for alignment is GenBank sequence was NG_009015.2 and NG_059281 for G6PD and HBB respectively. B) The linkage disequilibrium map of G6PD and HBB genes. The LD plot shows pairwise D' values given in the squares for each statistical comparison between the SNPs. The different shade of color represents D' values (between 0 and 1). An empty red square indicates that D' > 0.97. The Lewontin's D' coefficient correlate to level of recombination, useful for the finding of an haploblock.