High Level of Heterozygous Haplotype of Hemoglobin in Abidjan

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Research Article

Keywords: Sick cell anemia, haplotype, Ivory coast, Malaria

Posted Date: August 23rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-776734/v1>

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Abstract

Background.

Sickle cell disease (SCD) is an hemoglobin disorder that concerns 300,000 newborns each year around the world. Hemoglobin haplotypes can modulate SCD clinical expression. In Côte d'Ivoire, no study has yet investigated the distribution of hemoglobin haplotypes in the population. The goal of this study was to identify hemoglobin haplotypes for people attending dispensary with mild malaria in Abidjan (Ivory Coast) independently of their SCD status.

Methods

To determine haplotypes, specific restriction enzyme (RE) method is used after PCR amplification with different couples of primers. According to the digestion profile of PCR products, five hemoglobin haplotypes are found over the world.

Results

In Abidjan, four different "classical" haplotypes of hemoglobin were detected: Benin (56.5%), Bantou (28.5%), Senegal (4%), Cameroun (1%). In parallel 10% of atypical profiles were described. Heterozygous haplotype (69%) was more frequent than homozygous haplotype (31%).

Conclusions

Benin haplotype usually associated with more severe of SCD symptoms was predominant in the study population. However this preliminary study highlights also a high prevalence of atypical and heterozygous haplotypes in the population.

Background

Sickle cell disease (SCD) is a hemoglobin disorder that concerns 300,000 newborns yearly over the world (1). Sub-Saharan countries harbored the highest prevalence of SCD with S hemoglobin in Central Africa and C in Sahelian areas (2–4). However, haemoglobin haplotypes can modulate clinical expression of SCD. These haplotypes have different geographic origins (5, 6). The presence of some haplotypes can reduce the SCD symptoms, whereas other ones amplify clinical manifestations (7–14). To determine these haplotypes, specific restriction enzymes (RE) are used after PCR amplification. According to these digestion profiles five hemoglobin haplotypes can be described around the world: Bantou (CAR), Arabic, Senegal, Cameroun and Benin (15, 16). In Côte d'Ivoire, no study has yet investigated the distribution of haplotypes in population, and especially among people with SCD. This first study has been conducted retrospectively in Côte d'Ivoire to identify these haplotypes from peoples attending dispensaries with mild malaria independently to their SCD statute.

Materials And Methods

Sample collection

Abidjan is the economic capital of Côte d'Ivoire with five million inhabitants originating from a large number of neighboring countries. Participants were recruited at the CSUCOM Anonkoua-Kouté (Abidjan, Abobo) between 2013 and 2016 among patients attending the center with confirmed mild malaria (parasitemia more than 2000 parasites/ μ L blood).

The hemoglobin status of the patients was determined based on a standard acetate electrophoresis of hemoglobin using *Sebia® Hemoglobin electrophoresis*, following the protocol of the manufacturer. For molecular typing, 50 μ L of total blood was dried on *5M Whatman®* paper and stored in zip locked bag contained silicate gel until use in 2020.

Haplotype molecular typing

For molecular typing, DNA purification was performed on blood spots using the *Qiagen®Blood Minikit* as recommended by the manufacturer. For amplification, different programs were used according to the couple of primers used. Nine pairs of primers have been used following Sutton and al (15) and Doupa and al (17) (Table 1). After amplification, each type of PCR products was digested with a related restriction enzyme (RE). The haplotype profiles were identified according to Sutton and al (15) and Doupa and al (17). (Table 2)

Table 1
Primers used in this study

Primer name	Primer sequence	Source
5' G γ	AACTGTTGCTTTATAGGATTT T AGGAGCTTATTGATAACCTCAGAC	(17)
G γ	TGCTGCTAATGCTTCATTACA A AAGTGTGGAGTGTGCACATGA	
A γ	TGCTGCTAATGCTTCATTACA A TAA ATGAGGAGCATGCACACA C	
$\Psi\beta$	GAA CAG AAG TTG AGA TAG AGA ACT CAG TGG TCT TGT GGG CT	
3' $\Psi\beta$	TCT GCA TTT GAC TCT GTT AGC GGA CCC TAA CTG ATA TAA CTA	
3' δ	TGG ATT CTG CCT AAT AAA A GGG CCT ATG ACA GGG TAA T	
B	GCT GAG GGT TTG AAG TCC AA CAC TGA TGC AAT CAT TCG TC	
5' β	CTACGCTGACCTCATAAATG CTAATCTGCAAGAGTGTCT	(15)
3' β	TTCATACATAACAATACTCA GAGGAGAGCTTTACTTCCAA	

Results

Demographic results

Out of a total of 100 patients included in the study, 55% were women, with a mean age of 14.5 years.

Haplotype typing

Four different “classical” haplotypes of hemoglobin were detected, Benin (56.5%), Bantou (28.5%), Senegal (4%) and Cameroun (1%). The Arabic haplotype was not observed. In addition, 10% of atypical profiles were detected (i.e. 20 haplotypes). Atypic haplotypes were predominant in the of Benin (19/20) and Bantou (01/20) groups (Table 2, Fig. 1). Heterozygous (69%) were more frequent than homozygous (31%). For homozygote, women were more affected by SCD than men (40/69 and 29/69 respectively).

Table 2

Haemoglobin haplotype restriction profiles

(+) *presence of restriction enzyme site, (-) absence of restriction enzyme site; CAR = Central African Republic; (nbr of allele) for 20 atypic alleles.*

Gene and restriction enzyme									
	5gamma (Xmnl)	Gamma (HindIII)	Alpha (HindIII)	phiBêta (HincII)	3phiBêta (HincII)	3Delta (Hinfl)	Beta (Avall)	5Beta (Hinfl)	3Beta (Hpal)
Haplotype described (2,3)									
Senegal	+	+	-	+	+	+	+	+	+
Benin	-	-	-	-	+	-	+	+	-
Bantu (CAR)	-	+	-	-	-	-	+	+	+
Cameroon	-	+	+	-	+	+	+	-	+
Arabic	+	+	-	+	+	-	+	-	+
Atypic in this study									
With Bantu haplotype									
Atypic 1(1) *	-	-	-	-	+	+	+	+	+
With Benin haplotype									
Atypic 1(4) *	-	-	-	-	+	+	+	+	+
Atypic 2(10) *	-	+	-	-	+	+	+	+	+
Atypic 3(4) *	-	+	-	-	+	-	+	+	+
Atypic 4(1) *	-	-	-	-	+	-	+	+	+

Hemoglobin typing and malaria diagnostic

The AA genotype represented 87% of the samples. The other genotypes were AC, AS, SC and CC (8%, 2%, 2% and 1% respectively, Table 3).

Mean parasitemia was 46376 parasites/ μ L blood, without any significant difference between haplotypes (t-test, $p = 0,95$).

All participants with AC or CC genotypes were from the Benin group (homozygote Benin/Benin or Benin/Bantu) whereas AS was found in the Bantu group (Table 3). Atypic, Cameroun and Senegal haplotypes were observed

only in normal hemoglobin group (Table 3).

Table 3
Distribution of haplotypes according to malaria and haemoglobin type

	Mean parasitaemia (tpz/ μ L blood)	Hemoglobin typing (N*)					Total (N*)
		AA	AC	AS	CC	SC	
With Benin haplotype							
Benin/Atypic	44644	14	1				15
Benin/Benin	40342	20	1		1	1	23
Cameroon/Benin	43768	2					2
Senegal/Benin	31017	6	1				7
		42	3		1	1	47
With Bantu haplotype							
Atypic/Bantu	84307	1					1
Bantu/Bantu	75460	5		1			6
Senegal/Bantu	21866	1					1
		7		1			8
With Bantu and Benin haplotypes							
Bantou/Benin	48930	36	5	1		1	43
Another haplotype							
Atypic/Atypic	36279	2					2
Total	46376	87	8	2	1	2	100
*nbr of patients							

Discussion

Several authors have highlighted the interest of studying hemoglobin haplotypes for individuals with hemoglobin disorders as a modulation of the clinical profile of the disease can occur (7–14). In Côte d'Ivoire, not data was available on these haplotypes. This study updates these data for people living in Abidjan and experimenting mild malaria. During this work, women represented the highest proportion of people attending dispensaries. This is frequently observed as men use to practice self-treatment so are rarely visiting dispensaries. A prevalence of 13% of sickle cell disease was found in this population, which is in line with a previous study conducted in the same area (18).

The Benin haplotype was the most prevalent followed by the Bantu one, which can be attributable respectively to ethnic origin of the population in Abidjan, and the high level of migration from central Africa to Ivory Coast. Arab-Indian haplotype was not observed despite migration of populations across the Sahel (Peul and Toucouleur ethnics). A small prevalence of Senegal and Cameroon haplotypes was observed. These different prevalence are in line with the geographical origin of the different populations living in Abidjan (5, 6).

Similar other studies reporting 5 to 10% of atypical haplotypes (9, 19–21), this study reports 10% of atypical haplotypes in the population of Abidjan, mostly associated with the Benin haplotype. This association is important to explore as Benin haplotype is associated with a more severe form of expression of SCD.

Overall a high proportion of heterozygous genotype (61%) was found in Abidjan. That differs from the studies conducted by Doupa et al (32%) (17). This difference could be due to the selection of patients harboring SCD and to the use of only one single restriction site, by these authors. The emergence of these atypics haplotypes and the high proportion of heterozygous haplotypes could support a high level of mixed populations in Abidjan. Indeed, Abidjan is one of the major cities in West Africa, with a cosmopolite population. In addition, this important mix of populations would support a higher frequency of recombination between haplotypes leading to atypic profiles. Indeed, in line with other studies (19, 22, 23), the atypical haplotypes observed show strong similarities with the Benin and Bantu haplotypes. The high proportion of atypical haplotype and its similarity with the Benin haplotype could be explained by a selection pressure. Indeed, the association of an atypical haplotypes with the more severe Benin haplotype may modulate as well the clinical pattern of the disease, leading to an attenuated clinical expression.

Conclusion

In this preliminary study, a high prevalence of atypical and heterozygous haplotype was highlighted. Benin haplotype usually associated with severity of SCD was the most prevalent. Further studies involving a large number of SCD participants could help to estimate an accurate prevalence of hemoglobin haplotypes in Côte d'Ivoire.

Abbreviations

SCD: Sickle cell disease

RE: Restriction Enzyme

PCR : Polymerase Chain Reaction

Declarations

✓ Ethics approval and consent to participate

This study is a part of a protocol of survey of sickle cell anemia in Abidjan which received ethical clearance from the National Ethic Committee of Ivory Coast. All the participants gave their informed consent to participate

✓ **Consent for publication**

All the authors read the manuscript and agreed with the publication

✓ **Availability of data and materials**

The datasets used and/or analysed during the current study are available in the OneDrive repository https://1drv.ms/u/s!AnZdvsbBxIN05UlxWwj8x_1ldWKU?e=o1Aq7P

✓ **Competing interests**

The authors declare that they have no competing interests.

✓ **Funding**

This study and KOUÏ TOSSEA STEPHANE were supported by the Rotary International Foundation

✓ **Authors' contributions**

KOUÏ TOSSEA STEPHANE conducted the laboratory study and wrote the manuscript; GNONDJUI A. ALBERT, AKO ARISTIDE BERENGER and COULIBALY BABA participated in the collection of the blood sampling; ADJI A. ERIC participated to the PCR design; AKA A. DELPECHE realized the laboratory study ; GONEDELE BI S. E. and TOURE O. ANDRE participated to the study design and reviewed the manuscript ; RONAN JAMBOU supervised the study, obtained the ethic clearance and the funds, wrote the protocol and co-wrote the manuscript.

✓ **Acknowledgements**

We would like to thank all the people and institutions that have enabled us to carry out this work. This work and KS were supported by the Rotary international Foundation and the District of Paris (France). .

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Figures

Figure 1

Gamma PCR products digestion by HindIII (Restriction Enzyme) PCR product size for Gamma is 782 pb and 436 pb and 346 pb after digestion by HindIII, N°7, N°9, N°12, N°18 and N°24 are homozygotes samples without restriction site for HindIII. N°2, N°11, N°15 and N°23 are homozygotes with restriction site. All other are heterozygotes.

Supplementary Files

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