Scientific Research Publishing

Open Journal of Genetics, 2024, 14, 1-12
https://www.scirp.org/journal/ojgen
ISSN Online: 2162-4461
ISSN Print: 2162-4453

Influence of Hemoglobin S Haplotypes on the Responses to Hydroxyurea Treatment in Children with Sickle Cell Disease in Abidjan, Côte d'Ivoire

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How to cite this paper: Aye-Yayo, M., Yapo, V., Kouakou, B., Adjé, M.L., Adjambri, A.E., Kassi, E.H., Kamagate, T. and Sawadogo, D. (2024) Influence of Hemoglobin S Haplotypes on the Responses to Hydroxyurea Treatment in Children with Sickle Cell Disease in Abidjan, Côte d'Ivoire. *Open Journal of Genetics*, **14**, 1-12.
https://doi.org/10.4236/ojgen.2024.141001

Received: December 23, 2023
Accepted: March 18, 2024
Published: March 21, 2024

Abstract

Background: In Côte d'Ivoire so far, the circulating haplotypes have been inferred on the phenotypic profiling of SCD patients. The impact of the circulating haplotypes on the use of Hydroxyurea has not been assessed yet. Therefore the objective of this study is to identify in Abidjan the HbS haplotypes that modulate HU treatment responses. **Methods:** In a cross-sectional descriptive and analytical study, children aged 5 to 15 years with SCD, and carrying the hemoglobin phenotypes SSFA2 and SFA2, were recruited into a HU treatment cohort. Various parameters on the haplotypes and the outcomes of the treatment were analyzed. **Results:** Thirty nine children with SCD were included. The phenotypic profile of the cohort was 86.6% of SSFA2 and 15.4% of SFA2. Three haplotypes were found, the Benin haplotype, the Senegal haplotype, and an atypical one. The participants belonged to three genotypes, Benin/atypical (64.1%), Benin/Senegal (33.3%) and Senegal/Senegal (2.6%). Overall, HU treatment was successful in all haplotypes with 12 out of 39 patients failing treatment after 12 months in the Benin haplotype group. The association between HU treatment success and the Benin haplotype was found in terms of the decrease in the number of white blood cells and the students missing class. **Conclusion:** The study revealed that inferring haplotype based on the phenotypic profile could be inaccurate. The proportion of atypical haplotype that were not previously described in Côte d'Ivoire was

high. All the haplotypes seemed to be associated with HU treatment success but some patients with Benin haplotype did not respond well.

Keywords

Sickle Cell Disease, Children, Haplotype, Hydroxyurea, Côte d'Ivoire

1. Introduction

Sickle cell anemia is an inherited disease due to an abnormal hemoglobin (Hb) S that contains valine instead glutamic acid in its β subunit [1]. It is also referred to as Sickle Cell Disease (SCD). The presence of HbS results into polymerized hemoglobin under deoxygenated conditions leading to the sickling of the red blood cells (RBC). The abnormal RBCs make the blood more viscous and obstruct the blood vessels especially the small caliber ones causing pain and organ damages, events known as acute vaso-occlusion or AVO [1] [2] [3]. It is a common genetic disease in Sub-Saharan Africa with the majority of children born with it living there [1] [2] [4]. Its prevalence rate is around 12% of the general population in Côte d'Ivoire [3]. SCD has a solidly homogeneous genetic background even though it shows variability in its clinical expression, the severity of the disease and the life span of the people affected [1] [2]. Indeed genetic polymorphic variations exist around the main mutation in the β subunit gene. They are known as haplotypes and have shown influence on the clinical features of the disease [5] [6] [7].

The management of SCD aims to avoid pain crises, to relieve symptoms and to prevent complications. The tools used include blood transfusions, immunization against frequently encountered germs, malaria and bacterial infection prophylaxes, pain killers, SCD specific medicines such as hydroxyurea, L-glutamine, crizanlizumab, and voxelotor, bone marrow transplantation and gene therapy [8]. Treatment of SCD is still challenging despite the very few effective options including Hydroxyurea (HU) treatment. For instance, the handling of the maximum tolerated dose of HU in developing countries remains difficult which in turn results in the use of lower doses in studies conducted in Africa compared to the United States [9] [10] [11] [12]. Of note, HU was the first United States of America (USA) Food and Drug Administration (FDA) and European Medicines Agency (EMA)-approved therapy for clinical use in SCD patients respectively since 1998 in the USA and since 2007 in Europe, and the only one approved up until 2019 [13] [14] [15]. HU is a ribonucleotide reductase (RR) inhibitor first used to treat myeloproliferative diseases. Its use in SCD aims to induce HbF production by increasing the γ-globin gene expression and consequently reducing the proportion of HbA1 adult containing HbS [16] [17] [18] [19]. By doing so, HU reduces sickling and consequently the occurrence of the AVO events. It also reduces platelet, leukocyte and reticulocyte counts. It decreases the levels of

lactate dehydrogenase and cell adhesion molecules expression. And it increases nitric oxide levels. During HU treatment, HbF levels may range from 10% to 40% significantly improving the clinical manifestations of the disease such as the painful crisis and the bone necrosis. It leads to fewer episodes of acute chest syndrome, hospitalizations and blood transfusions, consequently reducing the mortality rate.

Though the most commonly used treatment worldwide, HU treatment shows great variability in the responses to treatment. Indeed a quarter of the SCD patients treated with HU are deemed poor respondents or non-respondents [13] [19]. That suggests that responses could also be a genetic trait of the SCD patients [16] [18] [19]. Therefore, it is necessary to identify the patients that are likely to favorably respond, and to switch to other available options those that have poor responses or who outright fail HU treatment. The current study intends to identify in Abidjan the HbS haplotypes that are associated with HU treatment responses, which might allow pinpointing HU treatment failure early and improving the management of the SCD in that context.

2. Methods

2.1. Study Design

A cross-sectional descriptive study was conducted to identify the HbS haplotypes of SCD children in Abidjan and their responses to HU treatment as they relate to their respective haplotypes.

2.2. Population, Variables and Definition

The participants to this study belong to a cohort of SCD children that were given HU treatment in Abidjan between November 2017 and April 2019. They had the homozygous SCD phenotype SSFA2 or the sickle-β°-thalassemia phenotype SFA2. They were aged 5 to 15 years, and their parents gave their informed consent to their participation to this study. The cohort was built at the Teaching Hospital of Yopougon, in Abidjan, Côte d'Ivoire. The clinical hematology ward of the aforementioned hospital provided medical care during the entire study period. All participants received HU medication upon inclusion. Biological analyses were performed at the central laboratory of the same hospital for hematological assays (HbS phenotyping and complete blood cells count) and biochemical assays (blood sugar, ASAT, ALAT, lactate dehydrogenase or LDH, total and conjugated bilirubin, urea and creatinine assessment). The haplotyping tests were run at the Molecular Biology Unit of the CeDReS at the Teaching Hospital of Treichville, Abidjan, Côte d'Ivoire. Data were collected from patients using an interview form administered by a research nurse. Eligible children who did not consent to the study and those who requested to quit after consenting were excluded from it. The participants did not stop taking their regular medication such as antibiotics, folic acid, malaria prophylaxis, and non-steroid anti-inflammatory drugs.

2.3. Ethical Consideration

The study was designed and conducted following the Declaration of Helsinki. It was reviewed and approved by the Ivorian National Ethical Committee for Health and Life Sciences Research under the registration number 108-23/MSHPCMU/CNESVSV-km.

2.4. Analytical Methods

Two types of Blood samples were collected per patient. One with ethylene diamine tetra-acetate (EDTA) as anticoagulant were drawn from fasting children for at least 10 hours at the inclusion and 12 months into HU treatment. The first part of the EDTA sample was used for the hematological assays on the automated blood cells counter XT of Sysmex, and for alkaline and acid electrophoreses of hemoglobin [20]. And the last part of it was used to perform a PCR-RFLP method that used five different primer sets amplifying specific regions within the human β gene cluster (Gγ, Aγ, the first and second sites inside $\Psi\beta$, δ), and three restriction enzymes that were HindIII, HindII and HincI [6]. A second blood sample was collected without an anticoagulant for the biochemical analyses of the aforementioned variables on the automated Cobas E411 of Roche.

2.5. Statistics

Probabilities of events were determined, and margins of error were calculated using GraphPad 6 v 2 software. Descriptive analyses described the profile of the studied population. The statistical parameters of the associations between the hemoglobin S haplotypes and the elements of HU treatment or the different phenotypes of SCD were the Pearson's chi-square test. A result was considered statistically significant for a p-value < 0.05.

3. Results

3.1. Social and Anthropological Description of the Population

In the cohort, more than 80.0% were male with sex ratio of 2.25 (**Table 1**).

That distribution was similar for the two hemoglobin phenotype groups recruited into the study. The average age was 9 ± 3 years. More than 90% was schooled children from preschool to senior high school. At least half of them had missed some school time due to SCD prior to their recruitment into the study. There were 19 out of 31 in the SSFA2 phenotype group and 3 out of 6 in the SFA2 phenotype group. Most of the participants with SSFA2 phenotype (51.5%) were born to a family that had another child with SCD. That proportion dropped to 33.3% for the children with the SFA2 phenotype.

3.2. Clinical Description of the Population

Of note, 74.3% was not immunized against frequently found infectious diseases for which a vaccine is available (**Table 1**). At the time of the recruitment, more

Table 1. Social and anthropological data of children with Hb phenotypes SSFA2 and SFA2

Parameters	Frequency of hemoglobin S phenotypes (%)		
	SSFA2 phenotype	SFA2 phenotype	Total
Male	22 (81.5%)	5 (83.3%)	27
Female	11 (18.5%)	1 (16.7%)	12
Sex ratio	2.00	5	2.25
5 to 10 years	18 (54.5%)	4 (66.7%)	22
11 to 15 years	15 (45.5%)	2 (33.3%)	17
Average age ± SD	9 ± 3 years	10 ± 2 years	9 ± 3 years
Minimum-Maximum age		5 years - 15 years	
Schooled child	31 (93.9%)	6 (100.0%)	37
No schooling	2 (6.1%)	0 (0.0%)	2
Out of classroom (before/after HU)	19/2	3/1	22/3
Other family cases of SCD	17 (51.5%)	2 (33.3%)	19
No-immunization	27 (81.8%)	2 (33.3%)	29
Total	33 (84.6%)	6 (15.4%)	39

than 60.0% of the participants had eating and sleeping disorders in both phenotype groups (Table 2).

After 12 months of HU, that proportion decreased 3-fold ($p < 0.0001$). At least half of the participants had abdominal and joint pains as well as jaundice at the inclusion that persisted over the 12 months of HU treatment. Hospitalization and blood transfusion were common among the SCD affected children at the inclusion (65.0%). The treatment almost eliminated those occurrences in both hemoglobin phenotypes ($p < 0.001$). Four participants died during the course of their HU treatment accounting for 10.2%. There were some side effects associated with the treatment. They were dominated by black and tarry stools and nausea in the participants to the study. However in terms of kidney and liver toxicity, the HU treatment was overall well tolerated after 12 months of treatment. The study participants' kidney and liver functions were not damaged. The most frequent organ damage encountered was the enlargement of the spleen in the children with a hemoglobin phenotype SSFA2 (54.5%). HU treatment reduced the proportion children with enlarged spleen to 6.9% after 12 months.

One of the main benefits of the HU is its ability to increase HbF gene expression resulting in more HbF in the blood. At the inclusion, half of the participants had HbF levels below 10%. HU treatment decreased that proportion to respectively 20.7% and 0% in the SSFA2 and SFA2 phenotype groups. With regards to the actual levels of HbF, the average was largely higher in the SSFA2 phenotype

Table 2. Clinical and biological data.

Parameters	SSFA2 phenotype—n (%)		SFA2 phenotype—n (%)	
	Inclusion	M12	Inclusion	M12
Sleeping and eating disorders	21 (63.6%)	4 (13.8%)	4 (66.7%)	1 (16.7%)
Joint and abdominal pains	19 (57.6%)	13 (44.8%)	3 (50.0%)	2 (33.3%)
Hospitalization/transfusion	26 (78.8%)	1 (3.4%)	4 (66.7%)	-
Jaundice	25 (75.8%)	21 (72.4%)	4 (66.7%)	3 (50.0%)
Liver damage	4 (12.1%)	-	1 (16.7%)	-
Kidney damaged	1 (3.0%)	1 (3.4%)	1 (16.7%)	-
Spleen damaged	18 (54.5%)	2 (6.9%)	-	-
Deaths	4 (12.1%)		-	
HbF levels below 10%	19 (57.6%)	6 (20.7%)	3 (50.0%)	-
HbF levels (average ± SD %)	9.0 ± 5.6	13.9 ± 7.2	17.6 ± 6.6	18.4 ± 6.8
Between 6 and 12 months age	4 (12.1%)		-	
Between 13 and 24 months age	7 (21.2%)		1 (16.7%)	
More than 24 months age	22 (66.7%)		5 (66.7%)	
Hb (G/dL)	6.9 ± 1.9	8.1 ± 1.6	6.7 ± 3.3	9.3 ± 1.0
Minimum-Maximum age	5.1 - 8.5	5.4 - 13.2	6.6 - 8.4	7.8 - 10.0
Hypochromia	17 (51.5%)	13 (44.8%)	3 (50.0%)	3 (50.0%)
Microcytosis	9 (27.2%)	6 (20.7%)	2 (33.3%)	4 (66.7%)
White blood cells—WBC count ($\times 10^3$)	12.8 ± 4.7	9.8 ± 3.1	12.5 ± 3.1	9.5 ± 2.1
Minimum-Maximum WBC count	8.2 - 30.0	4.1 - 15.1	11.8 - 16.2	7.6 - 12.4
Platelets count ($\times 10^5$)	371 ± 113	315 ± 109	400 ± 117	273 ± 95
Minimum-Maximum Platelet count	252 - 679	145 - 609	381 - 438	151 - 354
Total	33	30	6	5

group than in the SFA2 phenotype group (9.0% versus 17.6%). There were four cases of death under HU treatment, all in the SSFA2 phenotype group (12.1%). This corresponds to an overall mortality rate of 10.2% for the entire cohort.

3.3. Biological Description of the Population

The SCD diagnosis mainly occurred after the 2 years old mark accounting for 66.7% (Table 2). At the inclusion, the median hemoglobin level was just above 6.0 G/dL along with normochromia (around 50.0%) and normocytosis (around 30.0%). After 12 months of treatment, the hemoglobin level rose to just above 8.0 G/dL of blood. The median white blood cells (WBC) counts were around 12.5×10^3/dL of blood showing hyperleukocytosis in more than 70.0% of the participants. After the treatment, the median WBC counts was 9.5×10^3/dL of

blood indicating a decrease. Nine participants had high platelets count (PQ) above 4.5×10^5/dL of blood (23.1%). Overall, PQ was normal at inclusion, and remained unchanged after the 12 months of HU treatment.

3.4. Hemoglobin Phenotypes of the Population

At their inclusion into the study (Table 1), the distribution of the different hemoglobin phenotypes was 33 cases of the homozygous sickle cell disease phenotype SSFA2 (84.6%) and 6 cases of the sickle cell β^0-thalassemia phenotype SFA2 (15.4%).

3.5. Hemoglobin Haplotypes of the Population

Overall, 77 chromosomes were analyzed in this cohort (Table 3).

Out of the 39 participants, the distribution of the HbS haplotypes was 25 cases of heterozygous Benin/atypical haplotype (64.1%) and 13 cases of heterozygous Benin/Senegal (33.3%) and one case of homozygous Senegal (2.6%). Among the four participants who passed away during HU treatment three had the heterozygous Benin/atypical haplotype and one had the heterozygous Benin/Senegal haplotype (Table 4).

3.6. Interdependence of Studied Variables

Using adjusted logistic regressions; relationships were sought between hemoglobin haplotypes considered as a dependent variable and explanatory variables of the study such as increase in HbF levels, death of the participant, participant missing school time, increase in hemoglobin levels, and decrease in WBC counts (independent variables).

Factors Influenced by hemoglobin S haplotypes

Missing school time and decrease in the number of WBC were statistically associated with having the hemoglobin S haplotype Benin (Table 4). In univariate analyses, no relationships were found between the Hb phenotypes, HbF increase, the death of the participants and the hemoglobin levels (Table 4).

Table 3. HbS haplotype distribution.

Haplotypes	Frequency	Percentage (%)
Total number of chromosomes analyzed	77	100
Benin	38	49.3
Senegal	14	18.2
Atypical	25	32.5
Total number of genotypes uncovered	39	100
Benin/Atypical heterozygous	25	64.1
Benin/Senegal heterozygous	13	33.3
Senegal/Senegal homozygous	1	2.6

Table 4. Influence of the various parameters on HU treatment success.

Parameters		Hemoglobin S haplotypes (n)			p-value
		Benin/Atypical	Benin/Senegal	Senegal/Senegal	
Increased hemoglobin bF	Yes	18	8	1	0.1748
	No	7	5	0	
Death of participants	Yes	3	1	0	0.4958
	No	22	12	1	
Out of the classroom	Yes	13	9	1	0.0251
	No	12	4	0	
Increased hemoglobin levels	Yes	18	9	1	0.1964
	No	7	4	0	
Decreased white blood cell count	Yes	14	4	0	0.0357
	No	11	9	1	
Total		25	13	1	-

4. Discussion

4.1. About Analytical Methods

Association between hemoglobin F levels obtained with electrophoresis and specific hemoglobin S haplotypes was previously reported [21] [22] [23] [24]. However other reports have shown that among homozygous patients for a given haplotype, HbF levels varied up until the age of 10 years, and remain so for the entire adult life [25] [26] [27]. Therefore, the genotyping with PCR-RFLP conducted in this study allows for the accurate estimation of the proportions of HbS haplotypes within SCD children in Abidjan.

4.2. About the Studied Population

The average age of the participants to the current study is consistent with previously published data in children [2] [6] [28]. Some studies have tried to assess the impact of genetic modifiers on SCD mainly outside Western Africa [2] [5] [7] [14] [15] [23] [24]. They've shown that among other factors that modulate responses to treatment the hemoglobin haplotypes may play a significant role. The current study demonstrated that both the Benin and the Senegal haplotypes were likely to favorably respond to HU treatment in Abidjan. Although a small proportion of Benin haplotype did not respond to treatment. This is consistent with previous reports on HU therapy [8] [16] [17] [19].

SCD has proven quite deadly in children from Benin and Nigeria, two countries in Western Africa where respectively 50% and 90% of homozygous SSFA2 SCD children die [26] [27]. In the current study, the mortality rate was significantly lower than those rates. Indeed, it has been reported that morbidity and mortality are dependent on the quality of care and the phenotypic hemoglobin

profile of the specific child [4] [26] [27] [28]. The difference could be attributed to HU treatment. The time to the diagnostic of SCD indicated late SCD diagnosis compared to that observed in more advanced countries [28]. This points to the lack of perinatal genetic testing in Côte d'Ivoire. Previous reports on benefits of HU treatment in this category of patients have shown consistent improvement of the health of the SCD children with very little adverse effects of the drug itself [17] [18] [19] [24].

4.3. About Hemoglobin Haplotypes

More and more reports on hemoglobin S genotyping with the PCR-RFLP method detects the variants of the classically known hemoglobin haplotypes [5] [6] [16] [24] [28]. This study uncovered only one case of Senegal haplotype homozygote as described before in the literature whereas the others genotypes were associated with a variety of haplotypes that did match the generally accepted definition. Those new haplotypes were referred to as atypical haplotypes. These observations point to a molecular evolution of the populations that requires to gather more genetic data through sequencing in Africa in order to assess their impact on the clinical and biological presentation of SCD in that context. In Abidjan, SCD children are more likely to be of the Benin/atypical heterozygote haplotype. That piece of data is consistent with previous reports based upon inferences from phenotypic studies that suggest the Benin haplotype as the prominent haplotype in Côte d'Ivoire [21].

All the haplotypes seemed to favorably respond to the HU treatment. However, some children with the Benin/atypical heterozygote haplotype that had the lowest HbF levels before treatment, and those levels did not increase their levels above 10% after 12 months of treatment. This observation was previously described in Brasilia where live a considerable black population [19].

5. Conclusion

This report is the first study assessing hemoglobin haplotypes impact on HU treatment in children with SCA in Abidjan, Côte d'Ivoire. It shows that the disease was still deadly without appropriate medication. The Benin haplotype was still the most frequent haplotype and that it responded favorably to HU treatment at 12 months.

Acknowledgements

The authors thank the children and their parents for participating to this study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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