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AN ALGORITHMIC APPROACH TO THE MOLECULAR DIAGNOSIS OF ALPHA THALASSEMIA MUTATIONS IN GABONESE PATIENTS WITH SICKLE CELL TRAIT

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ABSTRACT

Background: Hemoglobinopathies represent a large group of quantitative and/or qualitative genetic disorders. The thalassemia syndromes are a diverse group of inherited disorders that can be characterized according to their insufficient synthesis or absent production of one or more of the alpha or β-globin chains. The aim of this study is to determine the presence of alpha-thalassemic mutations in Gabonese sickle cell trait carriers by using molecular biology technologies. **Methods:** The sample was composed of 450 patients from the 9 administrative provinces of Gabon. The strategy used a succession of procedures including DNA extraction, alpha GeneScan assay, multiplex Gap PCR and an analysis using the GeneMarker software. **Results:** The results showed deletions of one or more copies of the alpha gene present in both HbAA and HbAS genotypes. Regardless of the genotype considered, the types of alpha gene deletions exist to varying degrees -α/αα the silent carrier was most represented (45% and 52%); -α/-α thalassemia trait or minor (13%; 16%) and --/-α HbH disease (Thalassemia intermedia) at 1% in both phenotypes. The frequency of the α3.7 deletion was 21,42%, as homozygous (− α3.7/− α3.7) 35,71%, 14.28% homozygous αα/αα and 26.59% as heterozygous (− α3.7/αα). The heterozygous α-thal (−α4.2 deletion) was absent. **Conclusion:** The molecular biology strategy used here allowed us to detect the α3.7 deletion and homozygous form (− α3.7/− α3.7) mutation. Considering the role that thalassemic mutations play on the health of the sickle cell patients, this simple and direct strategy should be used to carry out systematic research within the sickle cell populations of Gabon for a better medical care and a follow-up adapted to each patient.

KEYWORDS: Sickle Cell Trait; α-Thalassemia, Gap-PCR, GeneScan, Gabon.

INTRODUTION

Hemoglobinopathies represent a large group of quantitative and/or qualitative genetic disorders (More than a thousand to date),[1] caused by mutations in the genes responsible for the synthesis of globin chains. Hemoglobinopathies represent an increasingly emerging problem for global health policy, with 7% of the world population carrying a hemoglobin disorder and 300,000-500,000 children born each year with a severe homozygous form of these diseases (83% sickle cell disease, 17% thalassemia).[1] The thalassemia syndromes are a diverse group of inherited disorders that can be characterized according to their insufficient synthesis or absent production of one or more of the globin chains. They are classified in to α, β, γ, δβ, δ, and εγδβ thalassemia depending on the globin chain(s) affected.[2] Sickle Cell disease (SCD) is an important cause of death in young children in Africa and in this continent it is a major cause of child ill-health and death.[3] Without treatment, which is rarely available in low-income, high-burden countries[4] the vast majority of these children die before the age of five.[5]

Indeed, in Africa, where SCD-related mortality and morbidity are severe childhood health problems, epidemiological studies are poorly developed, usually conducted in health centers, and records the prevalence of SCD only in hospitalized patients. Attempts to improve the medical care of patients with SCD in Africa have been scarce, reflecting the lack of concern from the African medical community.[6] Sickle Cell Cell Disease causes significant morbidity and mortality particularly in the early years unless prophylactic measures have been put in place.[7] Today, SCD is a major public health problem in the majority of African countries like the Republic of Gabon with a proportion of heterozygous HbAS, hereafter called Sickle Cell Trait (SCT) carriers of 21.1% in people over 15 years.[8]

The prevalence of SCD in Gabon is a serious public health problem and is a severe burden on its medical resources. There is increasing motivation to reduce the problem of SCD from both a medical, financial and social standpoint. The International Monetary Fund (IMF) reports that Gabonese authorities have achieved significant progress in restoring macroeconomic stability in the country since 1999. This provides Gabon with a unique opportunity to address the acute challenges confronting the country, one of which is SCD.

Since 2014, we know more on the epidemiology and localization of sickle trait in persons older than 15 years of age in the Gabonese territory. But the diagnostic of sickle cell disease can be under looked because of presence of many factors like alpha-thalassemia. Alpha-thalassemia (α-thal) is probably the commonest autosomal recessive hemoglobin (Hb) disorder with wide distribution, affecting around 5% of the world's population.[9] They can be classified, based on the genes involved and the type of defect, in thalassemia (alpha, beta, delta/beta) and hemoglobin variants (alpha, beta, delta). Alpha-Thalassemia (α-thal) is widely reported in the Arabian Peninsula as one of the main causes of asymptomatic microcytic hypochromic red blood cells with or without anemia in the pediatric population.[10] The most common cause of anemia is either iron deficiency or thalassemia trait.[11] Alpha-thalassemia is very common throughout all tropical and subtropical regions of the world.[12] Alpha-Thalassemia, the most common genetic disorder occurs widely throughout Africa, the Mediterranean countries, the Middle East and the Southeast Asia.[13,14] Coinheritance of α-thal influences the clinical and hematological phenotypes of beta-hemoglobinopathies (β-thalassemia and sickle cell disease) to variable degrees depending upon the degree of α-globin chain deficiency which in turn depends upon the type and location of the gene lesion.[9]

In the global and efficient management of the sickle cell patients in the Central African sub-region, several objectives have been defined such as: the establishment of molecular diagnostic screening techniques for SCD (newborn and mass carrier screening); providing genetic counselling to at-risk couples to help them make better reproductive choices; providing technical advice to the national health authorities to boost prevention, control and management of SCD. Majority of the carriers of beta-thalassemia and some forms of alpha-thalassemia are clinically asymptomatic and although their hematologic phenotypes (i.e., microcytic hypochromic red blood cells) with or without anemia are predictive markers, they are not specific and overlap with conditions such as iron deficiency. It is difficult to distinguish between alpha-thalassemia and iron deficiency, and DNA based diagnosis is essential. Furthermore, DNA diagnostics can provide insight into the commonly observed discrepancies between phenotypes and genotypes, especially in cases of sickle cell disease and beta-thalassemia, where alpha-thalassemia is known to act as a genetic modifier.[11] This study has two main aims: to determine the presence and types of alpha-thalassemic mutations in Gabonese sickle cell trait carriers by using molecular biology technologies and to facilitate the technology transfer that will be beneficial for a diagnosis of this type in Gabon as well as to train healthcare and research staff.

MATERIALS AND METHODS

Study Area and Sampling

Gabon, a Central African country crossed by the equator, is a country of 2 million inhabitants (5.6 inhabitants/km2), 73% of whom live in urban areas; divided into 9 administrative provinces (Estuaire, Haut-Ogooué, Moyen-Ogooué, Ngounié, Nyanga, Ogooué-Ivindo, Ogooué-Lolo, Ogooué-Maritime et Woleu-Ntem) with 2048 villages located mainly along roads and rivers. These different provinces have constituted our sampling place. In fact, about 450 samples collected between June 2005 and September 2008 during a project focused on the Ebola virus in Gabon, were selected for this study. Persons over 15 years of age and having been present in that locality for at least one year were selected in the different provinces for participation in the study.

Ethics statement

Written consent was secured from all participants. In the case of minors, consent was obtained from at least one parent. Our study received the approval of the Gabonese Ministry of Health, with a research authorization Nb. 00093, March 15, 2005.2.3.

Blood Collection and Processing

- ***Detection of abnormal hemoglobin***

Blood samples were usually collected in the village health care center into 7-ml vacutainer tubes containing EDTA (VWR International, France). The tubes were transported daily to the field laboratory for centrifugation (10 min, 2000g). Red blood cells were stored and were then processed for screening of abnormal hemoglobin.

The presence of abnormal hemoglobin was ascertained by the isoelectric focusing (IEF) method by which proteins are separated according to their isoelectric points. When an abnormal protein was detected, high-performance liquid chromatography (HPLC) was used to identify the exact variant: hemoglobin A (HbA), hemoglobin S (HbS) or hemoglobin C (HbC) as described by Délicat-Loembet et al.[8]

- ***DNA extraction***

Genomic DNA was isolated from 25 AA and 25 AS samples from each province using QIAgen DNA Mini Kit blood and tissue (QIAGEN, Germany) according to the manufacturer's instructions. The extracted DNA was stored at −20°C until use. The DNA quality and quantity were measured by Nanodrop 2000.[15] These Gabonese DNA samples were aliquoted and brought to the hematology department in Sultan Qaboos University of Oman.

- ***Molecular diagnostic of Alpha-thalassemia***

We performed many molecular techniques to diagnose the alpha thalassemia mutations in our samples. Protocols for the detection and molecular diagnosis of alpha-thalassemia were performed in the laboratory of hematology department in Sultan Qaboos University of Oman. The main requirements for methodologies providing molecular diagnosis are speed, cost, convenience, and the ability to test for multiple mutations simultaneously. For alpha-thalassemia the technique of multiplex Gap PCR is useful for targeting specific deletion mutations.[16] We will conduct our analysis according to the following analysis described in the figure a.

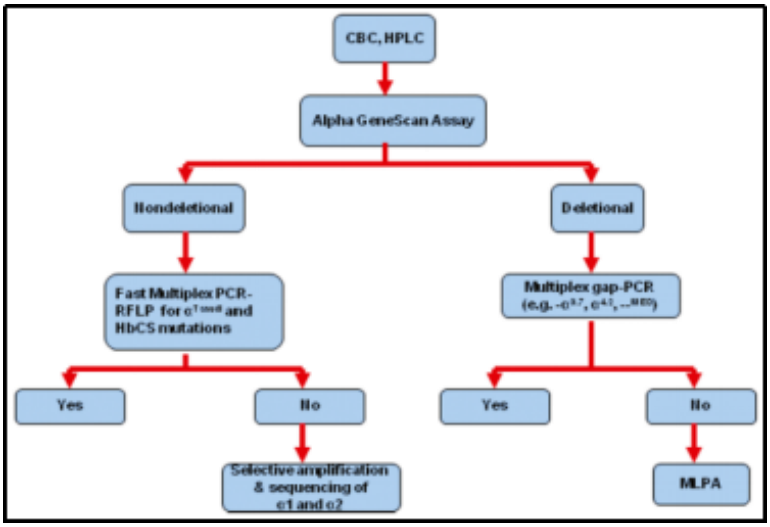

Figure a: Stepwise screening strategy for detecting the α-thalassemia alleles.

Alpha genescan analysis

In order to suggest if the alpha thalassemia mutation was deletional or non-deletional, the alpha GeneScan assay was performed. A multiplex polymerase chain reaction (PCR) assay was designed to co-amplify both α1, α2 and an internal control Gene (RNase P) with differences in size of their PCR products (Primers in table1).

Table 1 : Genscan primers sequences.

Alpha forward primer sequence	*GGACCCGGTCAACTTCAAGG*
Alpha reverse primer sequence	*GGTGCTCACAGAAGCCAGGA*
RNase P- Forward primer sequence	*CGGAGGGAAGCTCATCAGT*
Rnase P-Reverse primer sequence	*ACCTCACCTCAGCCATTGAA*

Thermal cycling parameters of amplification included: stage 1, at 96°C for 15 min, followed by stage 2, 25 cycles of, respectively, 45 sec at 98°C for denaturation, 1 :30 sec for 60°C for annealing temperature and 2 :09 sec at 72°C for extension and stage 3, 5 minutes at 72°C and 16°C to complete the reaction to infinity. 1µl (100 ng) of template DNA was analyzed by Fast PCR master mix.

The products were analyzed using the GeneScan v3.7 program (Applied Biosystems) by comparing the ratio of α-globin peaks versus internal control (RNase P). Details of the procedures are described in Alkindi *et al.*, 2013 publication.[9]

If the result of the GeneScan analysis showed the presence of a deletion of one or more alpha genes, the samples concerned were submitted to the multiplex Gap PCR protocol for confirmation of the result.

Multiplex Gap PCR for the study of α-globin gene deletions

The Gap PCR technique is a simple technique to detect the deletion with complementary primers of the flanking regions of this deletion. The presence of the expected amplification product reveals the existence of the deletion, as the primers are too far apart to successfully amplify the normal (non-deleted) allele. To confirm the alpha GeneScan results we used the GAP PCR to check the type of deletion **$-\alpha^{3.7}$; $-\alpha^{4.2}$; $-\alpha^{MED}$; $-\alpha^{THI}$; $-\alpha^{FIL}$; $-\alpha^{SEA}$.** Samples were amplified by PCR using the all primers mentioned (we use one tube containing a mix of different primers that amplify the common deletion of α-thalassemia like listed) in table 2.

Table 2 : Primers for Single-tube multiplex PCR analysis of common-thalassemia deletion. ***(Chong et al., 2008)***

Name	5' → 3' Sequence	GenBank ID: Nucleotides	Amplicon (Size)
LIS1-F	GTCGTCACTGGCAGCGTAGATC	HSLIS10:407 → 428	LIS1 3'UTR frag
LIS1-R	GATTCCAGGTTGTAGACGGACTG	HSLIS10:2909 → 2887	(2503 bp)
α2/3.7-F	CCCCTCGCCAAGTCCACCC	HUMHBA4:5676 → 5694	$-\alpha^{3.7}$jxn frag
3.7/20.5-R	AAAGCACTCTAGGGTCCAGCG	HUMHBA4:11514 → 11494	(2022/2029 bp)
α2/3.7-F	see above		α2 gene
α2-R	AGACCAGGAAGGGCCGGTG	HUMHBA4:7475 → 7457	(1800 bp)
4.2-F	GGTTTACCCATGTGGTGCCTC	HUMHBA4:3064 → 3084	$-\alpha^{4.2}$jxn frag
4.2-R	CCCGTTGGATCTTCTCATTTCCC	HUMHBA4:8942 → 8920	(1628 bp)
SEA-F	CGATCTGGGCTCTGTGTTCTC	HSGG1:26120 → 26140	$--^{SEA}$jxn frag
SEA-R	AGCCCACGTTGTGTTCATGGC	HSCOS12:3817 → 3797	(1349 bp)
FIL-F	TGCAAATATGTTTCTCTCATTCTGTG	HSGG1:11684 → 11709	$--^{FIL}$jxn frag
FIL-R	ATAACCTTTATCTGCCACATGTAGC	HSCOS12:570 → 546	(1166 bp)
20.5-F	GCCCAACATCCGGAGTACATG	HSGG1:17904 → 17924	$-(\alpha)^{20.5}$jxn frag
3.7/20.5-R	see above		(1007 bp)
MED-F	TACCCTTTGCAAGCACACGTAC	HSGG1:23123 → 23144	$--^{MED}$jxn frag
MED-R	TCAATCTCCGACAGCTCCGAC	HSGG1:41203 → 41183	(807 bp)

Thermal cycling parameters of amplification were at stage 1, at 96°C for 15 min, followed by stage 2, 30 cycles of, respectively, 45 sec at 98°C for denaturation, 1:30 sec for 60°C for annealing temperature and 2:09 sec at 72°C for extension and stage 3, 5 minutes at 72°C and 16°C to complete the reaction. The final product was separated on agarose 0.8% gel (Ultrapure™ Agarose) power supply 100 volt for 60 minutes.

Alpha Genes Sequencing with ABI 3130 Genetics Analyzer®

The negative result of the GeneScan analysis, i.e., did not reveal the presence of a deletion of one or more alpha genes, the samples concerned were submitted to sequencing for confirmation of the result or the detection of a possible mutation at the level of $\boldsymbol{\alpha_1}$ and $\boldsymbol{\alpha_2}$ genes by a selective amplification of the latter. In Oman automated direct nucleotide sequencing (ABI 3130; Applied Biosystems, Foster City, CA, USA) of the selectively amplified alpha1 and alpha2 globin genes was performed to characterize non-deletional α-thalassemia determinants using appropriate primers to ascertain the nature of the underlying molecular defect.[17] The sequencing results were analyzed using the GeneMarker software which is a genotyping software.

RÉSULTS AND DISCUSSION

Alpha GeneScan analysis

The normal result shows sample/control ratio allows almost equal peak heights of control and normal sample with four alpha-globin genes (αα/αα).

The number of alpha-globin genes is evaluated by the height (surface area) of both the α2 and α1 peaks. Equal intensity of both peaks (as compared with control) suggests either the presence of four alpha-globin genes or non-deletional mutation in the context of thalassemic phenotype (Figure b).

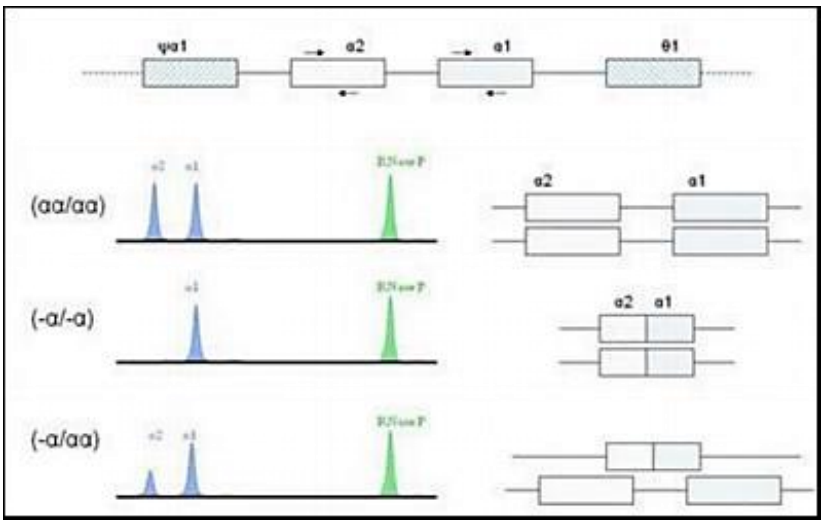

Figure b: Schematic diagram of Genescan studies for Alpha genes using control gene RNaseP.

The results of this analysis on our samples show that deletions of one or more copies of the Alpha gene are present in both HbAA and HbAS genotypes. Regardless of the genotype considered, the types of alpha gene deletions of varying degrees are -α/αα the silent carrier was most represented (45% and 52%); -α/-α thalassemia trait or minor (13%; 16%) and --/-α HbH disease (Thalassemia intermedia) at 1% in both phenotypes. The normal alpha gene profile is obtained in 41% and 31% (Figures c A and B). If both peaks are present and half of

the peak height of control, then a large deletion involving both α2 and α1 gene is suspected.

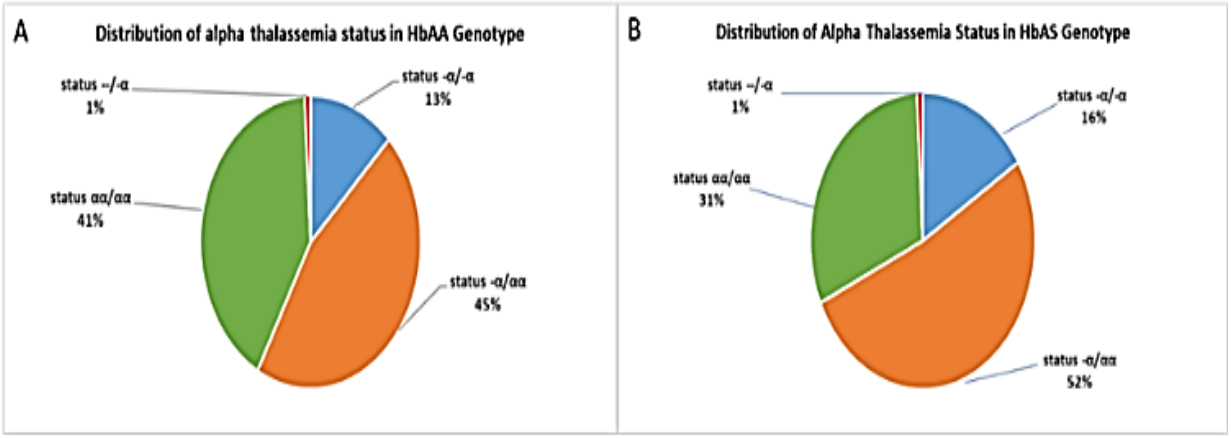

Figure c: Distribution of alpha-thalassemia in HbAA Génotype (A) and Distribution of Alpha-Thalassemia Statut in sickle cell carrier (HbAS) Genotype (B).

Multiplex Gap PCR for the study of α-globin gene Deletions and Alpha Genes Sequencing

Alpha-thalassemia is characterized by reduced or absence of α-globin chain synthesis and is mainly caused by deletions in the α-globin gene complex.[18] Gap-PCR amplifies the deleted DNA sequence using the primers flanking the deleted region.[19] Here Gap-PCR was performed to detect the common deletion of α-thalassemia. Alpha-globin GeneScan example profiles obtained in this study and the corresponding genotype is represented in figure d.

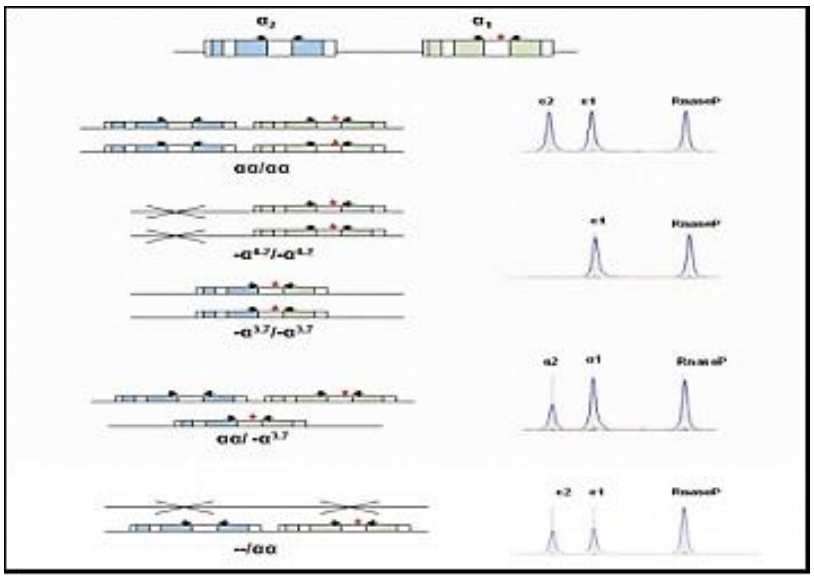

Figure d: Alpha-globin GeneScan example profiles obtained in this study and the corresponding genotype.

The result of the analysis performed by the Gene Marker V2.6.4 software (see figure e) shows different forms of loss of one or more copies of the alpha 1 and 2 genes. Samples with only one peak in the α1 position will indicate homozygous single-gene deletion that can be confirmed by subsequent gap-PCR which will define the nature of the deletion: $-\alpha^{3.7}$ or $-\alpha^{4.2}$. The various types of alpha gene deletions on varying degrees are -α/αα (sample 5 and 15) the silent carrier; -α/-α thalassemia trait or minor and --/-α (sample1) were observed. Sample 3 has a normal profile (αα/αα).

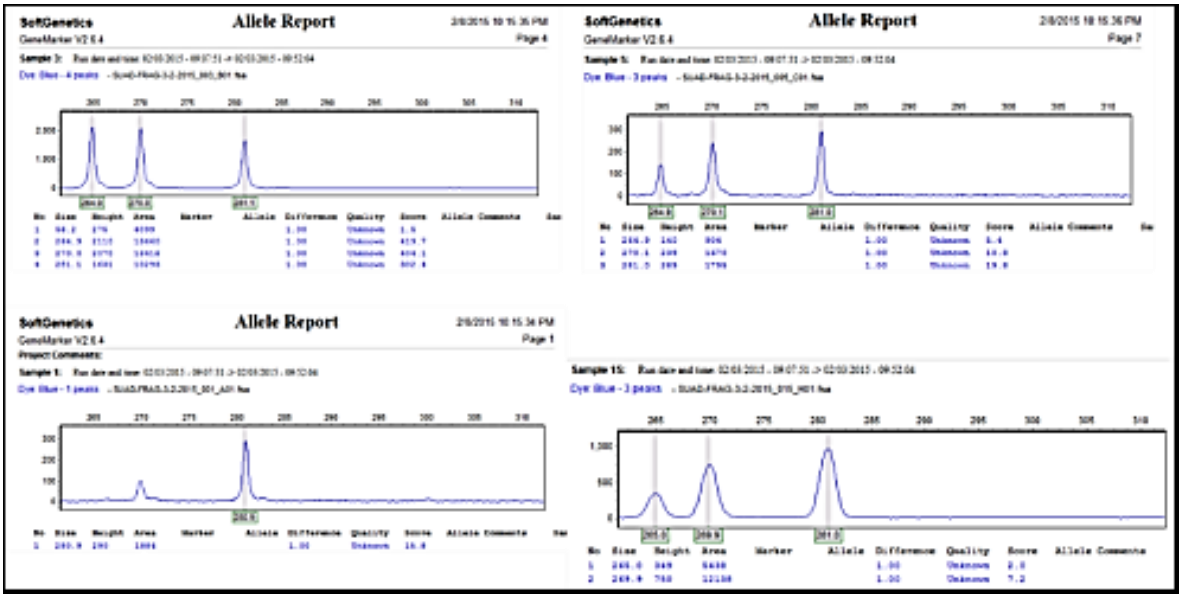

Figure e: Example of results observed during this study with Gene Marker V2.6.4.

Alpha-thalassemia is caused by deletion of a large fragment in the α-globin gene cluster DNA, that is the molecular biological mechanism of deletional α-thalassemia alleles.[20] In some areas, $-^{SEA}$, rightward deletion ($-\alpha^{3.7}$) and leftward deletion ($-\alpha^{4.2}$) are common deletional α-thalassemia alleles.[21] We can see an example of results observed on this agarose gel (Figure f). The frequency in this study of the α3.7 deletion was 21, 42% as homozygous (− α3.7/− α3.7) 35,71% and 14.28% homozygous αα/αα and 26.59% as heterozygous (− α3.7/αα).

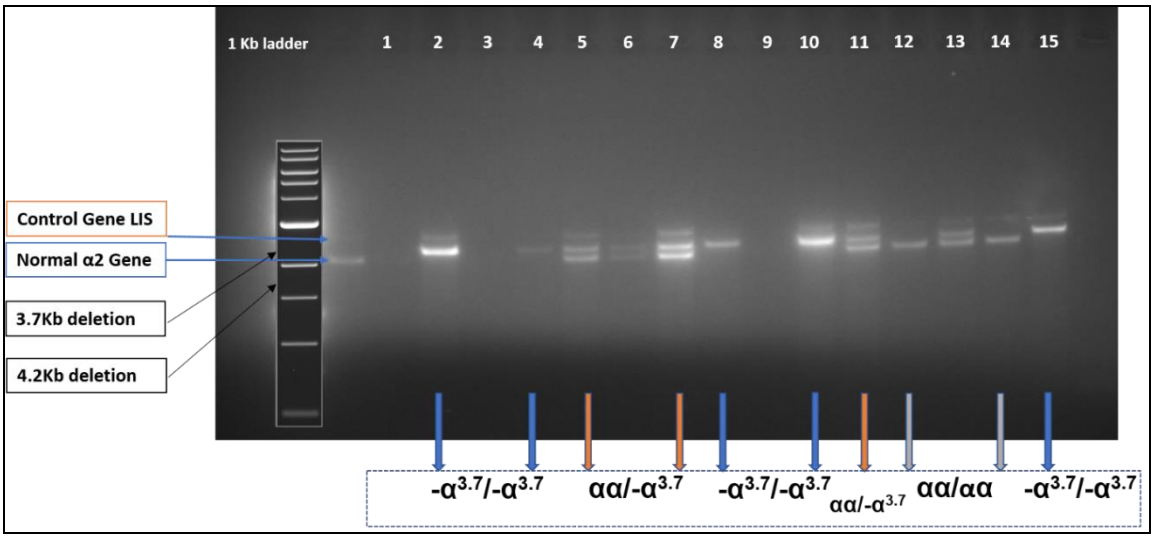

Figure f: PCR products of Alpha gene using control gene LIS seen on Ethidium sBromide-Stained Agarose gel. Lanes 2,4,8,10,15 shows one alpha gene deletion, lanes 5,7 & 11 shows 2 alpha gene deletion whereas lanes 12 & 14 shows normal alpha genes [No alpha gene deletion].

The two α-globin and β-globin chains that combine to form human hemoglobin are encoded by two α-globin genes (HBA1 and HBA2) and one β-globin gene (HBB).[22] The generic term α-thalassemia encompasses all of those conditions in which there is a deficit in the production of the α-globin chains of hemoglobin (Hb), a tetrameric molecule including two α-like and two β-like globin chains (α2β2).[23] Specifically, individuals described as having 'α-thalassemia' can have the loss of one or more α-globin allele(s); there is also HbH disease (loss of 3 α-globin alleles) and, finally, hydrops fetalis (loss of all 4 α-globin alleles and incompatible with life).[22] Geographically, the thalassemia can be found worldwide, with mild α-thalassemia reaching prevalence of 10–20% throughout a geographical belt spanning Sub-Saharan Africa, the Middle East, India, and Southeast Asia (and can even be found at much higher frequencies in selected parts of Southeast Asia).[22] Worldwide, the carriers of inherited Hb disorders are estimated to be about 270 million. Most of them live in the Southeast Asian region, where the thalassemia predominates. The African region comes next due to a high frequency of

sickle cell genes, α-thalassemia.[24] Individuals with the -α/αα genotype are not anemic, and usually have a normal mean corpuscular volume (MCV). The -α/-α genotype is associated with a microcytic erythrocytes and sometimes with a mild anemia.[25] The high frequency of α^{+}-thalassemia in the study population presumably results from this disorder having a selective advantage favoring survival due to tropical environmental factors.[26] The incidence of the association of heterozygous α-thalassemia with SCD is underestimated and probably high.[6] The co-inheritance of α-thalassemia and SCD is also associated with better hematological indices and lower consultation rates in Cameroonian patients.

The beta-globin protein forms part of the tetrameric hemoglobin complex; heterozygous persons have wildtype hemoglobin A as well as hemoglobin S, hence the term hemoglobin AS (HbAS). Sickle cell trait is most prevalent among persons with African ancestry. An estimated 7.3% of blacks, 0.7% of Hispanics, and 1.6% of U.S[27] residents overall have sickle cell trait. Sickle cell trait occurs in approximately 300 million people worldwide, with the highest prevalence of approximately 30% to 40% in sub-Saharan Africa.[28] Our study focused on a population of sickle cell trait carriers from the nine provinces of Gabon (Estuaire, Haut-Ogooué, Moyen-Ogooué, Ngounié, Nyanga, Ogooué-Ivindo, Ogooué-Lolo, Ogooué-Maritime and Woleu-Ntem). Because the detection of α-thalassemia in blacks is difficult, due to the mild hematological changes and minor alterations in hemoglobin composition that are present.[25] Also, because there are no characteristic alterations in hemoglobin composite which readily permits detection of the simple heterozygous. The presence of mild microcytosis, in the absence of iron deficiency or heterozygous β-thalassemia, suggests the presence of α-thalassemia. Moreover, alpha-thalassemia reduces the proportion of HbS in carriers of sickle cell trait.[25] Because of all the above-mentioned reasons, we chose to use molecular biology techniques for the realization of this study. The aim of this study was to determine the presence of alpha-thalamic mutations in sickle cell trait carriers by using molecular biology techniques in the Republic of Gabon. Thalassemia is a common monogenic recessive disease whose carriers primarily inhabit in the tropical and subtropical regions[20] According to the Haldane malaria hypothesis, heterozygous thalassemia is evolutionarily positively selected because of its protection against malaria.[29] In the case of α-thalassemia, the molecular biological mechanism is a defect in the α-globin gene cluster and the most severe form of α-thalassemia gives rise to Hb Bart's hydrops fetalis syndrome and HbH disease. Hb Bart's hydrops fetalis syndrome is commonly fatal to fetuses and newborns, which can also increase puerperal infection risk. Moreover, patients with severe HbH disease can suffer from anemia or hemolysis.[23] Currently, key detection methods for $-^{SEA}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ involve Gap-PCR,[30] quantitative fluorescent PCR (QF-PCR)[21] multiplex ligation-dependent probe amplification assay (MLPA)[31] and qPCR.[32]

We can note that the results obtained during this study shows that whatever the genotype considered HbAA or HbAS, we observe the absence of one, two or three alpha genes. Thalassemic disease, depending on the level, may or may not have symptoms but there are several factors that can aggravate this condition such as being sickle cell or carrying the sickle cell trait. In Gabon, the prevalence of SCT is 21.1% among people aged over 15 years, with the prevalence by region between 12.8 and 28.2%.[8] The individuals with SCT have minor α-thalassemia with 2 or 3 active genes of α-globin. This high rate of α-thalassemia in individuals with SCT implies a higher frequency of α-thalassemia in sickle cell patients.[33] Long considered a benign carrier state with relative protection against severe malaria, sickle cell trait occasionally can be associated with significant morbidity and mortality. Sickle cell trait is exclusively associated with rare but often fatal renal medullary cancer.[28] The protective effect of the HbAS genotype comes at a cost.[34] Homozygous HbSS individuals suffer from the physiological effects of sickling RBC deformations: microvascular obstruction and hemolysis leading to multiple downstream health deficits, frequent pain crises and reduced lifespan. Individuals with the HbAS genotype have a normal lifespan and are usually hematological asymptomatic. But during extreme circumstances, such as dehydration and high-intensity physical activity, complications including exertional rhabdomyolysis, splenic infarction and papillary necrosis, can occur.[28,22]

Deletional alpha (+)-thalassemia (-alpha (3.7)) was investigated in the two groups of unrelated individuals from samples (normal adults and sickle cells trait carriers) of Gabon, the presence of the (-alpha (3.7)) chromosome was observed in both normal adults and sickle cells trait carriers, while the (-alpha (4.2)) has not been observed. Figure f shows the different types of Thalassemia mutations and their combination detected mostly in individuals of the study. The frequency of the $\alpha^{3.7}$ deletion was 21,42%, as homozygous ($-\alpha^{3.7}/-\alpha^{3.7}$) 35,71%, 14.28% homozygous αα/αα and 26.59% as heterozygous ($-\alpha^{3.7}/\alpha\alpha$), the remaining 2% did not show any result. The malaria-rich environment, facilitating the positive selection of heterozygous α-thal.[24] The cases of absence of heterozygous α-thal ($-\alpha^{4.2}$ deletion) can be explained by the selection of the sample, or by a real absence of this type of mutation of the alpha gene within the Gabonese population. In a study conducted in Cameroon, the results showed a higher proportion of 3.7 kb a-globin gene deletion among SCA patients than controls, specifically HbAA individuals.[35] The principle of carrying out the experimental procedure has been established, and the results obtained showing the majority presence of the $\alpha^{3.7}$ deletion mutation and knowledge of its impact on the detection of sickle cell status or sickle cell trait carrier underscores the

importance of these techniques. In our context, where haematological parameters can vary according to exogenous factors, molecular biology techniques, given their specificity and sensitivity, can be a good method of confirming the diagnosis. Although the implementation and transfer of these techniques in our environment is a real challenge, it is not impossible to implement them, as more and more young people in the area are training in basic biological sciences with a view to integration into medical analysis laboratories. The creation of a center for the care of patients living with sickle cell disease could facilitate the acquisition of all the equipment needed for these experiments in a single location.

CONCLUSION

To evaluate the impact of the alpha thalassemic mutation in the genome of the Gabonese populations, it would be essential to carry out this study on a larger population while avoiding any mislabeling of the different samples. The molecular biology strategy used here allowed us to detect the α3.7 deletion and homozygous form (− α3.7/− α3.7) mutation. Considering the role that thalassemic mutations play on the health of the sickle cell patients, this simple and direct strategy should be used in order to carry out systematic research within the sickle cell populations of Gabon for a better medical care and a follow-up adapted to each Gabonese sickle cell patient.

Conflicts of interest: The authors declare that they have no competing interests.

Financial disclosure: These authors do not hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, now or in the future. All Authors reported no financial disclosures.

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Ethics approval: The Pharmacy and Medicine executive body of Gabon approved the procedure used for this medical screening initiative following ethical approval from both participating institutions' review boards and written informed consent from all participants.

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