Newborn Screening of Hemoglobinopathies in Bengo, Angola

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State of art

Sickle cell anemia (SCA) is caused by the presence of the sickle cell allele-HBB*S, in homozygosity. In sub-Saharan Africa, HBB*S typically reaches high frequencies, especially in regions where malaria is endemic. Epidemiologic evidence indicates that the burden of SCA, which currently represents a dramatic public health concern in sub-Saharan Africa, will predictably increase in the future.

In this sense, the determination of prevalence as well as markers of disease severity are necessary so that the Ministry of Health can develop correct programs to manage the disease.

Objectives

The objective of this work was to screen by direct sequencing the HBB gene in a sample of newborns from Bengo, Angola. Moreover, we aim to identify common haplotypes of SCA by Multiplex SNaPshot® system.

Methods

The sampling was performed at the General Hospital of Bengo between Abril 2014 and November 2016, involving the population living in the area covered by CISA's (the Centro de Investigação em Saúde de Angola) Health Demographic Surveillance System (Fig 1).

Children were selected from maternity, and all children born in the term in which mothers signed informed consent, where included.

A total of 359 blood samples, stored in FTA® (Whatman®), were analyzed.

Genomic DNA was extracted and purified using the QIAamp® DNA Investigator Kit (QIAGEN®). The 2 first exons of the HBB genic region were amplified and sequenced in a ABI PRISM 3130xl Analyzer.

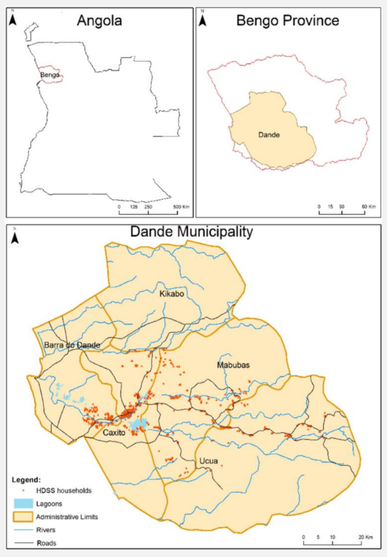

Fig 1. Study area in Bengo Province

In addition, in individuals harboring at least one *HBB*S* allele, a molecular characterization extended to variations across the β-globin cluster was performed through a Multiplex SNaPshot® system, aiming to identify the background haplotypes of HBB*S alleles (Fig 2.)

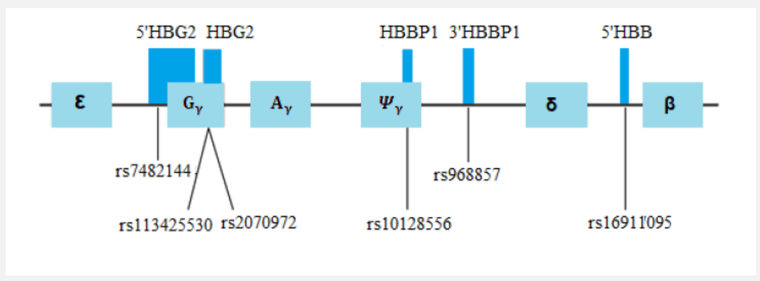

Fig 2. Beta Globin cluster´s representation and SNPs analysed by Multiplex SNaPshot® system

Results

Overall, this screening revealed the burden of SCA in Bengo is indeed worrying, with 3.3% of newborns analyzed homozygous for *HBB*S* (Table 1) and the estimated frequency of HBB*S in the sample was 0.146, with one in four being carriers.

Table 1. Observed and expected genotypic distribution for HBB*S (rs 334)

Genotype	Observed	Expected
AA	73.8	72.6
AS	22.9	25.2
SS	3.3	2.2

X^2 for HWE p= 0.091.

Results (cont.)

The typical SCA alleles *HBB*C*, *HBB*D* or *HBB*E* were not detected in this sample. Indeed other previous study didn´t found those alleles, or found in very low frequencies.

However, two polymorphisms associated with thalassemia phenotypes were detected, namely rs35004220/HbVar.827 and rs11549407/HbVar.845. The first variant was found in heterozygosity in 4 newborns 2 of them *HBB*S* carriers, and the second in 5 newborns 2 of them *HBB*S* carriers. The combine frequency of carriers of these two β thalassemia variants was 2,5% in this sample. Considering as a whole all newborns molecularly diagnosed as SCD, they accounted to 4,5% of newborns.

The method for analysis of HBB*S haplotypes was based on a Multiplex SNaPshot® system, which includes 6 SNPs (Fig. 3)

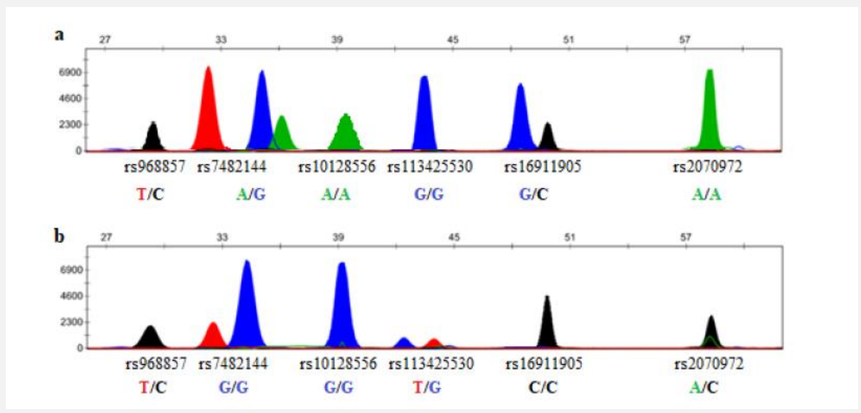

Fig 3. Electropherograms examples of SNaPshot® reaction results. a) sample 860 b) sample 886

By the analysis of the 6 SNPs in 94 samples (12 *HBB*S* homozygous and 82 heterozygotes) the Arlequim 3,5v software deduced 8 haplotypes. From those, 4 haplotypes were anchored in HBB*S allele (Table 2).

Table 2. Deduced haplotypes in 94 studied samples.

Haplotype ID	Haplotype definition	Frequency	Observation
1	G G A G C C **T**	88	CAR
2	G G A A C C A	6	
3	A G A A T G **T**	7	SEN
4	G G A G C C A	25	
5	G T C G T C A	25	
6	G T C G T C **T**	4	BEN
7	G T A G T C A	24	
8	G T A G T C **T**	9	BEN

Order of SNPs: rs7482144, rs113425530, rs2070972, rs10128556, rs968857, rs16911905, rs334

The *HBB*S* haplotype distribution was: 82.93% Cameroon CAR, 9.76% Benin and 7.31% Senegal. As for the homozygous for the sickle gene, 83.33% were homozygous for the Cameroon haplotype while the remaining 16.67% were homozygous for the Benin haplotype. This haplotype distribution has implications in terms of the clinical course of SCA, since the Cameroon haplotype was correlated with the lowest levels of fetal hemoglobin and the more severe SCA phenotypes, being next followed by the Benin haplotype.

Conclusions

Overall, the Bengo newborns molecularly diagnosed with SCD, including SCA, summed up 4.5%. Moreover, the most prevalent haplotypes are the ones with more severity of the disease (Cameroon and Benin). Further studies are in progress.

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