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Modulator Effect of XmnI Polymorphism Concerning 50 Mauritanian Sickle Cell Disease Patients

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Abstract: ***The present study shows that the XmnI polymorphism (rs7482144) at -158 C → T of Gγ gene is well associated with increased expression of HbF among sickle cell homozygotes in Mauritania. The prevalence of homozygous and heterozygous XmnI polymorphism is respectively of 36% and 34%, whereas negative individuals for this polymorphism are of 30% in the Mauritania population. This XmnI polymorphism induces increased HbF synthesis. The most found haplotype in Mauritania is the Senegalese type(77.7%) which was described as a relative protector by the presence of HbF followed by the beninese haplotype (8.8%) , the Arab-Indian (5.5%), the Bantu (4.4%) and the two atypical haplotypes 1 and 2 (2.2 and 1.1%) reflecting the multiethnic character of Mauritania.***

Keywords: XmnI; sickle cell disease; Mauritania

1. Introduction

Sickle Cell Disease (SCD) is a monogenic genetic disease related to a mutation in hemoglobin (Hb) at amino acid 6 of the β-globin chain (β6 Glu-Val). This hemoglobin tends to polymerize leading to sickling the red blood cells in case of oxygen desaturation. It is favored by various factors like stress, fever, cold, oxidative stress, endothelial dysfunction, infections and dehydration. The combination of this deformation to the increase in viscosity and the adhesion to the vascular walls causes the blocking of the flow in the capillaries, especially in bones. The clinical expression of this sickle cell disease is heterogeneous with numerous and very varied manifestations, mainly reflecting genetic and environmental influences [1.2.3]. Different hematological markers, which themselves depend on the earlier influences, change the nature and frequency of SCD complications. The reasons for this heterogeneity are not yet well understood; however, fetal hemoglobin (HbF) seems to be the best known and most studied regulator of SCD. This HbF, by inhibiting polymerization of HbS, is often associated with a reduction of some clinical manifestations and thus to a reduction in the severity of the disease and to the improvement of the patient's survival.

The objective of this work is the study of the regulatory role of the XmnI polymorphism and its effect in SCD.

2. Materials and Methods

Study Population

The study included 50 patients (27 males and 23 females), with major sickle cell syndrome. Monitoring of the in disease was achieved in the Maurilab laboratory (Nouakchott, Mauritania), for a period of one year.

The samples were collected in EDTA/K3 containers, and then analyzed by an automatic counter, Cell-Dyn Rubby™, (Abbott) for complete blood count to determine the parameters suggestive of a hemoglobinopathy, such as anemia, microcytosis and hypochromia. Hemoglobin electrophoresis was done by capillary electrophoresis on Capillarys Flex Piercing™ analyzer (Sebia).

To prevent interference on the study, we excluded all individuals with hemoglobinopathies other than SCD and we have ruled out the factors likely to increase HbF, such as hemolysis.

DNA analysis

DNA extraction was performed from peripheral blood leukocytes. Qiagen™ kit was used for this extraction. The evaluation of the quality of the DNA was done by electrophoresis on 0.8% agarose gel in 0.5x TBE buffer (4.45 mM Tris; 4.45 mM Boric acid, 0.1 mM EDTA; pH = 8). The choice of the nature and composition of the gel depends on the relative size of the fragments to be analyzed. The quantification was carried out by spectrophotometry at 260 nm using the following formula, (knowing that one unit absorbance (A260) corresponds to a concentration of 50 µg/ml) [4]: DNA (µg/ml) = A260 x 50 x dilution factor

The DNA amplification was performed by PCR (Polymerase Chain Reaction) in a thermal cycler Biometra™ Whatman. RFLP was used to determine the XmnI C/T polymorphism in the -158 position of the Gγ gene on chromosome 1.

For sequencing, we used an automatic sequencer ABI 373A™ and a reaction kit, Prism Big Dye Terminator Cycle Sequencing Ready Reaction ™ kit (Perkin Elmer).

The statistical analysis was performed using SPSS software, version 20.

3. Results and Discussions

The 50 identified homozygous SCD patients had an average age of 12 and a half years, (from 4 to 35 years) without any female or male predominance (M/F = 1.17) (Table1).

The prevalence of XmnI polymorphism site among these homozygous sickle cell patients is shown in Table 2. 36% of XmnI polymorphism were homozygous patients (+/+) and 30% were XmnI (-/-). Table 1 also shows that the level of hemoglobin was significantly higher among XmnI individuals (+/+) compared to other polymorphisms ($p < 0.05$).

The most found haplotype is the Senegalese type (77.7%). This haplotype has been described as a relative protector, by the presence of HbF [5]. It is followed by the Beninese haplotype (8.8%), the Arab-Indian (5.5%), the Bantu (4.4%) and finally be two atypical haplotypes 1 and 2 (2.2 to 1.1%) [5] that reflect the multiethnic character of Mauritania. The Beninese haplotype is the major one in the Tunisian population [6].

The association of XmnI and HbF rate shows that the XmnI polymorphism induces an increase of HbF synthesis. These results agree with those in the literature where the expression of HbF has been correlated with the presence of CTG polymorphism in the Arab-Indian haplotype and the Senegalese one [2,4,7,8]. The average value of HbF found in sickle cell XmnI (+/+), 15.75 ± 6.56%, is significantly higher than that found in the Tunisian population (7.6 ± 2.9) [6,9], which is likely related to the exclusive presence of the Beninese haplotype in this almost homogeneous population in difference of a very heterogeneous population we studied.

The presence of the XmnI polymorphism sites in the 5' area of the Gγ gene influences the expression of HbF. We have observed a significant positive correlation in some SCD, thereby creating a restriction site XmnI in Senegalese and Arab-Indian haplotypes (β^S) and the increased expression of Gγ and HbF.

Clinical characteristics in XmnI function (Table 3) noted evidence of the protective role of HbF in the sickling of red blood cells and the clinical severity of the sickle cell disease. Among patients with high HbF level, leg ulcers was less frequently, while the frequency of pain attacks and the occurrence of acute chest syndrome are inversely proportional to the HbF level [10]. Morbidity and mortality rates among patients with sickle cell disease is also much improved in adults with a high level of HbF [11-14]. The age and gender do not seem to have influence on the state of our patients, in contrast to results found recently in India showing that women had HbF expression level higher than men due the presence of a QTL X-linked (quantitative trait locus) [15].

Our results also objectify significant protection ($P < 0.05$) among homozygous and heterozygous patients for the polymorphism site XmnI compared to negative individuals for this site. These results also show a reduction in anemia, splenomegaly, and the frequency of the vaso - occlusive crises among patients with homozygous polymorphism compared to heterozygotes. Homozygous patients for the XmnI polymorphism site are free from signs of strokes, of priapism, the dactylie and cholelithiasis in perfect agreement with other authors [10].

Other studies [3,5] did not find a correlation between the haplotype and the HbF level . Indeed, Italia et al. [16] found severe clinical complications among adults of Arab Indian haplotype despite the high level of HbF. This difference in results leads us to think that modulating factors may be fully or partly genetic, ethnic, environmental or other.

Analysis of hematological data relative to the composition of hemoglobin in sickle cell patients from different countries (Table 4) [9] shows that the Senegalese haplotype is found with a sharp increase in HbF levels and that holders Beninese haplotype have the lowest levels of HbF and Gγ chains. Senegalese haplotype is strongly correlated with increased synthesis of Gγ chains.

The association between the level of expression of fetal genes (ratio Gγ and Aγ), of HbF and some sickle haplotypes and β-thal was established.

According to Nagel et al. [17], the level of HbF in sickle cell patients aged over 5 years was dependent on the CT mutation at position -158 Gγ in the Gγ promoter globin gene (Known as the XmnI-Gγ) [15]. These authors also found average concentrations of HbF 6.4% and 12.4%, respectively, in the groups having a level of Gγ < 38% and ≥ 38%.

In many studies, it was suggested that the haplotype may be a marker for phenotypic heterogeneity of SS patients [18,19]; homozygous for the Senegalese haplotype and Arab-Indian, with the most important HbF levels (15-30%) compared to Beninese, Cameroon and Bantu homozygous haplotypes (from 1 to 10 %) (Table 4) [9], agree with our results.

4. Conclusion

Sickle cell disease is a major public health problem in Mauritania. Sickle cell patients are exposed to numerous complications, in particular vaso-occlusive crisis and infectious, can compromise the vital or functional prognosis. The HbF rate proved its protective role with respect to the chronic manifestations of the disease. Thus, patients with high HbF in Senegalese haplotype and Arab-Indian have a silent clinic evolution with fewer complications compared to populations with HbF levels generally low or Bantu or Beninese type.

We suggest that the determination of HbF and study of the haplotype of the β-globin gene namely the XmnI polymorphism (C/T Gγ -158) constitute an additional exploration at sickle cell disease. This polymorphism seems

clear that is a powerful modulator of clinical manifestations in SCD.

5. Competing interests

The authors declare no competing financial or other relationship with other people or organizations interests.

6. Author's Contributions

All authors read and approved the final manuscript.

7. Contributorship

These authors contributed equally to this work

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Table 1: Characteristics of the study population

	Number	Average age (Years)	Extremes (Years)
male	27	13.6	4-35
Female	23	11.4	6-31
Total	50	12.5	4-35

Table 2: Hematological characteristics according to the XmnI polymorphism

hematological parameters	Xmn1 (+ /-) 18 (36%)	Xmn1 (+ /-) 17 (34%)	Xmn1 (- /-) 15 (30%)
RBC (millions/µL)	3,33 ± 0,70	3,04 ± 0,47	2,6 ± 0,95
Hb (g/dL)	8,41 ± 1,54	7,92 ± 0,75	7,23 ± 4,91
MCV (fL)	80,82 ± 7,35	84,8 ± 10,42	85,98 ± 14,28
MCH (pg)	25,49 ± 1,54	27,25 ± 5,15	26,66 ± 4,91
HbF (%)	15,75 ± 6,56	13,36 ± 6,92	9,21 ± 3,79

Table 3: clinical characteristics based on XmnI

hematological parameters	Xmn1 (+ /-) 18 (36%)	Xmn1 (+ /-) 17 (34%)	Xmn1 (- /-) 15 (30%)
Splenomegaly	2 (11%)	2 (11,7%)	2 (13,3%)
vaso-occlusives crisis	3 (16%)	6 (35%)	6 (40%)
Strokes	0	2 (11,7%)	0
Foot and hand syndrome	0	3 (17%)	1 (6%)
Priapism	0	1 (5%)	1 (6%)
cholelithiasis	0	1 (5%)	0

Table 4: blood data and hemoglobin in sickle cell patients from different countries [9]

	countries	*Hb (g/dL)*	*RBC (10^{12}/L)*	*MCV (fL)*	*HbA2 (%)*	*HbF (%)*
Beninese Haplotype	USA	8.8 ± 1.0	3.0 ± 0.6	84.0 ± 9.0	2.9 ± 0.5	11.7 ± 5.2
	Surinam	8.3 ±1.4	2.8 ± 0.3	102.5 ± 8.7	3.2 ± 1.0	9.3 ± 4.4
	Nigeria	7.5 ± 1.4	2.8 ± 0.6	103.7 ± 13.3	2.9 ± 1.0	9.2 ± 5.6
	Italy	7.5 ± 1.6	2.5 ± 0.4	97.2 ± 7.5	2.9 ± 0.7	9.0 ± 5.6
	Turkey	8.4 ± 1.8	2.7 ± 0.6	91.9 ± 7.0	2.7 ± 0.6	9.8 ± 4.0
	Syria	9.2	2.6	98.0	3.1	9.2
	Tunisia	8.2 ±1.2	2.5 ± 0.3	98.8 ± 8.1	2.9 ± 1.1	7.6 ± 2.9
Arabo-Indian Haplotype	USA	10.1 ± 1.4	3.78 ± 0.1	78 ± 4	1.2 ± 0.3	27.8 ± 5.3
	India	9.7 ± 1.8	3.17 ± 0.7	88.6 ± 7	1.9 ± 0.3	23.2 ± 5.9
Senegalese Haplotype	Notre étude	7.93±1.6	2.88 ± 0.66	83.27 ± 9.46	2.8 ± 0.6	15.75 ±6.56