The *XmnI* polymorphic site 5' to the gene $^{G}\gamma$ in a Brazilian patient with sickle cell anaemia – fetal haemoglobin concentration, haematology and clinical features

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Abstract

We report a 20-year-old female with sickle cell anaemia and with an HbF concentration of 15.8%. The patient was not using hydroxyurea and was not receiving regular blood transfusions. The patient never had chronic manifestations of sickle cell anaemia, only pain crises of a mild intensity. After laboratory tests, we found that she was homozygous for HbS with the Bantu/atypical haplotype, and was heterozygous for the *XmnI* site. The influence of the *XmnI* site on the expression of HbF can explain the amelioration in clinical features in this haplotype association in a case of sickle cell anaemia.

Key words: sickle cell disease, HbF expression, clinical manifestation.

Introduction

Sickle cell anaemia (SCA) is a genetic disorder characterized by a multifaceted pathophysiology that involves changes in erythrocytes, vaso-occlusive processes, chronic inflammatory states, oxidative stress and endothelial dysfunction. Therefore, there is decreased blood flow and obstruction of the microcirculation, which leads to a variety of clinical complications [1].

The reasons for this heterogeneity are not fully understood; however, fetal haemoglobin (HbF) is the best-known and most frequently studied genetic regulator of SCA, because it inhibits HbS polymerization. The individual variation in HbF levels is probably one of the main factors contributing to the clinical heterogeneity observed in these patients [2].

We considered the genotype of SCA and the haplotypes of the beta globin gene cluster in 50 patients with clinical features of sickle cell disease from the São Paulo state, south-eastern Brazil. We found one patient with HbF levels above the expected and reported values. The presence of the *XmnI* polymorphic site in the 5' $^{G}\gamma$ gene influences HbF expression, and a few studies have been conducted to explain the genetic factors involved in HbF levels in genetically mixed populations, such as that of Brazil. We evaluated the *XmnI* site and correlated it with HbF values, haplotype and haematological data, and clinical features from a patient with SCA.

Case report

This study analyses a 20-year-old woman of African descent, diagnosed with SCA through electrophoresis tests. She was receiving clinical follow-up care at the Blood Centre of the Santa Casa Medical School in São Paulo, Brazil. She was not receiving hydroxyurea, or any other drug that induces the expression of HbF. She was not receiving regular blood transfusions, and the only medication in use was folic acid.

According to the haematological data of the patient, she presented with: RBC (2.6×10^6/µl), Hb (7.8 g/dl), Hct (24%), MCV (94.1 fl), MCH (28.9 pg), WBC (8.3×10^3 mm^3), neutrophil (61.3%), lymphocyte (19.0%), monocyte (3.0%), eosinophil (2.2%), platelet (539 103 mm^3), reticulocyte (10.8%).

The patient never had any chronic manifestations of sickle cell disease, such as leg ulcers, osteonecrosis, retinopathy, stroke, cholecystectomy or acute chest syndrome. But in the 3 years prior to our study, she had 5 painful crises with mild intensity that did not require hospitalization.

After the patient gave her consent to participate in this study, we collected blood samples and performed tests.

In order to characterize the genotype of sickle cell disease, we performed cytological tests, electrophoresis in alkaline pH, acid pH and neutral pH, high performance liquid chromatography (HPLC) (VARIANT^{TM}, Bio-Rad Laboratories, CA, USA) and molecular analysis using PCR-RFLP. Next, the amplificated fragment was digested using the FastDigest^{TM} DdeI restriction enzyme (Fermentas, ON, CA) [3]. Haplotypes were determined through the analysis of the following polymorphic restriction sites: $^{G}\gamma$ (*Hind*III), $^{A}\gamma$ (*Hind*III), ψβ (*Hinc*II), 3'ψβ (*Hinc*II) e 5'β (*Hinf*I), following Sutton *et al*. [4].

The identification of the polymorphic *XmnI* site in $^{G}\gamma$ (rs7482144) was performed through PCR, followed by restriction analysis using the *XmnI* enzyme (New England Biolabs) [4].

The test results are shown in Table I. According to the genotype of sickle cell disease, the patient was homozygous for HbS, and she had not inherited the α-thalassaemia gene. Her HbF concentration was 15.8%. Because the presence of the *XmnI* site was observed in only one allele, we investigated other polymorphic sites to verify whether the SNP patterns presented were related to haplotypes listed in the literature.

Based on these findings, we concluded that the patient was HbSS positive with the Bantu/atypical haplotype (+ – – + + –). The atypical allele has a combination of individual polymorphic sites that do not fit the classification described in the literature, 5' $^{G}\gamma$ (*Xmn*I) = +, $^{G}\gamma$ (*Hind*III) = –, $^{A}\gamma$ (*Hind*III) = –, ψβ (*Hinc*II) = +, 3' ψβ (*Hinc*II) = +, 5'β (*Hinf*I) = –.

Discussion

The association between the *XmnI* polymorphism (rs7482144) in the proximal promoter of the $^{G}\gamma$-globin gene and HbF levels is well documented in sickle cell disease patients. In 1985, a polymorphism (C/T at position –158 of $^{G}\gamma$-globin, later named *XmnI*-HBG2 or rs7482144), was

Table I. Laboratory tests performed to characterize the haemoglobin profile

Test	Result	Normal values	Method
Chromatography analysis	SF	AA	Eastman
Alkaline pH electrophoresis	SF	AA	Marengo & Rowe
Acid pH electrophoresis	SF	AA	Vella
Neutral pH electrophoresis	Absent	Absent	Dacie & Lewis
Erythrocyte morphology	++	Normocytosis	Bonini-Domingos
Heinz inclusion bodies	Absent	Absent	Papayannopoulos
HbH inclusion bodies	Absent	Absent	Papayannopoulos
Quantification of HbA_2	2.30%	2.5–3.5%	HPLC
Quantification of HbF	15.80%	0–1.0%	HPLC
Quantification of HbS	83.80%	0%	HPLC
Osmotic resistance	Negative	Negative	Silverstoni & Bianco
Sickling test	Present	Absent	Dacie & Lewis
Solubility test (HbS)	Positive	Negative	Itano
Molecular analysis HbS	SS	absence of mutation	PCR-RFLP
–158 (C → T) 5' $^{G}\gamma$ (*XmnI*)	(+/–)	–	PCR-RFLP

SF – homozygous of HbS (sickle cell anaemia + HbF), AA – homozygous of HbA (normal adult), SS – homozygous of HbS (sickle cell anaemia)

identified and proven to promote the expression of $^{G}\gamma$-chain and to contribute to HbF variability. Other studies in the same decade characterized SNP patterns in and around β-globin cluster genes, and found the β^{S} chromosome to be associated with five major haplotypes originating from different geographical regions of Africa, the Middle East, and the Indian subcontinent [5].

Of the main haplotypes, the one carrying the Senegal and Arab-Indian characteristics exhibits the polymorphism *XmnI*, and this is strongly associated with increased levels of Hb F and, consequently, more minimal clinical manifestations, unlike the Bantu haplotype, which is associated with low levels of HbF and haematocrit, and which is clinically more severe [6].

In our study, the SCA patient carried a Bantu allele and an atypical allele (+ – – + + –). She also had an *XmnI* site, and an HbF concentration of 15.8%. Even with the low haematocrit values, the clinical complications were minimal when compared to other SCA patients and to those with the Bantu haplotype.

An increase in HbF has been associated with minimal clinical manifestations in SCA patients. Leg ulcers are less common in patients who have high levels of HbF [7]; the occurrence of acute chest syndrome and the frequency of painful crises are inversely proportional to HbF levels. Buchanan *et al.* [8] suggested an association between high HbF levels and a low frequency of leg ulcers. Powars and Hiti [9] and Nagel and Steinberg [10] showed that high levels of HbF were associated with milder SCA symptoms. They also found that a higher expression of HbF in adulthood improves the rates of morbidity and mortality in SCA patients [11]. These results support our observations, in which the patient has a high level of HbF and a milder phenotype, therefore requiring fewer blood transfusions.

We would like to highlight that the *XmnI* site's contribution to the variation in HbF levels in sickle cell patients is low when compared in isolation; however, Lettre *et al.* [12] showed that the rs7482144 polymorphism in homozygotes accounted for 2.2% of the variation in levels of HbF in 1275 SCA patients from the African American Cooperative Study of Sickle Cell Disease. However, the sum of five different SNPs – rs4671393 in the BCL11A gene; rs28384513, rs9399137, and rs4895441 in the intergenic region between HBSL1 and MYB on chromosome 6; and rs7482144 in the 5' gene $^{G}\gamma$ – explain 20% of the variation of HbF in SCA patients. In this study, it was not possible to evaluate the effect of the polymorphism in the group of patients in Brazil, because the Senegal and Arabic-Indian haplotypes are rare in the Brazilian population.

The polymorphism rs7482144 acts to increase the expression of HbF. Carriers who have the *XmnI* site also respond better to treatment with hydroxyurea [13], and this shows that new drugs can act in this region in the search for different therapies for the treatment of SCA.

In conclusions, the presence of the *XmnI* polymorphic site (rs7482144) in patients who are heterozygous for the 5' gene $^{G}\gamma$ was responsible for the increased expression of HbF. Although one of the alleles was of the Bantu haplotype, the clinical manifestation was minimal. These results reveal the importance of investigating factors that regulate HbF in sickle cell patients in a genetically mixed population such as that of Brazil.

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