Effects of Quantitative Trait Loci (QTLs) of BCL11A and HBS1L-MYB genes on clinical-biological variability of sickle cell disease in a Senegalese pediatric population

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High levels of hemoglobin F (HbF) could reduce the severity of sickle cell disease (SCD) by inhibiting hemoglobin S (HbS) polymerization. HbF expression is modulated by the Quantitative Trait Loci (QTLs) located on the HBS1L-MYB intergenic region (HMIP) (rs28384513) and intron 2 of the B-cell lymphoma/leukemia 11A (BCL11A) gene (rs4671391, rs1427407). To assess the impact of QTLs of HbF on clinical and biological parameters associated with the severity of sickle cell disease in a Senegalese pediatric population, 301 children with SCD not treated with hydroxyurea were recruited. The numbers of hospitalizations and VOC were estimated over 2 years. HbF levels were determined by HPLC. Three QTLs of HbF were genotyped by High Resolution Melting (HRM), two single nucleotide polymorphisms (SNPs) of BCL11A and one SNP of HMIP. Data analysis was performed using SPSS. The mean frequency of hospitalizations was 0.84 ± 1.29. The mean number of vaso-occlusive crises (VOC) episodes was 2.73 ± 1.98. The mean Hb concentration was 7.76 ± 1.05 g/dl. The mean HbS was 82.28 ± 4.78% and the mean HbF was 9.49 ± 5.12%. BCL11A (rs1427407) was associated with fewer hospitalizations. Both BCL11A SNPs were associated with increased HbF levels. BCL11A SNPs were associated with increased HbF levels, decreased HbS levels, and decreased hospitalizations (rs1427407). However, no association was noted between these SNPs and the number of VOC episodes. Thus, HbF QTLs are not the only genetic factors modulating the clinical severity of sickle cell disease, which suggests the involvement of other genetic factors such as alpha-thalassemia.

Key words: sickle cell disease, hemoglobin F, quantitative trait loci of HbF, HBS1L-MYB intergenic region, B-cell lymphoma/leukemia, vaso-occlusive crises.

INTRODUCTION

Sickle cell disease is an autosomal recessive inherited disorder characterized by the presence of mutated hemoglobin (Hb) in the red blood cells (RBCs) called hemoglobin S (HbS). The latter results from a point...
mutation in the seventh codon of the β-globin gene, GAG, which is replaced by GTG. At the protein level, this mutation will cause the replacement of glutamic acid by valine in the β chain of globin. Sickle cell disease is the most common genetic disease in the world, with nearly 7% of the world’s population carrying a sickle cell or thalassemic gene (Gueye Tall et al., 2017). It is a real public health problem in Africa where its prevalence varies from 10 to 40% of heterozygous carriers depending on the region. In Senegal, 1 person in 10, regardless of ethnicity, geographical origin, or social class, carries the sickle cell gene (Thiam et al., 2017).

Pathophysiologically in deoxygenated conditions, HbS polymerizes, leading to the sickling of red blood cells. The RBCs formed falciform, are fragile and rigid. This explains the chronic hemolytic anemia of the patients on the one hand and the occurrence of painful vaso-occlusive crises on the other. The pro-oxidant, pro-adherent and pro-inflammatory context modulates the clinical severity of the disease.

Although sickle cell disease is a monogenic disease, it is characterized by inter-individual clinical variability. This is partly related to the existence of genetic factors. Indeed, various modifier genes have been identified or suspected to have a direct or indirect influence on the clinical severity of the disease (Dahmani et al. 2016; Gueye Tall et al., 2017). HbS polymerization is the main driver of sickle cell disease pathophysiology and high levels of fetal hemoglobin may reduce the severity of sickle cell disease due to its ability to inhibit HbS polymerization and also reduce the mean concentration of corpuscular HbS (Steinberg and Sebastian, 2021). HbF is therefore, the main genetic modulator of the hematological and clinical features of sickle cell disease (Makani et al., 2011).

Many studies in sickle cell disease patients have demonstrated that genetic variations at loci influencing HbF levels modify the clinical course of sickle cell disease. These advantages and recent information on HbF regulation have prompted new efforts to provide additional information on the regulation of the genes that code for HbF. Given these findings, this study aims to assess the frequency and influence of three SNPs found at the BCL11A (rs4671393 G>A; rs1427407 T>G) and HMIP (rs28384513 A>C) loci on HbF levels and to study the effect of these three SNPs on the clinical severity of sickle cell disease.

**MATERIALS AND METHODS**

**Clinical follow-up of the cohort**

The study population consisted of 301 children and adolescents with homozygous sickle cell disease SS (169 boys and 132 girls; median age of 9.1 years). These children were all followed at the Albert Royer Children’s Hospital in Dakar, Senegal. Parents and guardians were interviewed; patients’ medical records were reviewed to define their clinical characteristics over the past two years. These medical files were therefore consulted to find the age of diagnosis of the disease, the number of vaso-occlusive crises (VOC), and the number of hospitalizations for reasons other than a VOC during the last 2 years. All painful VOC events were observed by a clinician during an acute outpatient visit or hospital admission. Non-specific complications of sickle cell disease such as fever or pneumonia were not taken into account and the malaria crisis was systematically excluded. In addition, the patients did not benefit from neonatal screening or treatment with hydroxyurea. They have also not been transfused during the last three months preceding the study.

The study was approved by the Ethics Committee of Cheikh Anta Diop University in Dakar. All patients’ parents and legal caregivers gave informed consent for the genetic diagnosis of sickle cell disease, including modifier genes.

**Hematological and biochemical parameters**

Hematological parameters consisting of the hemogram and reticulocytes (RET), were measured using a Sysmex XT 4000i device (System Corporation, Tokyo, Japan). Biochemical parameters were determined using the Mindray BA88 analyzer (Mahwah, NJ). These were lactate dehydrogenase (LDH), total and direct bilirubin (BIL), and C-reactive protein (CRP). The different hemoglobin fractions were also quantified by high-performance liquid chromatography using the short program VARIANT II β-thalassemia (Biorad, Hercules, CA).

**Genetic analysis**

DNA was extracted from peripheral blood stained with EDTA using the QIAmpVR DSP DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany) in Dakar. Genotypic analyses were performed in Lyon, France. Differential diagnostic between B0, B+3, B0/B+3 (HBB: c.15G>A), B0/B+3stat or B+3/B+3 genotypes was performed with a dedicated amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method (Gilman et al., 1985). For
patients suspected to carry \( \beta^A / \beta^A \) or \( \beta^A / \beta^S \) by ARMS-PCR, a direct Sanger sequencing of the HBB gene was performed on the ABI PRISMVR 3130x1 (Applied Biosystems, Foster City, CA, USA) apparatus to confirm the diagnosis and to identify the b-thal mutation or the hemoglobin (Hb) variant at the molecular level. Genotyping of the 2 SNPs in intron 2 of the BCL11A gene (rs4671393 G>A; rs1427407 T>G) and the SNP in the HMIP region (rs28384513 A>G; C), was performed using the HRM technique on the Light Cycler R 480 (Roche Diagnostics, Meylan, France) (Lederman, 1999).

**Statistical analysis**

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 22 (SPSS Inc., Chicago, IL). The significance level was defined at \( p < .05 \). Continuous variables were reported as mean ± standard deviation (SD) and categorical variables as number (N) or frequency. The normality test of the distribution of biological parameters was calculated using the Kolmogorov-Smirnov test. The comparison of means was done with the one-sample analysis of variance test (one factor ANOVA) between the three genotypes of each QTL and the post hoc test (Bonferroni goodness-of-fit test) between two genotypes of each QTL.

For each genetic variant, the allelic frequency was calculated and the balanced distribution of the three possible genotypes in the population (homozygous wild type, heterozygous and homozygous mutant) was checked by the Hardy Weinberg equilibrium.

**RESULTS**

**Clinical and biological characteristics of the cohort**

The mean number of VOC episodes over 2 years was two with extremes ranging from 0 to 12 episodes, while the frequency of hospitalizations ranged from 0 to 10 with a mean of 0.84. All these characteristics are reported in Table 1.

The blood count parameters studied were hemoglobin, leukocytes, reticulocytes, and platelets. For Hb, we noted that its concentration varied between 5.50 to 12.3 with a mean of 7.76 g/dl. The mean white blood cell count (WBC) and reticulocyte values were 14.30 and 325.03 G/L respectively. The mean platelet count was approximately 449.56 G/L. The HbS level, with a mean of 82.28%, ranged from 62.60 to 91.80%. The hemolysis parameters were LDH and bilirubin (B). The mean enzyme activity of LDH was 940.43 with extremes ranging from 148 to 3318 IU/L. As for total bilirubin (TB), the values varied between 7.21 and 106.90 with a mean concentration of 43.98. All these characteristics are reported in Table 2.

**Molecular characteristics of the cohort**

The prevalence and allelic frequencies of the QTLs are reported in Table 3. The SNP of the HMIP gene was less frequent in our study population with an allele frequency of 0.18. The two polymorphisms of the BCL11A gene had similar allele frequencies. Indeed, the allele frequency of rs1427407 T>G was 0.27 and that of rs4671393 G>A was 0.28. Furthermore, all three polymorphisms studied were homogeneously distributed according to the Hardy-Weinberg equilibrium (\( p > 0.05 \)).

**Effects of QTLs on clinical parameters**

The results of the association studies between clinical parameters (number of hospitalizations and number of VOC episodes over 2 years) and QTLs are reported in Figures 1 to 3. VOC episodes were not associated with the two SNPs of the BCL11A gene (rs1427407 T>G, rs4671393 G>A) (Figure 1). However, the rs1427407 polymorphism of the BCL11A gene was significantly associated with hospitalizations (\( p = 0.03 \)) (Figure 2). The SNP rs28384513 of the HMIP gene was not associated with either VOC episodes or hospitalizations (Figure 3).

**Effects of QTLs on biological parameters**

**Variations of HbF level as a function of QTL**

HbF level was associated with the rs467139 and rs1427407of BCL11A with p-values of 0.001 and 0.004 respectively (Figure 4).

For the HMIP QTL (rs28384513) no statistically significant association was found between this polymorphism and the HbF level (Figure 5).

**Variations of HbS level as a function of QTL**

The HbS level was associated with the QTLs BCL11A, rs1427407, and rs4671393 with p-values of 0.006 and 0.014 respectively. For the SNP rs28384513 of the HMIP gene, no statistically significant association was noted (Figure 6).

**DISCUSSION**

**Clinical and biological characteristics of the cohort**

In this study, the mean number of VOC episodes for the last two years was equal at 2.73 ± 1.98. For hospitalizations, the mean frequency was 0.84 ± 1.29. These results were different from those reported in Cameroon by Wonkam et al. (2014). Indeed, these authors found an average of 2 episodes of VOC and 2.3
Table 1. Means of clinical variables.

| Clinical parameter                  | Mean (n) ± SD | Max-Min |
|-------------------------------------|---------------|---------|
| Number of VOCs over 2 years         | 2.73 ± 1.98   | 0.00 - 12.00 |
| Number of hospitalizations over 2 years | 0.84 ± 1.29  | 0.00 - 0.00 |

VOC: Vaso-occlusive crises. The mean number of VOC episodes over 2 years was 2 with extremes ranging from 0 to 12 episodes, while the frequency of hospitalizations ranged from 0 to 10 with a mean of 0.84.

Source: Authors

Table 2. Means of biological variables.

| Biological parameter                  | Mean ± SD       | Min-Max            |
|---------------------------------------|-----------------|--------------------|
| Lactate dehydrogenase (LDH) (UI/L)    | 940.43 ± 499.48 | 148.00 - 3318.00   |
| Total bilirubin (mg/L)                | 43.98 ± 24.11   | 7.21 - 106.90      |
| Direct bilirubin (mg/L)               | 23.02 ± 15.11   | 2.07 - 68.30       |
| Indirect bilirubin (mg/L)             | 20.96 ± 13.18   | 0.20 - 73.51       |
| CRP (mg/L)                            | 4.55 ± 5.43     | 0.08 - 34.6        |
| White blood cells (G/L)               | 14.30 ± 4.34    | 4.21 - 28.4        |
| Reticulocytes (G/L)                   | 325.03 ± 159.9  | 28.12 - 954.00     |
| Reticulocytes (%)                     | 12.00 ± 5.59    | 0.60 - 36.5        |
| Platelets (G/L)                       | 449.56 ± 135.87 | 135.00 - 945.00    |
| Hemoglobin (g/L)                      | 7.76 ± 1.05     | 5.50 - 12.3        |
| HbS level (%)                         | 82.28 ± 4.78    | 62.60 - 91.80      |
| HbF (%)                               | 9.49 ± 5.12     | 1.10 - 26.80       |

CRP: C-reactive protein; HbS: hemoglobin S; HbF: hemoglobin F.

Source: Authors

Table 3. Prevalences and allelic frequencies of QTLs.

| Numbers (n) | Prevalences (%) | Allelic frequencies | p* |
|-------------|-----------------|---------------------|----|
| **BCL11A (rs1427407 T>G)**                |                 |                     |    |
| Wild        | 158             | 52.5                |    |
| Heterozygous| 120             | 39.9                |    |
| Mute        | 23              | 7.6                 |    |
| **BCL11A (rs4671393 G>A)**                |                 |                     |    |
| Sauvages    | 151             | 50.2                |    |
| Heterozygous| 126             | 41.9                |    |
| Mute        | 24              | 8.0                 |    |
| **HMIP (rs28384513 A>C)**                 |                 |                     |    |
| Wild        | 197             | 65.4                |    |
| Heterozygous| 96              | 31.9                |    |
| Mute        | 8               | 2.7                 |    |

* The Hardy-Weinberg equilibrium was verified by the chi-square test.

Source: Authors

Hospitalizations per year in their study population. These differences would be because the Benin and Cameroon haplotypes which are in the majority in the Cameroonian population correspond to more severe phenotypes than the Senegal haplotype which would be in the majority of our study population (Gueye Tall et al., 2017). In addition to clinical parameters, hematological and biochemical parameters were also studied. Thus, the blood count...
performed systematically revealed anemia in all children, with a mean total Hb concentration of 7.76 ± 1.05 g/dL. This result confirms the idea that anemia in sickle cell disease is constant (Dahmani et al., 2017). The low mean total Hb concentration in our cohort was comparable to that already reported in Senegal by Thiam et al. (2017), which was 8.6 g/Dl. Many other studies confirmed the anemia and low Hb concentrations in sickle cell disease. This is the case of the study conducted by Muszlak et al. (2015) on the island of Mayotte. Indeed, these authors reported a mean Hb concentration of 7.83 ± 0.91 g/dL (Muszlak et al., 2015). Mean total Hb levels of 7.98 g/dL

**Figure 1.** Association between *BCL11A* gene SNPs and vaso-occlusive crises. VOC: Vaso-occlusive crises. VOC episodes were not associated with the two SNPs of the *BCL11A* gene (rs1427407 T>G, rs4671393 G>A).

Source: Authors

**Figure 2.** Association between *BCL11A* gene SNPs and frequency of hospitalization. The rs1427407 polymorphism of the *BCL11A* gene was significantly associated with hospitalizations (p = 0.0381).

Source: Authors
Figure 3. Association between HMIP gene polymorphism and vaso-occlusive crisis and hospitalization. VOC: vaso-occlusive crises. The SNP rs28384513 of the HMIP gene was not associated with either VOC episodes or hospitalizations.

Source: Authors

Figure 4. Mean HbF levels as a function of BCL11A SNPs. HbF: Hemoglobin F. HbF level was associated with the rs467139 and rs1427407 of BCL11A with p-values of 0.0019 and 0.0046 respectively.

Source: Authors

(Belisário et al., 2010) and 8.1 g/dL (Bernaudin et al., 2018), which are similar to ours, have been reported respectively in studies carried out in Brazil and France, precisely at the pediatric center for sickle cell disease. The mean reticulocyte count in our series was 325.03 ± 159.9 G/L, suggesting that most patients in our series had regenerative anemia.

Indeed, during sickle cell disease, erythropoiesis is stimulated to compensate for the destruction of red blood cells linked to hemolytic crises. Our results are similar to those reported in the study of Dahmani et al. (2017) who reported a mean reticulocyte value equal to 363 ± 195.5 G/L (Dahmani et al., 2018). We also noted that in our patients, the mean leukocyte count was 14.30 ± 4.34 G/L, indicating a tendency to hyper-leukocytosis. Indeed, hyper leukocytosis is physiological in sickle cell disease and can be explained by hyperactivity of the bone marrow and inflammatory phenomena (Chies and Nardi, 2001). The hypothesis of any inflammation would be improbable, especially as our patients were all in the stationary phase at the moment of inclusion. This hyper-leukocytosis could therefore be the consequence of bone
Figure 5. Mean HbF levels as a function of HMIP gene SNP. HbF: Hemoglobin F. No statistically significant association was found between the HMIP polymorphism (rs28384513) and the HbF level. Source: Authors

Figure 6. HbS level as a function of QTLs (BCL11A, HMIP). HbS: hemoglobin S. The HbS level was associated with the QTLs BCL11A, rs1427407, and rs4671393 with p-values of 0.006 and 0.014 respectively. For the SNP rs28384513 of the HMIP gene, no statistically significant association was noted. Source: Authors
marrow hyper production. However, the absence of a leukocyte formula does not allow us to exclude cases of false hyper-leukocytosis secondary to erythroblastosis. Indeed, in acute hemolysis, the strong regeneration of the bone marrow is responsible for an erythroblastosis at the origin of a false hyper-leukocytosis, since the erythroblasts, because of their nucleus, are counted as leucocytes by the automates. This uncorrected leukocytosis is often very important in homozygous patients. The mean platelet count was 449.56 ± 135.87 G/L. The thrombocytosis observed could be explained by the occurrence of functional or organic asplenia or could be the consequence of hyposplenism (Tchotokou, 2004). The strong regeneration could also lead to a stimulation of thrombopoiesis. This tendency to thrombocytosis in patients with sickle cell disease was described by Mounkaila et al. (2015) with a mean platelet count of about 554 ± 25 G/L (Boutchi, 2015).

Concerning CRP, we noted an average plasma concentration of 4.55 ± 5.43 mg/L, attesting that our patients were all in the stationary phase at the time of inclusion, i.e., absence of any fever, vaso-occlusive crisis, and inflammation. Indeed, an increase in CRP (> 6.00 mg/L) would be in favor of an inflammatory reaction. Our result was lower than that of the study by Doupa et al. (2017) which reported a mean CRP value of 12.87 ± 21.9 mg/L (Doupa et al., 2017).

The results of the hemolysis parameters of our study revealed an average catalytic concentration of LDH of 940.43 ± 499.48 IU/L, reflecting the context of chronic hemolysis in our patients. However, according to the literature, LDH activity is considered a marker of severity when its value is higher than 1,000 IU/L as is the case in VOC (Mattioni et al., 2016). As for bilirubinemia, it was increased with a mean concentration of 43.98 ± 24.11 mg/L in our patients. This particular indirect increased hyperbilirubinemia (unconjugated) which, in our cohort, varied between 0.20 and 73.51 with a mean concentration of 20.96 ± 13.18 is a known biological sign associated with hemolysis in patients with SS sickle cell disease. According to the literature, there exists generally an increase in markers of hemolysis such as free bilirubin, AST, reticulocytes, and the appearance of free hemoglobin in the plasma in the case of VOC (Mattioni et al., 2016). However, our patients were all in the stationary phase at the time of inclusion, that is, in the absence of any vaso-occlusive crisis. This increase in hemolysis markers could therefore be the consequence of chronic hemolysis.

In this study, the mean hemoglobin S level was 82.28 ± 4.78%. SS homozygous subjects have major sickle cell syndrome. Their total hemoglobin concentration is between 6 and 10 g/dl and the red blood cells contain mainly hemoglobin S, with an increased hemoglobin F fraction (in the range of 5 to 20%) and a low hemoglobin A2 fraction. Hemoglobin A is absent (Jeanne, 2010). Hemoglobin S and hemoglobin F would therefore be the parameters that can be considered sensitive and found in significant proportions in cases of homozygous sickle cell disease SS. High concentrations of hemoglobin S are found in particular when the sickle cell trait is associated with β-thalassemia. This is the reason why a differential genotypic diagnosis was performed for the selection of SS homozygous subjects in our cohort. This result is corroborate the study by Somerville et al. (2001), which reported a rate of between 80 and 95% (Somerville et al., 2001).

The mean HbF level was 9.49 ± 5.12%. Elevated HbF levels are found in newborns, but also in β-thalassemia major, in HbF persistence syndrome, and in sickle cell disease patients treated with hydroxyurea, which induces an increase in hemoglobin F. Thus, the differential genotypic diagnosis and the absence of treatment with hydroxyurea would make it possible to exclude these hypotheses. The high level of HbF would be the consequence of compensation of HbA, absent in the SS homozygous subject. Studies in Senegal have shown a mean hemoglobin F level of 6.8 ± 5.9% in children (Diagne et al., 2000) and 8.2 ± 5.8% in adults of the same group (Diop et al., 1999). This difference in our results could be explained by the age of the patient. In healthy subjects, HbF is the major constituent expressed during fetal life and is progressively substituted during the perinatal period by HbA. Substitution is normally complete by 6 months of age, with HbA becoming the major component, and HbF usually represents less than 1% of total Hb (Labie, 2000). In sickle cell disease, since HbA is not expressed, residual HbF continues to be synthesized during adult life and highly variable levels have been observed. It is now accepted that these quantitative variations are related to polymorphisms and this is the general objective of this study.

**Molecular characteristics of the cohort**

The results of our study revealed variable allele frequencies in the Senegalese population according to the type of QTLs. These frequencies were 0.276 for rs1427407 (BCL11A), 0.289 for rs4671393 (BCL11A) and 0.186 for rs28384513 (HMIP). Other authors have also evaluated the frequencies of these polymorphisms in different population groups of our cohort according to the haplotypes frequently found. For example, Bernaudin et al. found that rs1427407 had a lower allele frequency (about 0.22) in children who were homozygous for the Bantu haplotype compared to those who were homozygous for the Benin haplotype (about 0.40) (Bernaudin et al., 2018). Pule et al. (2015) found an intermediate allele frequency for this same SNP (0.26) in
a very large cohort (n = 541) of patients of Beninese and Cameroonian origin. The allele frequency of rs28384513 was about 0.20 in Tanzanian (Makani et al., 2011) and Cameroon cohort (Wonkam et al., 2018), with a majority of Benin and Cameroon haplotypes, while it was significantly higher in Mayotte (Muszlak et al., 2015), with a majority of Bantu haplotype subjects (0.55) suggesting that the Bantu haplotype would be associated with rs28384513 of HMIP.

**Effects of QTLs of HbF on clinical parameters**

The association between genotype and phenotype in sickle cell disease is interesting to investigate as it is not well described in the literature. Although several studies have demonstrated the influence of the two loci BCL11A and HMIP on HbF levels (Lettre et al., 2008, Creary et al., 2009, Mtatiro et al., 2014) few authors have focused on their direct effects on the severity of sickle cell disease.

Only the SNP rs1427407 of the BCL11A gene was associated with a decrease in the frequency of hospitalizations (p = 0.035). Indeed, the presence of the mutation seems to reduce the number of hospitalizations since patients carrying the wild type had a mean number of hospitalizations of 1.02 ± 1.49 whereas these means were respectively 0.62 ± 1.00 and 0.74 ± 0.96 for mutated heterozygotes and mutated homozygotes. These results differ from those reported by other authors. Indeed, Muszlak et al. (2015) showed that the rs4671393 polymorphism of the BCL11A gene was associated with a lower number of hospitalizations in a group of 82 children with sickle cell disease on the island of Mayotte (Muszlak et al., 2015). Similarly Wonkam et al. (2018) reported that in a Cameroonian cohort rs4671393, as well as rs11886868 of the BCL11A gene and two other SNPs located on the HMIP gene (rs28384513 and rs9494142), were associated with a low frequency of hospitalizations related to painful events (Wonkam et al., 2018). This difference between our results would suggest a possible implication of haplotypes on the effect of these polymorphisms on the frequency of hospitalizations.

About VOCs, our results revealed that none of the polymorphisms of interest studied were associated with VOC episodes. These results were superposable with those of a study by Wonkam et al. (2014), whose population genotyping included all three SNPs in our study (Wonkam et al., 2014) Lettre et al. (2008) found a significant link between the association of 5 SNPs (BCL11A rs4671393, HMIP rs28384513, rs9399137 and rs4895441, and XmnI rs7482144) and reduced VOC in a cohort of sickle cell disease patients (Lettre et al., 2008). These SNPs are also associated with a less severe clinical phenotype in another hemoglobin disorder, beta-thalassemia (Danjou et al., 2015). This results show a stronger gene-phenotypic correlation when several SNPs are associated (Lettre et al., 2008).

**Effects of QTLs of HbF on biological parameters**

**Variations of HbF level according to QTL**

The BCL11A SNP rs4671393 was associated with increased HbF levels (p=0.004) and carriers of the genotype including this polymorphism in the homozygous state had significantly higher HbF levels (11.66 ± 4.46). This polymorphism is located in an intron of the BCL11A gene and is involved in lymphoid malignancies and is expressed in erythrocyte precursors (Liu et al., 2003). This could explain its impact on HbF expression. Our results are consistent with those reported in Brazilian and African-American patients (Lettre et al., 2008) but also in Tanzanian, British, Afro-Caribbean, and West African patients (Makani et al., 2011), all with homozygous SS sickle cell disease.

Similarly, the SNP rs1427407 still in the BCL11A gene was also associated with increased HbF levels (p = 0.001) and it was the mutated homozygotes that had significantly higher HbF levels (11.84 ± 4.47). This polymorphism is commonly found in patients with sickle cell disease (Bhanushali et al., 2015).

The SNP rs28384513 of the HMIP-1 gene was not correlated with increased HbF levels (p = 0.268) and instead, mutated homozygotes had significantly lower HbF levels (8.23 ± 3.68). This result is corroborated by that of a study in North Brazilians, where of three genotyped SNPs (rs28384513, block 1; rs4895441 and rs9399137, block 2), only the rs4895441 polymorphism was significantly associated with increased HbF levels (Cardoso et al., 2014). According to a Cameroonian study, all the HMIP SNPs explain 8.3% of the variations in HbF levels in sickle cell patients (Wonkam et al., 2014). It should also be noted, that of the common alleles of the three haplotype blocks of this gene region associated with HbF expression, block 2, 24 kb in size, accounts for the majority of the variability in HbF levels. As a reminder, HMIP polymorphisms are divided into three blocks of imbalance, called blocks 1, 2, and 3 of the HBS1L-MYB intergenic polymorphism. The discrepancy noted between our different results could also be explained by the ethnic-geographical origin. Indeed, HMIP-2 is characterized by eleven SNPs, which all showed a strong association with HbF levels in European patients, but only some of these SNPs showed a significant association in patients of African origin (Thein et al., 2009). The association of rs28384513 with HbF levels was first described in a sample of Northern European descendants (Thein et al., 2009). Subsequent studies have shown that this SNP was associated with
elevated HbF levels in sickle cell patients from other African-American, Brazilian, and Tanzanian populations as well as from African-British populations (Creary et al., 2009). In summary, we note that while polymorphisms in the HBS1L-MYB intergenic region are associated with HbF in sickle cell disease patients of African origin, they are much less associated with HbF in European and Chinese patients due to their much lower allelic frequency (Bernaudin et al., 2018).

**Variations of the HbS level according to the QTL**

In our series, the study of HbS levels according to QTL type showed statistically significant associations only with rs1427407 and rs4671393 of the BCL11A gene. The BCL11A SNP rs4671393 was indeed associated with lower HbS levels (p = 0.014) and carriers of genotypes containing this mutated allele in the homozygous state had significantly lower HbS levels (80.58 ± 4.02) (Figure 6). The SNP rs1427407 in the BCL11A gene was also associated with lower HbS levels (p=0.006) and it was again the genotypes containing the mutation in the homozygous state that had significantly lower HbS levels (80.46 ± 4.06) (Figure 6). In homozygous SS sickle cell disease, HbA is not expressed and HbF and HbS are the two types of hemoglobin synthesized during adult life. Thus when the fraction of one increases, the fraction of the other decreases. To this end, any QTL that would induce an increase in HbF will be associated in the same way and with the same statistical strength with a reduction in HbS.

**Variations of other biological parameters according to QTLs**

In our study, no significant difference was found between the QTLs and the means of the other biological parameters (LDH, BT, BD, BI, CRP, and Hb, leukocytes, platelets, and reticulocytes). These results could be explained by the young age of our study population (pediatric population; median age 9 years) and the relatively high levels of fetal hemoglobin (HbF).

**Conclusion**

The two SNPs of BCL11A would induce an increase in the HbF level and a decrease in the HbS level, but no association was noted between these SNPs and the number of VOC episodes. However, the rs1427407 polymorphism of the BCL11A gene was associated with a decrease in the frequency of hospitalizations. On the other hand, no variation in clinical parameters was associated with the presence of the rs28384513 SNP of the HMIP gene. Thus it follows from these results that HbF-QTLs are not the only genetic factors modulating the severity of sickle cell disease, which suggests the involvement of other factors. These markers interact with other parameters such as alpha thalassemia or G6PD deficiency, which must be explored to explain the inter-individual variability of the sickle cell disease clinic. Furthermore, the absence of association between the phenotypes studied and the SNP of HMIP would not exclude the involvement of this inter-gene region, rich in polymorphisms, in the clinical heterogeneity of this condition.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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