Blood Cells, Molecules and Diseases 54 (2015) 4–8

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Genetic variant in the BCL11A (rs1427407), but not HBS1-MYB (rs6934903) loci associate with fetal hemoglobin levels in Indian sickle cell disease patients

Aparna A. Bhanushali [a], P.K. Patra [b], D. Nair [a], H. Verma [b], B.R. Das [a,*]

[a] Research and Development, SRL Ltd, Prime Square Building, Plot No 1, S. V. Road, Goregaon (W), Mumbai 400 062, India
[b] Department of Biochemistry, Pt. J.N.M. Medical College, Raipur (C. G.), India

ARTICLE INFO

Article history:
Submitted 18 September 2014
Revised 24 September 2014
Accepted 11 October 2014
Available online 6 November 2014

(Communicated by M. Narla, DSc,
11 October 2014)

Keywords:
Sickle cell Disease
Fetal hemoglobin
India
BCL11A
HBS1-MYB

ABSTRACT

India along with Nigeria and DRC contribute to 57% of the world sickle cell anemia population. The annual number of newborns in India with SCA was estimated at 44,000 in 2010. Even with this high prevalence there is minimal information about genetic factors that influence the disease course in Indian patients. The current study was conducted on 240 patients with SCD and 60 with sickle cell trait, to determine the association of genetic variants at the BCL11A (rs1427407) and HBS1-MYB (rs6934903) loci with fetal hemoglobin levels (HbF). Both these loci have been implicated with influencing HbF levels, a powerful modulator of the clinical and hematologic features of SCD.

Our results indicate the BCL11A rs1427407 G > T variant to be significantly associated with HbF levels {19.12 ± 6.61 (GG), 20.27 ± 6.92 (GT) and 24.83 ± 2.92 (TT) respectively} contributing to ~23% of the trait variance. Interestingly no association of the HBS1L-MYB rs6934903 with the HbF levels was seen.

The present study indicates the BCL11A (rs1427407) but not HMIP (rs6934903) to be associated with elevated HbF levels in Indian patient. Further interrogation of additional variants at both the loci; as also a GWAS which may help uncover new loci controlling HbF levels.

Introduction

Sickle cell disease [SCD] is a hemoglobinopathy that affects millions of people worldwide [1,2]. India is estimated to be home to over 50% of the world's patients with SCD [3]. It is a debilitating monogenic disorder caused by a point mutation within the gene encoding the β-subunit of adult hemoglobin (HbA, $\alpha_2\beta_2$). The single base pair change causes the codon to change from GAG to GTG; this gives rise to a corresponding amino acid change at the sixth position of the β-globin chain, resulting in hemoglobin that is designated as HbS or sickle hemoglobin [4].

The phenotypic manifestations of sickle cell disease show great variability. This heterogeneity is evidenced in the form of several clinical outcomes, such as stroke, vaso-occlusive episodes, acute chest syndrome, avascular necrosis, leg ulcers, priapism and retinopathy [5,6]. These outcomes cannot be explained by the single mutation in the beta-globin gene alone but may be attributed to genetic modifiers and environmental effects [7–9].

Several studies have revealed fetal hemoglobin as a major genetic modulator in sickle cell disease [10,11]. Fetal hemoglobin (HbF) has been shown to have an ameliorative effect as it prevents the polymerization of deoxy sickle hemoglobin [12–14].

The HbF levels are controlled by a number of genetic determinants, resulting in variable HbF concentrations, which in turn may be responsible for the phenotypic variability seen in SCD [15–18]. Haplotypes of the beta-globin gene cluster are among the most extensively studied in this respect [19–24].

Recently, genome wide association studies (GWAS) have revealed three major quantitative trait loci (QTLs) (Xmn-HBG2, HBS1L-MYB intergenic region on chromosome 6q23, and BCL11A on chromosome 2p16) that account for 20–50% of the common variation in HbF levels in patients with Sickle Cell anemia (SCA), and β-thalassemia, and in healthy adults [25,26].

SNPs have been identified within the 14 kb intron 2 of *BCL11A* that correlate most strongly with the HbF expression [25]. The *BCL11A* genotype that is associated with high HbF is associated with reduced *BCL11A* expression.

Several genetic variants associated with the HBS1L-MYB intergenic (HMIP) region have also been identified that affect HbF levels. Genetic variants that show the strongest effects are concentrated in 24 kb of *HMIP* block 2, located 33 kb upstream of *HBS1L* and 65 kb upstream of *MYB* [27,28].

* Corresponding author at: SRL Ltd, Prime Square Building, Plot No 1, S. V. Road, Goregaon (W), Mumbai, 400 062, India. Fax: +91 22 66922303.
E-mail addresses: aparna.bhanushali@srl.in (A.A. Bhanushali), pkp1964@yahoo.co.in (P.K. Patra), devika.nair04@gmail.com (D. Nair), henu.verma@yahoo.com (H. Verma), brdas@srl.in (B.R. Das).

http://dx.doi.org/10.1016/j.bcmd.2014.10.003
1079-9796/

In India, the prevalence of the sickle cell gene is high both in tribal, and non-tribal populations [2,29–31]. Though SCD in India has a milder phenotype, which is attributable to the Arab–Indian haplotype in the beta-globin gene cluster, nevertheless it is prudent to determine the effect of other variants on the backdrop of this genetic information.

In the current study which is one of the first report from India we have attempted to determine the frequency of specific variants in the HBS1L-MYB intergenic loci (rs6934903) and BCL11a (rs1427407) in a cohort with sickle cell disease and sickle cell trait from Central India and to correlate the association of this SNP with HbF levels. This may aid in the determination and prediction of the severity of the complications in sickle cell anemia, which can in turn have important implications for genetic counseling and clinical management.

Materials and methods

Subject group

The current study population included a total of 300 individuals which consisted of 240 patients with sickle cell disease and 60 patients with sickle cell trait confirmed by solubility testing and HPLC (High-performance liquid chromatography) variant analysis. Blood specimens were collected from the tribal population of district of Raipur, Chhattisgarh under 'Sickle cell project' carried out by Department of biochemistry (Pt. J.N.M. Medical College) and funded by the Government of Chhattisgarh. An informed consent and detailed case record form pertaining to information on demographics, medical history and risk factors such as occurrence of pain crisis and its frequency, were obtained from each participant through perusal of their medical records. The study was approved by the local ethical committee and is performed in accordance with the Helsinki declaration.

DNA extraction and genotyping

Genomic DNA was extracted from the collected EDTA whole blood using QIAamp® DNA extraction Kit following the manufacturer's instructions (Qiagen, Germany). Genotyping was performed by ARMS-PCR [32].

Statistical analysis

Allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. Differences in HBF levels based on genotypes were determined by ANOVA. SNPStat online software tool [33] was applied to determine the association of genotypes with HbF using linear regression with a genotypic genetic model. In all regression models we used the natural logarithmic transformation of HbF to satisfy the normality assumption.

Results

Demographic details of the study population are indicated in Table 1. The mean age of SS patients was found to be 13.19 ± 7.44 years and that of patients carrying sickle cell trait (AS) was 16.03 ± 8.38.

The genotypic and allelic frequencies for both loci are indicated in Table 2. For HBS1L-MYB rs6934903 the allelic frequencies were 0.87 for the "T" allele and 0.13 for the "A" allele, for the BCL11A were 0.88 for the "G" allele and 0.12 for the "T" allele.

The mean HbF levels for rs6934903T > A were 19.03 ± 6.74 (TT), 19.97 ± 6.98 (AT) and 24.73 ± 4.01 (AA) shown in Table 3. Association with HbF levels was determined only for the SS (sickle cell diseased) patients. ANOVA indicated that there was no association of the HbF levels with the rs6934903 variant. However it was significant ($P = 0.045$) in the case of the rs1427407 G > T variant with values 19.12 ± 6.61 (GG), 20.27 ± 6.92 (GT) and 24.83 ± 2.92 (TT) respectively as indicated by Table 3, Fig. 1.

Table 1
Description of sickle cell disease (SS) and sickle cell trait (AS) cohorts.

General characteristics	SS (n = 240)	AS (n = 60)
Age (mean ± SD)	13.2 ± 7.44	16.6 ± 9.98
Sex, n (%)		
a. Males	110 (45.8%)	39 (65%)
b. Females	130 (54.2%)	21 (35%)
Category		
a. General (n = 7)	5 (2.08%)	2 (3.3%)
b. OBC (n = 204)	166 (69.2%)	38 (63.4%)
c. SC (n = 44)	36 (15%)	8 (13.3%)
d. ST (n = 36)	27 (11.25%)	9 (15%)
e. Unknown	6 (2.5%)	3 (5%)
Hematological parameters		
[a]Hb g/dl	10.49 ± 2.79	
[a]RBC x10^6	3.92 ± 1.17	
[a]MCV	81.20 ± 13.21	
[a]MCH	22.58 ± 9.93	
[a]WBC	10.83 ± 5.39	
[a]Plt count × 10^6	347.39 ± 162.52	
[b]HbF % (mean ± SD)	19.5 ± 6.59	–
[b]HbA_2%(mean ± SD)	3.5 ± 1.92	–

SS, sickle cell disease; AS, sickle cell trait, OBC, other backward classes; SC, schedule castes; ST, schedule tribes.
[a] Data on 162.
[b] Data on 216 individuals.

Further multivariate analysis using different genetic models indicated that rs1427407 variant was significant for the recessive genetic model with the TT genotype resulting in significantly higher HbF levels (Table 4).

Discussion

Sickle cell anemia is a clinically heterogeneous disease for which multiple factors influence a particular patient's disease outcome [9,34,35]. Fetal hemoglobin is the most powerful genetic modulator of sickle cell disease [5] and 3 major quantitative trait loci have been identified in this respect, namely; Xmn1-HBG2, HBS1L-MYB intergenic region on chromosome 6q23, and BCL11A on chromosome 2p16 [11,25,27,36–39].

The BCL11A QTL has shown the strongest effect on HbF/F cell levels to date, BCL11A polymorphisms have been strongly associated with HbF concentrations in normal persons and several different populations of patients with sickle cell anemia/thalassemia [37,38]. By its effects on HbF concentration, *BCL11A* modified the clinical features of both thalassemia and sickle cell disease [5]. BCL11A is indicated to be a repressor of HbF expression [40–43]. BCL11A protein is developmentally regulated and is required to maintain HbF silencing in human adult erythroid cells [40,41].

Table 2
Frequency of HBS1L-MYB rs6934903 & BCL11A rs1427407 genotype in SCD and sickle cell trait cases.

	Genotypic frequencies n (%)			Allele frequency	
				Major	Minor
HBS1L-MYB rs6934903	TT	AT	AA	T	A
Total (n = 298)	228 (76.51%)	65 (21.81%)	5 (1.68%)	0.87	0.13
SS (n = 239)	178 (74.48%)	58 (24.27%)	3 (1.26%)	0.87	0.13
AS (n = 59)	50 (84.75%)	7 (11.86%)	2 (3.39%)	0.91	0.09
BCL11A rs1427407	GG	GT	TT	G	T
Total (n = 267)	211 (79%)	47 (17%)	9 (3.37%)	0.88	0.12
SS (n = 215)	171 (79.5%)	36(16.74%)	8 (3.72%)	0.88	0.12
AS (n = 52)	40 (76.9%)	11 (21%)	1 (1.92%)	0.88	0.12