ORIGINAL ARTICLE

Genetics of fetal hemoglobin in tribal Indian patients with sickle cell anemia

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India tops the list of countries with sickle cell disease (SCD) with an estimated 44,000 live births in 2010 and a prevalence of 10%–33%. In the present study, the first from India, we have investigated the effect of genetic variants in the *BCL11A*, the *HMIP* (*HBS1L-MYB* intergenic polymorphism) locus, in addition to the *HBB* locus, which are known to be associated with fetal hemoglobin (HbF) levels, a major modulator of the disease phenotype. The present study was conducted on 240 individuals with SCD and 60 with sickle cell trait. Genotyping was performed for the *BCL11A* rs11886868 and rs34211119; *HMIP* rs9399137, rs189600565, rs7776196, rs34778774, and rs53293029; *HBG2* Xmn1 polymorphism rs7482144; and −68C > T *HBD* promoter polymorphism. All the 3 quantitative trait loci were associated with HbF levels in Indian patients with SCD. The highest difference was seen in the Xmn1 single-nucleotide polymorphism, which accounted for 11% of the trait variance, the *BCL11A* rs11886868 for 3.65%, whereas the *HMIP* rs9399137 for 3.8%. The present study indicates the *BCL11A*, *HMIP*, and β-globin region to be associated with increased HbF levels in Indian patient. Further interrogation of these genotypes with respect to pain crisis is warranted in this population, which may help in prognostication, as also a genome-wide association study, which may help uncover new loci controlling HbF levels. (Translational Research 2015;■:1–8)

Abbreviations: AI haplotype = Arab-Indian haplotype; ANOVA = Analysis of variance; ARMS-PCR = Amplification refractory mutation system-polymerase chain reaction; *BCL11A* = B-cell lymphoma/leukemia 11A; CSSCD = Co-operative study of sickle cell disease; DNA = Deoxyribonucleic acid; EDTA = Ethylenediaminetetraacetic acid; GWAS = Genome wide association studies; *HbA2* = α-globin gene; *HBB* = β-globin gene; *HBD* = δ-globin gene; HbF = Fetal hemoglobin; *HbG2* = γ-globin gene; *HBS* = Sickle hemoglobin; *HBS1L* = *HBS1*-like translational GTPase; *HMIP* = *HBS1L-MYB* intergenic region; HPLC = High Performance Liquid Chromatography; HWE = Hardy-Weinberg Equilibrium; LD = Linkage disequilibrium; *MYB* = oncogene; OBC = Other backward class; PCR = Polymerase chain reaction; QTL = Quantitative trait loci; SC = Scheduled caste; SCD = Sickle cell disease; SNP = Single nucleotide polymorphism; SS = Sickle cell disease patients; ST = Scheduled tribe

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AT A GLANCE COMMENTARY

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Background

India tops the list of countries with sickle cell disease (SCD); however, there is minimal information about genetic factors influencing the disease course in Indians. Fetal hemoglobin (HbF) is the most powerful modulator of the clinical features of SCD. The present study is the first report in this population investigating the effect of genetic variants affecting HbF levels.

Translational Significance

Elucidation of genetic variants responsible for the phenotypic variability in SCD will have important implications for genetic counseling and clinical management. In future it may help in the development of individual therapeutic strategies and preventive cures to produce better outcomes.

INTRODUCTION

Sickle cell disease (SCD) a common genetic disorder results in the annual loss of several millions of disability-adjusted life years, particularly in the developing world.[1,2] In India, the sickle gene frequency is found to vary from 2% to 34%.[3,4] It is highly prevalent in central India and among the tribal belts in western, eastern, and southern India.

SCD caused because of a single amino acid substitution glutamic acid (GAG) to valine (GTG) in the β-globin gene is a potentially devastating disease.[5] The disease expression is highly variable with different clinical outcomes such as stroke, avascular necrosis, leg ulcers, priapism, and retinopathy. Thus, although SCD is a monogenic disorder, at the phenotypic level it is a multigenic disease.[6]

Several modifiers of the disease have been elucidated, which include β-globin cluster haplotypes, α-globin gene number, and fetal hemoglobin (HbF) expression.[7] Of these HbF levels have received the most attention.[8-10] HbF as its name implies is the primary hemoglobin present in the fetus from mid to late gestation.[11] HbF is hereditable[12-14] and is the most powerful modulator of the clinical and hematologic features of sickle cell anemia. Higher HbF levels were associated with a reduced rate of acute painful episodes, fewer leg ulcers, less osteonecrosis, less frequent acute chest syndromes, and reduced disease severity.

Initial studies mapped a quantitative trait locus (QTL) controlling F cells in Asian-Indian kindred with β-thalassemia to a 1.5 kb locus on chromosome 6q23.[15] In another study, a new F cell QTL was mapped to BCL11A protein on chromosome 2p15. The 2p15 *BCL11A* QTL accounts for 15.1% of the trait variance.[16] More recently genome-wide association studies and family studies have shown that other regions outside the β-globin gene cluster, including 2q16, 6q23, 8q, and Xp22.2, are implicated in the regulation of HbF levels.[17,18] However, almost 45% of variations in HbF levels are associated with the presence of 3 main quantitative trait loci (QTLs), including the Xmn1 polymorphism on chromosome 11(11p15), the *HMIP* (*HBS1L-MYB* intergenic polymorphism) loci on chromosome 6 (6q23), and the *BCL11A* loci on chromosome 2.[10,16,19,20] Moreover, not only are variants at *BCL11A*, *HMIP*, and β-globin gene associated with HbF levels but also with pain crisis rate.[21]

Because, in India, the prevalence of sickle cell gene is high in both tribal and nontribal populations, it becomes very important to evaluate genetic markers that are associated with amelioration of SCD in the populations. It would be imperative to first evaluate the presence and impact of the 3 known major loci on the HbF trait. In the present study, which is the first report from India, we have attempted to determine the frequency of specific variants in the *BCL11A*, *HBS1L-MYB* loci, and *HBB* gene in a cohort with SCD and sickle cell trait from central India and to correlate the association of these single-nucleotide polymorphisms (SNPs) with HbF levels.

PATIENTS AND METHODS

Subject group. The present study was conducted on 300 individuals, which consisted of 240 patients with SCD and 60 patients with sickle cell trait. These individuals are a subset of the total cohort identified during a screening survey of 2094 villages from the district of Chhattisgarh in central India. The blood specimens were collected from the general castes, schedule castes (SC), schedule tribes (ST), and other backward classes (OBC) under "Sickle cell project" was carried out by Department of biochemistry (Pt. J.N.M Medical College) and funded by Government of Chhattisgarh. The nomenclature of social classes was derived from Articles 340(1) and 340(2) of the Constitution of India. The relative proportions of these social groups in the State of Chhattisgarh are general castes (17%), SC (25%), ST (8%), and OBC (50%).[22]

The study was approved by the local ethical committee and is performed in accordance with the Helsinki declaration. An informed consent and detailed case record form pertaining to information on demographics,