Genetic Modulation on the Phenotypic Diversity of Sickle Cell Disease

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Abstract

Sickle cell hemoglobin is a β chain structural variant where valine is substituted for glutamic acid in the sixth amino acid position. A point mutation (A→T) in codon 6 of the β globin gene which is located on the short arm of chromosome 11 is responsible for this abnormality. Despite the fact that all sickle cell disease patients have an identical single base change in their DNA, the severity in the clinical manifestations varies between and within different population groups. Advances in DNA technology have enabled us to characterize the b^S mutation by restriction fragment length polymorphism (RFLP) and also became possible to identify many variations (DNA polymorphism) in the non coding regions of globin genes. Studies in different population groups have revealed that the clinical severity may be ameliorated by higher fetal hemoglobin (HbF) expression, inheritance of a particular β^S haplotype along with some other DNA sequence polymorphisms in the β-globin gene cluster and the co-inheritance of associated α-thalassemia.

Our study in the tribal and non-tribal population groups of western India with a diverse clinical manifestations revealed that the β^S gene was linked to the typical Arab-Indian haplotype with a higher HbF levels in those who were homozygous for Arab-Indian haplotype. However, homozygosity for Arab-Indian haplotype had no effect on the clinical severity. The presence of other polymorphisms like β-silencer and β LCR motif within the Arab-Indian haplotype in each of these populations were found to be identical. The prevalence of α -thalassemia was found to very high and varied between 24 to 97% in these groups. The clinical manifestations were less frequent in the patients with homozygous α-thalassemia as compared to normal α-globin gene. It is interesting to note that the presence of excess α genes ($\alpha\alpha\alpha^{3.7}$ / αα) in sickle heterozygotes resulted in unusually severe clinical symptoms. Hence, the phenotypic expression of sickle cell disease linked to the Arab-India haplotype is not uniformly mild and α-thalassemia is a powerful and additional genetic factor in ameliorating the severity of the disease.

Introduction

Sickle cell hemoglobin is a β chain structural variant where valine is substituted for glutamic acid in the sixth amino acid position. A point mutation (A→T) in codon 6 of the β globin gene which is located on the short arm of chromosomes 11 is responsible for this abnormality (Marotta et al, 1977).The β^S mutation is one of the commonest single gene mutations in man and has a very widespread geographical distribution including most of the Africa, the Middle East, India and parts of the Mediterranean (Bodmer and Cavalli, 1976). Despite the fact that all sickle cell disease patients have an identical single base change in their DNA, the severity in the clinical manifestations especially the morbidity pattern varies between and within different population groups (Mukherjee et al, 1997). The diversity of the clinical course is believed to be due to some environmental as well as the interaction of various genetic factors (linked or unlinked). In the last few

years, remarkable progress has been made in the understanding of the gene structure and function and in our capacity to unravel the molecular basis of genetic diseases. By using different molecular biological techniques, it is possible to identify many variations (DNA polymorphism) that occur in non coding regions of globin genes and these variations are now used as genetic markers. Studies in different population groups have revealed that the clinical severity may be ameliorated by following factors:

1. Higher fetal hemoglobin (HbF) expression.
2. Inheritance of a particular β^S haplotype and some other DNA sequence polymorphisms in the β-globin gene cluster.
3. The co-inheritance of associated α-thalassemia.

HbF expression in sickle cell disease

It has been well established that HbF interferes with the polymerization of HbS and therefore high levels of HbF have the potential to ameliorate the clinical course of sickle cell disease. Many studies have been done on HbF levels and the clinical severity of sickle cell disease in different population groups (Serjeant, 1992). In India, it has been reported by Kar et al (1986) that sickle cell disease is associated with higher HbF levels and patients tend to have less anemia and generally milder clinical manifestations. However, no significant correlation between HbF levels and clinical severity of the disease has been observed by Mukherjee et al (2000). Nevertheless, the levels of HbS were significantly lower when the HbF were high (>10%) as reported by Kadam et al (1996). Splenomegaly is more common in Indian patients and is found to be associated with high HbF levels by Kar et. al (1986). It is possible that the higher HbF levels seen in Indian patients help reducing the episodes of painful crisis but allow the spleen to enlarge as a result of compensatory ongoing mild hemolysis.

A point mutation (C→T) at –158 5' to the Gγ gene is believed to be responsible for the expression of high Gγ levels. Homozygosity for this mutation was found to be associated with high Gγ expression and higher HbF levels in sickle cell disease patients. However, among the sickle heterozygotes and normal individuals, the homozygosity for the – 158 Gγ C→ T mutation had no effect on HbF production (Miller et al, 1987). Thus, this mutation may not be singly responsible for high levels of HbF, but it may have an important role in regulating its production and requires interaction with other unknown genetic determinants for increased γ gene expression possibly linked to the β^S gene.

β-globin Gene Cluster Haplotypes

The pioneering discovery of the HpaI restriction endonuclease site on the 3' side of the β globin gene by Kan and Dozy (1978) and subsequently other polymorphic restriction sites in the b globin gene cluster opened up new vistas of research into the genetic and clinical variants of the β^S gene. By using HpaI and other polymorphic sites, different haplotypes linked to β^S gene have been observed. However, the most common haplotypes were 7, 19, 20 and 31 also now known as Senegal, Cameroon, Benin, Bantu (CAR) and Arab-Indian haplotypes respectively (Nagel and Ranney, 1990). In India, Kulozik et al were the first to report the β^S haplotype from the tribal population of Orissa and Pune and found that the β^S gene was strongly associated with the Arab-Indian haplotype. Subsequently, other studies from Gujarat, Maharashtra, Orissa, Tamilnadu, Madhya Pradesh and Andhra Pradesh confirmed these finding (Mohanty and Mukherjee, 2002).

β-globin gene cluster haplotypes believed to be a marker for the phenotypic heterogeneity of sickle cell disease because of its association with variable HbF levels. In a study by Nagel et al, (1985), it was found that the Mean HbF level was significantly higher in homozygous for Senegal haplotype than Benin haplotype while Mukherjee et al, (2004) in their study observed that the Mean HbF levels in homozygous for Arab Indian haplotype was also higher than that for Benin haplotype. Patients bearing the Senegal haplotype have fewer dense and irreversible sickled red cells in the circulation and associated with a more benign clinical condition. However, homozygosity for Arab-Indian haplotype had no effect on clinical severity (Mukherjee, 1997).

DNA sequence polymorphisms, other than haplotypes, in the b globin gene cluster

Inheritance of other DNA sequence polymorphisms like β-silencer and β-LCR HS2 regions are also known to play an important role in the expression and regulation of globin gene transcription (Mukherjee, Immuno Bull).

β-Silencer Region

This particular region located 0.5 kb 5' to the β-gene cap site and may influence the expression of the β -globin gene by binding a putative repressor protein called BP1 (β protein 1). Five different sequence motifs linked to the b^S haplotypes have been reported and also have variable affinity for binding the BP1 protein. The Indian type motif $[(AT)_9 (T)_5]$ binds the putative BP1 protein more strongly than the Benin and Bantu type motifs respectively and has been postulated that this could be the reason for lower β^S expression in sickle heterozygotes with a normal a globin gene complement among Indians as compared to Blacks in Africa

β -globin Locus Control Region (LCR)

β LCR HS 2 is located 6 to 18 kb upstream of the ε-globin gene and appear to be the most important regions of the LCR for the developmental regulation and expression of the γ -globin genes. It has been shown that five different sequence configurations of a simple sequence repeat motif in the β LCR HS 2 segment are in strong linkage with each of the five major β^S haplotype. Additional point mutation (AT→GT) is unique to Benin haplotype. Interestingly, recombinant chromosomes on which a Senegal haplotype motif was linked downstream to a Benin haplotype were observed in two patients from the same family together with a high level of HbF. This has led to the hypothesis of a possible correlation between the 5' HS 2 sequence configuration of this motif and HbF expression.

Interaction of α-thalassemia with sickle cell anemia

The clinical significance of the interaction of α-thalassemia with sickle cell disease is controversial. Painful crisis has been reported to be more common whereas acute chest syndromes and leg ulceration were less frequent among the patients with α-thalassemia. It has also been reported by Sergeant (1992) that co-existing α-thalassemia increases the incidence of aseptic necrosis and proliferative retinopathy. Data on HbF levels are conflicting. A small study from USA suggested a significant increase in HbF levels whereas a larger Jamaican study conducted by Nagel et al (1985) indicated significantly lower HbF levels in sickle homozygotes having a deletion of 2 α genes.

In India, the prevalence of α-thalassemia was found to be very high and varied between 24 to 97% in different population groups by Mukherjee et al (2000). Sickle cell

anemia patients with homozygous α-thalassemia (- α / α) had significantly increased mean levels of Hb, HCT, RBC counts, HbA_2 and decreased MCH and MCV as compared to those with a normal a genotype (αα / αα). The lower MCH and MCV levels are likely to diminish the amount of intravascular sickling and this reduction is reflected in the lowered b^S expression associated with a-thalassemia in sickle cell heterozygotes. The clinical manifestations like painful crisis, acute chest syndromes, hospitalization, infections and blood transfusions were less frequent in the patients with homozygous a-thalassemia whereas splenomegaly was significantly more common in this group (Mukherjee, Immuno Bull). It is interesting to note that the presence of excess a genes ($\alpha\alpha\alpha^{3.7}$ / αα) in sickle heterozygotes resulted in unusually severe clinical symptoms (Mukherjee, 2001). Previously it has been shown that splenomegaly was more common in Indian sickle cell anemia patients as compared to Jamaican patients and was associated with higher HbF levels and a higher incidence of α- thalassemia with a milder clinical course (Kar et al 1986).

Hence, severity of sickle cell anemia should dispel the notion that the sickle cell disease in India is universally benign. The phenotypic expression of sickle cell disease linked to the Arab-India haplotype is not uniformly mild and α-thalassemia is a powerful and additional genetic factor in ameliorating the severity of the disease.

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