

## Contribution of Genetic Factors in Variation of Clinical Severity Among Siblings with Homozygous $\beta$ -Thalassemia in Two Indian Families

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**ABSTRACT** We report two Indian families with a variable degree of anemia in two  $\beta$  thalassemia homozygous siblings from each family. In both the families the siblings with delayed presentation had co-inherited an  $\alpha$  thalassemia 2 determinant as well as a gene for increased Hb F production. As against this, the severely affected siblings from both the families had shown either absence or presence of only one ameliorating factor. It appears that contribution of two ameliorating factors ( $\alpha$  thalassemia 2, Xmn 1 polymorphism) could synergistically compensate for lack of  $\beta$  globin chains in the homozygotes leading to a milder presentation in the form of thalassemia intermedia.

### INTRODUCTION

Homozygous  $\beta$ -thalassemia is usually characterized by severe anemia, requiring regular blood transfusions for survival from early childhood. However, a subset of about 10% of cases may have a milder clinical presentation and a transfusion independent survival and they are grouped as thalassemia intermedia cases (Weatherall and Clegg 1981). The clinical manifestation of the disease may be modulated by genetic factors like the nature of the  $\beta$ -thalassemia mutation, co-inheritance of  $\alpha$ -thalassemia or the presence of a substitution in the  $\gamma$  gene promoter region (-158 G $\gamma$  (C  $\rightarrow$  T)) increasing the expression of HbF (Camaschella et al. 1995). We present genetic interactions in two Indian families having  $\beta$ -thalassemia homozygous siblings differing in clinical severity.

### METHODS

Hematological indices were measured on an

Erma cell counter(PC-608). HbA<sub>2</sub> estimation was done by elution after cellulose acetate electrophoresis (pH – 8.6) (Marengo-Rowe 1965) and HbF by alkali denaturation (Singer et al. 1951). Globin biosynthesis was done according to the method described by Clegg (1983).  $\beta$ -thalassemia mutations were characterized by Covalent reverse dot blot hybridization and DGGE analysis (Colah et al. 1997; Mayers et al. 1987). The 619 bp deletional mutation was characterized by PCR across the breakpoints of the deletion (Colah et al. 1997). Deletional  $\alpha$ -thalassemia ( $-\alpha^{3,7}$  and  $-\alpha^{4,2}$ ) was characterized by Southern blot hybridization of Bam H1 and Bgl II digests hybridized to an  $\alpha^{32}$ P dCTP labelled  $\alpha$  Pst probe (Hsia et al. 1988). The G $\gamma$  globin gene promoter region polymorphism (Xmn 1) was studied by digesting the PCR product containing the polymorphic site with the restriction enzyme Xmn1 (Sutton et al. 1989).

### CLINICAL REPORT

In the first family, the proband (II-2) clinically presented with anemia at the age of 4 years. Later on, for 2 ½ years he was intermittently transfused (10 transfusions in 2 ½ years). After the age of 6 ½ years, he required a regular transfusion regime (12 times/ year) for survival. He had hepatosplenomegaly with spleen and liver of 3 cm. His sister (II – 1) aged 13 years had moderate anemia but was untransfused. She had typical thalassemic bony changes with pronounced hepatosplenomegaly (liver – 6 cm, spleen – 13 cm).

In the second family the proband (II-2) showed clinical manifestations of severe homozygous  $\beta$ -thalassemia. He presented clinically at the age of 6 months and received regular blood transfusion therapy. He had hepatosplenomegaly with a liver of 3 cm and a

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spleen of 4 cm. His sister (II-1) had a thalassemia intermedia like phenotype and presented clinically at the age of 6 years. Subsequently for 2 years she required intermittent transfusions ( 5 transfusions in 2 years), but is now regularly transfused. She had a liver size of 3 cm and spleen of 5 cm. She required transfusions possibly because of hypersplenism and is at present being evaluated for splenectomy

Both these families were referred to us for prenatal diagnosis.

The clinical, hematological and molecular analysis in these families is shown in figures 1 and 2. The hematological findings are post transfusion levels. However blood samples were collected after a gap of 25-30 days after the last transfusion. The pre-transfusion hematological parameters were not available. The hemoglobin levels at examination in the severely presenting transfusion dependent sibs in the two families (Family I II-2, Family 2 II-2) were 8.3 g/dl and 8.1 g/dl, respectively, indicating that they were not maintained on a hyper transfusion regime.

The HbF level was 57.0 % in case II-2 from family 1 as measured by alkali denaturation. In

view of two  $\beta^0$  mutations it should have been 100% . This reflects that the alkali denaturation method is not accurate for measuring very high values of HbF

MOLECULAR ANALYSIS

$\beta$ -thalassemia mutations characterized in the parents in family 1 were IVS 1 nt 1 ( G $\rightarrow$ T) and 619 bp deletion. Both these mutations were present in the 2 homozygous children and are known to produce a severe  $\beta^0$  phenotype. Globin chain synthesis done in these 2 siblings also showed the absence of  $\beta$  chain synthesis ( $\beta/\alpha = 0$ ). The  $\gamma/\alpha$  ratios were 3.2 and 4.1 in the milder and severe case respectively (Fig. 1). The lesser imbalance in the milder sib is probably due to a decrease in  $\alpha$  chain synthesis because of the single  $\alpha$  gene deletion coupled with increase in  $\gamma$  chain synthesis (Xmn1 +/+). The 13 year old untransfused  $\beta$  – thalassemia homozygous girl (II – 1) from this family showed a single  $\alpha$  gene deletion along with the – 158 G $\gamma$  ( C  $\rightarrow$  T) substitution which was present in the homozygous state ( Xmn 1 +/+). The propositus

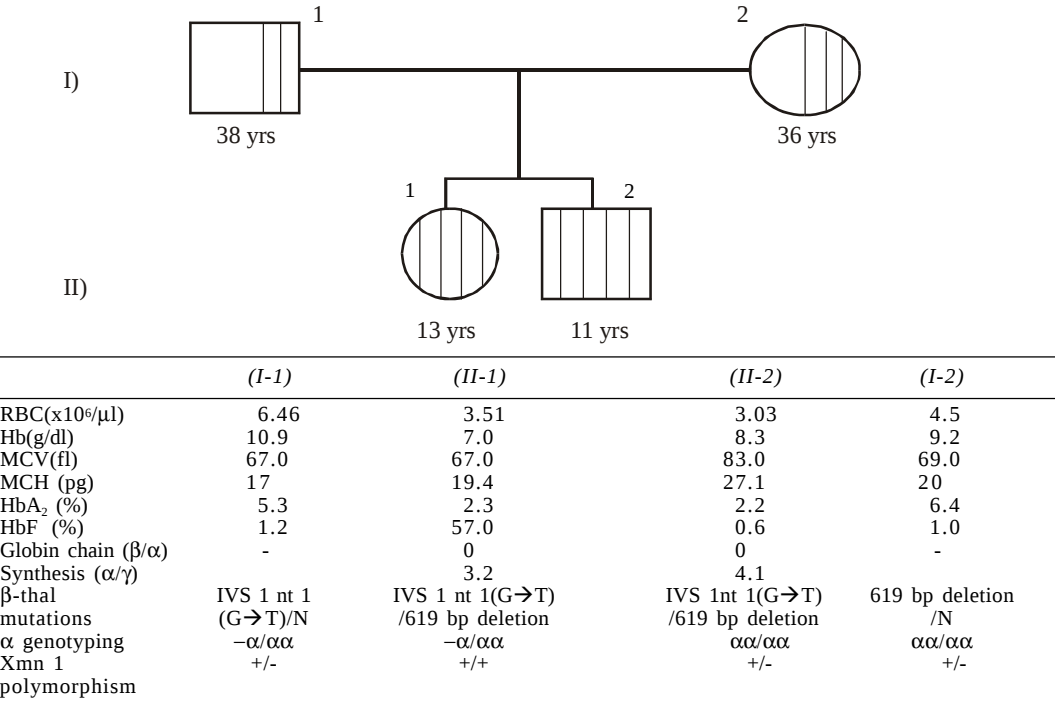
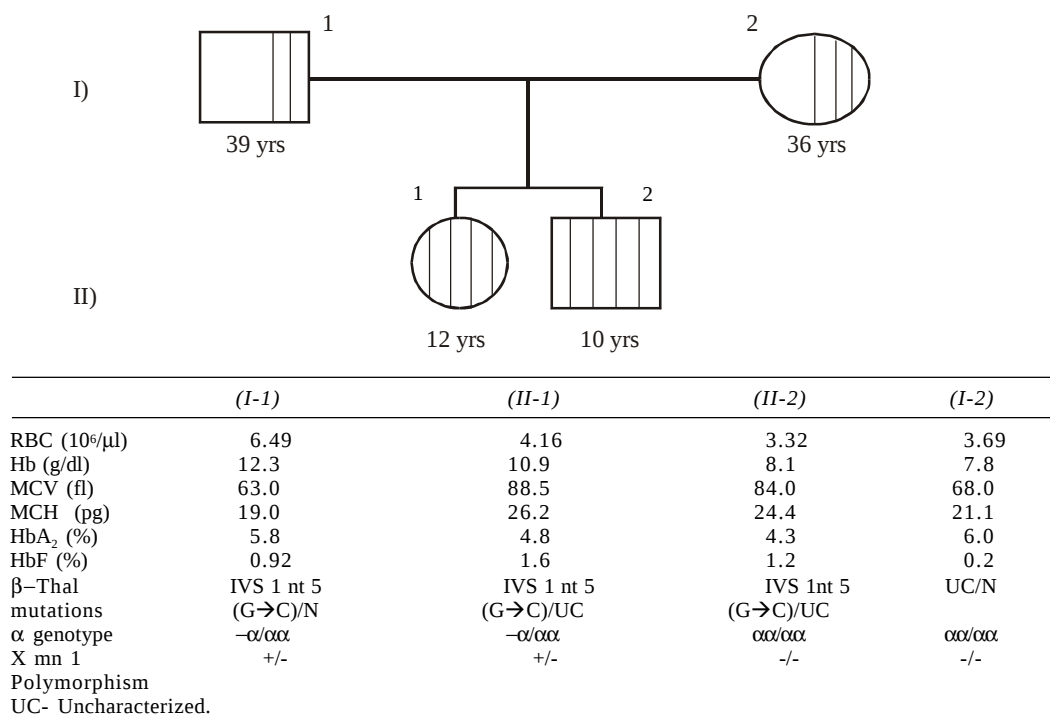


Fig. 1. A  $\beta$ -thalassemia intermedia family with clinical diversity among 2 homozygotes



**Fig. 2. A  $\beta$ -thalassemia intermedia family with clinical diversity among 2 homozygotes**

(II-2) who had a severe disease had 4 intact  $\alpha$  globin genes and was heterozygous for the Xmn 1 polymorphism (+/-).

In the second family, the father showed the common IVS 1 nt5 (G $\rightarrow$ C) mutation while in the mother, no mutation could be detected after scanning the entire  $\beta$  globin gene by DGGE analysis and her  $\beta$  thalassemia mutation remained uncharacterized. Both the homozygous children also showed the presence of one mutation only [IVS 1 nt5 (G $\rightarrow$ C)].

The proband II-2 had a normal  $\alpha$  genotype ( $\alpha\alpha/\alpha\alpha$ ) and the Xmn 1 site in the  $\gamma$  globin gene promoter region was absent on both the chromosomes (-/-). His sister who presented as a thalassemia intermedia showed deletion of one  $\alpha$  gene ( $-\alpha^{3.7}/\alpha\alpha$ ) and was heterozygous for the Xmn 1 polymorphism in the  $\gamma$  gene (+/-).

## DISCUSSION

The primary pathophysiological factor leading to severe homozygous  $\beta$  thalassemia is an excess of  $\alpha$ -chains in red cell precursors. Any mechanism that reduces the imbalance

would be beneficial to the affected individual. There are individual family reports suggesting that the clinical course of homozygous  $\beta$  thalassemia siblings may be modified by interaction of various genetic factors.

Winichagoon et al. (1987) have reported a family from Thailand, where they showed that the variable clinical severity of homozygous  $\beta$  thalassemia among 3 siblings could be related to the  $\alpha$  genotype of the homozygotes. In their studies, the homozygotes with a two  $\alpha$  gene deletion ( $-\alpha/-\alpha$ ) had a mild anemia and did not require any blood transfusions, the one with a single  $\alpha$  gene deletion ( $-\alpha/\alpha\alpha$ ) had moderate anemia and mild jaundice, while the most severely affected sibling who was on regular blood transfusions had 4 intact  $\alpha$  genes ( $\alpha\alpha/\alpha\alpha$ ). Similarly Galanello et al. (1989) had encountered two Italian families with homozygous children differing in clinical manifestations. They had reported that the presence of two  $\alpha$  gene deletions or non-deletional  $\alpha$  thalassemia could contribute to the development of a mild clinical picture. A similar effect of  $\alpha$ -globin gene deletions in this condition has been reported in a

Chinese homozygous  $\beta^0$ -thalassemia (Lie-Ingo et al. 1982). In contrast, homozygotes with three  $\alpha$  gene deletions have severe clinical manifestations (Melis et al. 1983). The effect of an  $\alpha$ -thalassaemia-2 determinant in the severity does not seem to be consistent.

In our study, in family 1, both the homozygous children have inherited 2 severe  $\beta$ -thalassemia mutations, but the clinically milder sib has associated  $\alpha$ -thalassemia ( $-\alpha/\alpha\alpha$ ) as well as the (C  $\rightarrow$  T) substitution in the  $\gamma$  promoter region on both chromosomes (+/+), which could increase the  $\gamma$  chain production. A combination of both these factors may be contributing to amelioration of the clinical manifestations. On the other hand, the sibling with severe manifestations had the normal complement of 4  $\alpha$  genes leading to a greater imbalance between  $\alpha$  and  $\beta$  chain synthesis and the  $\gamma$  substitution although present, was in the heterozygous state (+/-).

In family-2, the  $\beta$ -thalassemia chromosome of the mother (I-2) remained uncharacterized after scanning the entire  $\beta$ -globin gene and its flanking regions by DGGE analysis. It is estimated that the  $\beta$ -thalassemia gene may remain uncharacterized in about 1% of the world population and it is postulated that in such cases the mutation may lie upstream in the locus control region (Kazazian 1990). Hence, we were able to identify only one severe  $\beta^+$ -thalassemia mutation in both the homozygous children. Here too, the clinically milder sib had a single  $\alpha$  gene deletion and  $Xmn1$  (+/-) and the sib with more severe manifestations did not have  $\alpha$ -thalassemia and the substitution in the  $\gamma$  promoter region was also absent. It is also interesting to note that both the mildly affected sibs were females and severely affected ones were males. As it is known that there are some genes on X chromosomes whose transacting product influence HbF production, it is tempting to speculate that some X-linked factors may also have additionally contributed to the milder nature of the disease in our cases (Dover et al. 1992).

Thus, it appears that in our two families, the genetic mechanism leading to amelioration of the disease or a delayed presentation include a combination of an  $\alpha$ -thalassaemia-2 determinant and inheritance of a gene for increased HbF production. Both these factors could synergistically compensate for the lack of  $\beta$ -globin chains in the homozygotes with a thalassemia intermedia like presentation.

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