Disorders of Erythropoiesis

Molecular characterization of thalassemia intermedia in Indians

Thalassemia intermedia shows considerable heterogeneity. The purpose of this study was to evaluate the prevalence and effect of common molecular determinants in thalassemia intermedia. In 73 cases of thalassemia intermedia, the possible molecular basis was co-existent α-deletions (n=16/50), homozygous *Xmn*I polymorphism (n=17/50), both factors (n=3/50), and milder β-alleles (n=9/50) in homozygous β-thalassemia (total 50 cases). In heterozygous β-thalassemia, ααα$^{anti-3.7}$ triplication was the predominant factor (14/23 cases).

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In this prospective study we studied 73 subjects with thalassemia intermedia attending clinics in two specialized centers in 2004. Informed consent to the study was given by patients or parents (for children <10 years old). The ethical committee approved the study.

The classification into heterozygous and homozygous β-thalassemia was based on high performance liquid chromatography (HPLC): (i) homozygous: HbF >20%, HbA_2<3.5%; (ii) heterozygous: HbF < 10%, HbA_2> 3.5%. Three cases with HbF 10-20% and HbA_2 >3.5% were not included. Heterozygous and homozygous β-thalassemia cases maintaining a mean Hb of 7-9g/dL with occasional (≤4/year) or no transfusions were included as cases of thalassemia intermedia. Family studies were done to confirm the homozygous or heterozygous state and to exclude high HbF due to hereditary persistence of fetal hemoglobin (HPFH). Red cell indices was estimated using an automated counter (Sysmex K-4500). HPLC was done on a VARIANT™ instrument (Biorad Laboratories, Hercules, CA, USA). Common α determinants studied were α^+ (-$\alpha^{3.7}$and -$\alpha^{4.2}$), α^0 (--^{SEA} and --^{SA}), and ααα$^{anti-3.7}$ triplication.[3-6] β-globin genotyping was done by reverse dot blotting[7] and gap polymerase chain reaction (PCR) (for the 619bp deletion). The XmnI polymorphism was studied by digestion of the PCR product[8] with XmnI (Bangalore Genei, Bangalore, India). Data were analyzed using SPSS software, version 10.00 running the unpaired t-test, Kruskall Wallis and χ^2 tests. A *p* value of <0.05 was taken as statistically significant.

A total of 73 patients with thalassemia intermedia [homozygous-β-thalassemia (n=50) and heterozygous-β-thalassemia (n=23)] were studied. Five cases with HbF 10-20% were excluded. Most of patients (52/73, 71%) came from Delhi and adjoining states.

Seventeen of the 73 patients (23.3%) had -$\alpha^{3.7}$ deletions of whom three were homozygous for this deletion. The –$\alpha^{4.2}$ deletion and – –^{SA} deletion were seen in one patient each. α triplication was found in 15/73 (20.5%) cases; among the 23 cases of heterozygous-β-thalassemia, 14 (60.9%) had the α triplication. There were no significant differences in most clinical and hematologic parameters (Table 1).

The IVS1-1(G-T) was the commonest β-mutation (Figure 1), occurring in a homozygous state in 10 cases. One patient was a compound heterozygte for Cd16(-C) and Cd30(G-C). *Xmn*I polymorphism homozygosity was seen in 20 of the 73 patients (27.4%); three had concurrent α-deletions. In one family, the mother had IVS1-1 and cap site mutations, while the son was an IVS1-1 homozygote with –$\alpha^{3.7}$ deletion. In another family, one sibling (22 years old) had splenomegaly, and an average hemoglobin of 9 g/dL, without any blood transfusions. The other sibling (19 years old), who had thalassemia minor, had jaundice, no splenomegaly, and a hemoglobin concentration of 11.8 g/dL. The percentages of HbF and

Table 1. Clinical and hematologic characteristics of 73 individuals with thalassemia intermedia.

Clinical parameters		*Hematologic parameters*	
Age of onset (<10 years)	49/73 (67.1%)	Hemoglobin (Mean±SD)	7.5±1.3 g/dL
Blood transfusions	40/73 (54.8%)	Mean cell volume (Mean±SD)	75.1±8.4 fL
Intermittent jaundice	44/73 (60.3%)	Mean cell hemoglobin (Mean±SD)	23.9 ± 3.04 pg
Splenectomy	4/73	Mean cell hemoglobin concentration (Mean±SD)	32±2.3 pg/dL
Age of onset (Deletion cases)	7.6±6.7 years	Average bilirubin	2.3 mg/dL (range 1.5-15.2)
Age of onset (Triplication cases)	11.9±8.5 years	Hemoglobin (triplication cases)[c]	8.1±1.21 g/dL
Jaundice (Triplication cases)	12/14 (85.7%)	Hemoglobin (Deletion cases)[d]	7.67±1.9 g/dL
Jaundice (Deletion cases)	9/17 (52.9%)	Average bilirubin (Triplication cases)[e]	5.8±3.4 mg/dL
BT requirement/year[a] (Deletion cases)	2.8±2.6 (mean±SD)	Average bilirubin (Non-triplication cases with jaundice)[f]	3.9±2.1 mg/dL
BT requirement/year[b] (Triplication cases)	1.3±1.7 (Mean±SD)		

On statistical analysis of parameters listed above: [a] vs [b], [c] vs [d], and [e] vs [f], the p value was non-significant.

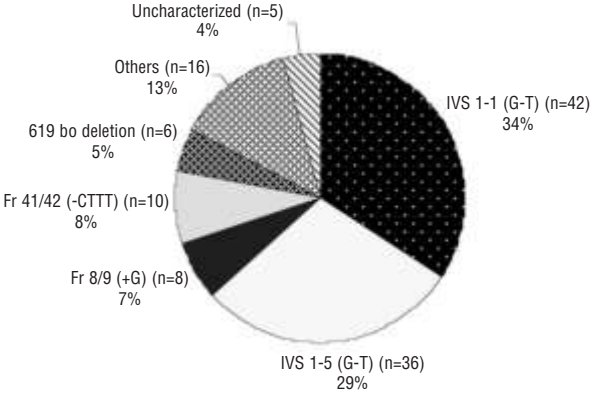

Figure 1. The distribution of β-globin gene mutations in thalassemia intermedia cases. No. of chromosomes studied=123 (50 homozygous β-thalassemia cases and 23 heterozygous β-thalassemia cases). *Others* include Cd 30 (G→C) (β^+) (n=3), Cd 16 (-C) (β^+) (n=3), Cd 15 (G→A) (n=2), -88(C→T) (β^{++}) (n=5), and Capsite [+]

HbA_2 were, respectively, 6.0 and 4.2% for the elder sibling and 0.7 and 4.6% for the younger one. The α-genotype was normal for the elder sibling whereas the younger one had α triplication. The β-globin gene mutation could not be identified.

Among the cases of homozygous-β-thalassemia, there was no significant difference in presentation (those with [α]-deletions *vs.* homozygosity for *Xmn*I). Interestingly, three patients with both determinants had onset of clinical manifestations at 9-11 years old and maintained hemoglobin concentrations of 8-9 g/dL with occasional transfusions. All three were over 25 years old at the time of this study and had been splenectomized.

The average frequency of the β-thalassemia gene in Indians is reported to be 3.3%.[1] The frequency of α thalassemia is 1 to 80%.[1,2] We found a high incidence of $\alpha\alpha\alpha^{anti\text{-}3.7}$ triplication (20.5%) in thalassemia intermedia. There is an earlier report of a 5% incidence.[9] The Maharastrian study[10] did not find any α triplications but found a 33% frequency of deletions. The differences are statistically significant (p<0.001). The $-\alpha^{4.2}$ deletion, Hb-Constant-Spring and Hb-Koya-Dora have been reported in eastern states. The $--^{SA}$ deletion was previously reported from South India in HbH disease.[5] There is considerable regional variation in α mutations.[2,9,10]

The familes illustrated show that multiple genetic factors may interact in the same family. One family had different β-globin mutations. In a second family, there is a possibility of dominant β-thalassemia. The elder sibling may have some form of HPFH in addition, whereas in younger, α-triplication allele may be acting functionally as a deletion allele. We were able to determine a possible molecular basis of thalassemia intermedia in 60 of the 73 cases (82.2%). Among the cases of homozygous-β-thalassemia, 16 were due to α deletions, 17 due to homozygosity for *Xmn*I polymorphism, 3 due to a combined effect and 8 due to very mild (β^{++}) thalassemia mutations. In one patient, the phenotype was probably due to two β^{+}alleles [Cd16(-C) and Cd30 (G-C)] and in another case, the α-triplication allele was possibly acting functionally as a deletion allele. Although there are conflicting views on the *Xmn*I polymorphism, previous reports[11] and previous Indian experience[12] (*Raina A et al., presented at PEDICON 2006, Delhi*) suggest a definite beneficial effect. The $\alpha\alpha\alpha^{anti3.7}$ triplication was an important factor in the causation of thalassemia intermedia in heterozygous-[β]-thalassemia (14/23). Intermittent jaundice was a prominent feature (85.7%); this could be due to the triplication *per se*, because of inherent instability of the α chain obtained from the triplication allele. In the remaining cases, there may be some other form of α triplication or quadruplication. In one patient with homozygous-β-thalassemia, α triplication may have decreased disease severity, acting functionally as a deletion allele. Other sequence variations in the globin genes are linked to some δ- and β- thalassemia mutations. In the Hellenic population,[13] the nucleotide variations in the $^{A}\gamma$ genes, i.e., –588 A-G, –499 T-A, and a 4bp deletion (-225 to – 222 AGCA) in the *cis* position are suggested to constitute an important genetic repository upon which the thalassemia mutations occur. In another study in thalassemia intermedia,[14] the T-haplotype in the $^{A}\gamma$-δ globin intergenic region, a motif in the locus control region, and TAG pre-$^{G}\gamma$ haplotype were found to be associated with high HbF in the absence of HPFH syndrome. These genetic alterations contribute to the milder phenotype in patients who are compound heterozygotes for severe β-thalassemia mutations. In conclusion, the study of common globin gene mutations can help to identify most cases of thalassemia intermedia in Asian Indians.

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