

Beta-Globin Gene Mutations in India and Their Linkage to β -Haplotypes

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ABSTRACT A total of 124 chromosomes of 64 unrelated Indian β -thalassemia and β -thalassemia patients along with their family members were studied for their haplotype pattern and mutations. These included, 35 with β -thalassemia major, 4 with thalassemia trait, and 25 with β -thalassemia. Fourteen mutations were detected by PCR and Sequencing. The most common mutation IVS1-5 (G-C) was linked with 8 different haplotypes. Nineteen haplotypes were found on β -thalassemia mutations, with haplotype (+ - - - + -) being the most widespread and was found associated with 39 chromosomes of IVS1-5 (G-C), 2 of HPFH and 1 each of CD41/42(-CTTT) and CD16 (-C).

INTRODUCTION

The technique of RFLP depends on the property of restriction endonuclease to fragment DNA at specific recognition sites and then electrophoresis to separate the resulting fragment according to their size. Variations from the pattern seen in "normal" DNA samples constitute restriction fragment length polymorphisms or RFLP's.

In β -thalassemia, a heterogeneous genetic disorder of β -globin synthesis, a strong association between particular β -globin gene haplotype and specific mutation was suggested (Orkin et al. 1982). It was only later elucidated that the same haplotype may be associated with different mutations, while the same mutation may be associated with many different haplotypes (Cheng et al. 1984; Orkin and Kazazian 1984; Wong et al. 1986). The aim of this study is to investigate the degree of correlation between different β -globin gene mutations and haplotypes

MATERIALS AND METHODS

Subjects: Sixty-four unrelated Indian families with at least one index case with thalassemia were included in this study. These families were referred to our center from hospitals in 3 different states of India [viz 47 from Uttar Pradesh (U.P.), 7 from West Bengal and 10 from Gujarat].

Methods: Hematological analysis was performed on Sysmex automated cell counter.

HbA2 was estimated by column chromatography (Schleider et al. 1977) while HbF was determined by alkali denaturation test (Bette et al. 1959) DNA was extracted by the phenol-chloroform method (Poncz et al. 1982). The samples were screened for the 5 common, 5 less common, and few rare mutations reported among Asian Indians (Gupta et al. 2003) first by Reverse dot blot (Gorashaker et al. 1997) and then by Amplification refractory mutation system-PCR (Newton et al. 1989) These samples were then subjected to nested PCR followed by single-stranded conformational polymorphism (SSCP) (Gupta and Agarwal 2003) and sequencing. Haplotype analysis was performed by RFLP-PCR (Sutton et al. 1989). Seven restriction endonuclease sites within the β -globin gene cluster (Fig. 1) were used to construct a haplotype (*Hind*II5'e, *Xmn*I5'Gg, *Hind*IIIg, *Hind*IIIg, *Hinc*II5'jb, *Hinf*I5'b, and *Ava*IIb).

DNA was amplified using 15.0pM of each primer, 2.5mM of $MgCl_2$ (Bangalore Genei), 10.0mM of dNTPs (Bangalore Genei) and 1Unit of *taq* polymerase (Bangalore Genei). This sample mix was subjected to hot start consisting of denaturation at 94°C for 7 min, annealing at 65°C for 1 min and extension at 72°C for 1.30 min and finally to 24 PCR cycles. Each cycle was 94°C for 1 min, 1 min annealing at 65°C and an extension of 1.30 min at 72°C (Fig. 2).

The digestion reaction was performed in an overnight incubation of 10mg of each PCR product with the correspondent endonuclease according to the manufacturer recommendation.

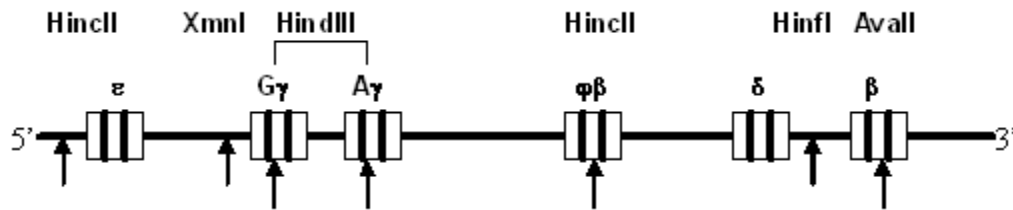


Fig. 1. Diagrammatic representation of 7 RFLP sites in β -globin gene cluster.



Fig. 2. Amplicons for different RFLP sites in β -globin gene cluster. Left to right marker, Xmn I, Hind III G, 3'Hinc II $\gamma\beta$, Hinf I, 5'Hind III $\gamma\beta$, Ava II, 5' Hinc II

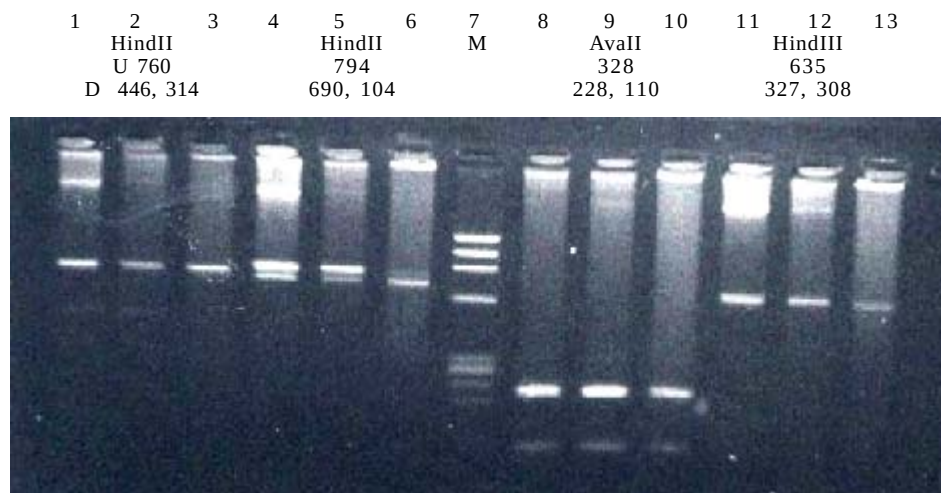


Fig. 3. Gel photograph showing PCR products after digestion with respective enzymes. 1,2,3 – HindII site absent (-/-), 4,5-(+/-), 6-(+/+), 8, 9, 10- (+/+), 11, 12, 13-(-/-).

The digestion profiles were obtained by agarose gel electrophoresis with subsequent staining with ethidium bromide and visualization in a UV transilluminator (Fig. 3).

RESULTS

Characterization of Mutations

Of the 124 chromosomes studied, 72 were of IVS1-5(G-C), 6 of IVS1-1(G-T), 5 of CD41/42(-CTTT), 3 of CD8/9(+G) and 2 each of CD15(-T), CD16(-C) and HPFH, 1 each of 619 bp deletion, CD15(G-A), -29(A-G), CD 30(G-C), Ini (ATG-ACG), a novel 8bp deletion, a 17 bp deletion at CD126-131 and 25 of CD26.

The five most common Asian Indian mutations accounted for 70% of the total allele with IVS1-5 (G-C) being the predominant mutation (58%).

In 10 β -thalassemia chromosomes and 6 HbE chromosomes, haplotypes could not be assigned because of heterozygosity at one or more polymorphic site and nonavailability of family members.

Haplotype Study

A total of 19 haplotypes were found on β -thalassemia chromosomes (Table 1). The most common mutation IVS1-5 (G-C) was associated with 8 different haplotypes with haplotype {+ - - - - +} being the predominant (54%). It was found on 32 chromosomes of UP origin, 6 of W. Bengal and 1 of Gujarat. This haplotype was also associated with CD16 (-C) and CD41/42 (-CTTT). The second haplotype associated with the mutation was {+ - - - - +} that was found in 8 chromosomes (1 U.P. and 7 Gujarati). The third most common haplotype associated with IVS1-5 was {- - + + - -} and was present on 5 chromosomes. The 3 major haplotypes associated with IVS1-5 accounted for a total of 72.2%.

IVS1-1 (G-T) mutation was found on 2 haplotypes, while CD8/9 (+G) and CD41/42 (-CTTT) were found on 3 haplotypes each. IVS1-1 (G-T) and CD8/9 (+G) are present on the same chromosomal background. CD15 (G-A), CD16 (-C), 619bp del, HPFH and CD26 were found on the same haplotype as IVS1-5 (G-C). CD30 (G-C) and novel 8bp deletion shared the same haplotype. Ini (ATG-ACG), 17 bp del, CD15 (-T)

Table 1: Association of β -thalassemia/HbE mutations and haplotypes.

Mutations	Haplotype	U.P.	W.Bengal	Gujarat
IVS1-5(G-C)	+ - - - - +	32	6	1
	- - + + - -	5	-	-
	- - - - - +	3	-	1
	+ - - - - -	-	-	2
	+ - - - - +	1	-	7
	+ - - - - +	1	-	-
	- - + - - +	-	-	1
	- + - - - +	-	-	1
IVS1-1(G-T)	- + + - - +	4	-	-
	- + + + + +	2	-	-
CD8/9(+G)	+ + + - - -	-	-	1
	- + + - - +	1	-	-
	- + + + + +	1	-	-
CD15 (-T)	- - + + - -	2	-	-
CD15 (G-A)	+ - - - - +	-	-	1
-29 (A-G)	+ - + - - -	1	-	-
CD30 (G-C)	+ + - + + +	1	-	-
CD16 (-C)	+ - - - - +	1	-	-
	+ - - - - +	1	-	-
CD41/42 (-CTTT)	- - - - - +	-	-	2
	+ - - - - -	-	-	1
	- - + - - +	2	-	-
Ini (ATG-ACG)	- - + + + +	1	-	-
-8bp del	+ + - + + +	1	-	-
-17bp del	- - + - - +	1	-	-
-619bp del	+ - - - - -	-	-	1
HPFH	+ - - - - -	2	-	-
CD26	- + - - - +	1	3	-
	+ - - - - +	13	0	-
Normal	+ - - - - +	41	-	-
	+ + - - - -	12	-	-
	- + + - - +	1	-	-
	- + + - - -	2	-	-
	- - + - - +	4	-	-

and -29 (A-G) were found on different chromosomal backgrounds. Both the chromosomes with CD15 (-T), a rare mutation in Indians was found to have the same haplotype pattern.

A total of 25 b^E chromosomes were analyzed for the β -globin gene cluster haplotypes (18 of U.P. and 7 of W. Bengal origin). Strong linkage of b^E mutation with one particular haplotype {+ - - - - +} in 13 chromosomes (72.2%) of U.P. origin was observed. However, in the W. Bengal group haplotype {- + - - - +} significantly contributed to linkage with the b^E mutation (42.8%)

Among the 73 normal chromosomes, 5 different β -globin cluster haplotypes were found. With the exception of one {+ - - - - +}, none of the β -thalassemia chromosomes were represented among the normal haplotypes. The predominant haplotype {+ - - - - +} was found associated with IVS1-5 (G-C) but with lower frequency.

DISCUSSION

Analysis of DNA polymorphic sites is a valuable tool in relating human genetic variation to disease and for understanding human evolutionary history. The beta-globin locus contains several single-base restriction fragment length polymorphism (RFLP) sites throughout chromosome 11. In addition to these polymorphic sequence repeats, others are being studied in order to expand our knowledge concerning the role between haplotype-genotype and phenotype associations (eg., presence of XmnI leads to elevation of HbF and thereafter buffering the severity of the disease).

β -thalassemia alleles found among our patients are typical of Asian Indians. Our results reveal that the five most common mutations are IVS1-5(G-C), IVS1-1(G-T), CD8/9(+G), CD41/42(-CTTT) and -619bp constitute 70.0% of the allele.

The IVS1-5 is the most frequently observed mutation present in β -thalassemic chromosomes represents 58.0% of the 124 β -thalassemic chromosomes studied.

It has been reported in high frequency in Indonesians and Melanesians but in low frequency in Chinese and Thais. In Indonesians and Melanesians this variant is mostly associated with the haplotype {+ - - - - - + + +} (Lie-Injo et al. 1989), in Chinese with haplotype {- - - - - + + + + +}. In our case we found that eight different haplotype harbored IVS1-5 (G-C) mutation, with {+ - - - - + -} being the most common. There are two plausible explanations for such extreme heterogeneity associated with this mutation. (1) The mutation arose more than once in the population, the chances of which are very rare. (2) multiple haplotypes are result of simple recombination events, which is more likely as 5' region to beta-globin gene is a hotspot for recombination. The different and vast number of haplotypes associated with this mutation, its high frequency and omnipresence is suggestive its independent as well as most ancient origin among Asian Indians.

CD 41/42 is another prevalent mutation found in Southeast Asian countries. This mutation was first reported in Taiwanese (Kimura et al. 1983), later in Chinese (Wong et al. 1986) and in Asian Indians (Kazazian et al. 1984). Its widespread distribution, high frequency and association with different haplotype and framework suggest a separate/early origin. In our patients it was found on three chromosomes of IVS1-5. Unlike what

was reported by Varawalla et al. (1992) and Thein et al. (1984), in our patient group -619 bp, CD8/9 and IVS1-1 are not confined to a particular region or group and are not in strong linkage disequilibria with their haplotypes. Infact CD8/9 and IVS1-1 were found on the same chromosomal background. The reason for this could be small sample size. Both the chromosomes with CD15 (-T), having the same haplotype pattern can be explained on the grounds that the couple in which it was found was first cousins. -29 (A-G) mutation is not reported in Indians but has been found in Malay (Tan et al. 2004), Chinese (Mo et al. 2004), American Blacks (Antonarakis et al. 1984), Guadeloupeans (Romana et al. 1996), Moroccans (Lemsaddek et al. 2004), Tunisians (Chouk et al. 2004) and Cubans (Muniz et al. 2000). The mutation was found associated with different haplotypes in different population suggesting an independent origin in those populations.

The high mutational heterogeneity observed in this study has provided useful information about the types and geographic distribution of β -thalassemia mutations in India, thus allowing rapid detection of mutations in couples at risk.

In addition, presence of 19 different haplotype on β -thalassemia chromosomes proves that this genetic diversity has originated from both new mutational events and gene flow due to population migration.

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