

Beta Globin Gene and Related Diseases: A Review

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ABSTRACT In the present paper an attempt has been made to review the variations Clinical or phenotypic diversity of β -thalassaemia and other β -haemoglobinopathies suggest that it is determined by layer upon layer of complexity. A wide variety of primary mutations at the β -globin gene; two well-defined secondary modifying loci (δ and α gene) and several less well characterized tertiary modifiers interact with strong environmental component.

STUDY OF HUMAN BETA GLOBIN GENE

Use of combination of somatic cell hybridization and hybridization using DNA probes permitted assignment of the β globin locus to chromosome 11. Using translocation cell line [t(7;11)(q22;p15)] coming from a erythroleukemia patient, Morton et al.⁹³ stated that β -globin gene was situated at 11p15^{78,86}. Others confirmed this observation. With the help of high resolution cytogenetics and *in situ* hybridization Lin et al.⁸² placed the beta gene in the 11 p15.4-11p15.5 segment. Genetic studies and structural analysis of haemoglobin fusion proteins^{6,7} predicted linkage of the γ , δ and β genes. Physical linkage between the δ and β genes was demonstrated by genomic blotting experiments⁵⁴ by analysis of recombinant bacteriophage containing both δ and β genes⁷⁶. Linkage of the $G\gamma$ and $A\gamma$ genes was determined by genomic blotting experiments⁸⁴ and confirmed by analysis of clones containing both of these genes^{55,56}. Linkage between the $A\gamma$ and δ genes was established using a cloned hybridization probe from the $A\gamma$ - δ intergenic region and a well defined deletion located in this region^{55,124}, by mapping DNA fragments from the $A\gamma$ - δ intergenic region generated by nonlimit restriction enzyme digests of genomic DNA and ultimately by cloning overlapping DNA fragments that span the $A\gamma$ - δ intergenic region⁵⁶. Analysis of overlapping cloned DNA fragments also made it possible to demonstrate physical linkage between the ϵ gene and remainder of

gene cluster⁵⁵ the isolation of which was initially reported by Proudfoot and Baralle.¹⁰⁸ The five transcriptionally active β -like globin genes are found within a 45 Kb cluster on chromosome 11 in the following order: 5' - ϵ - $G\gamma$ - $A\gamma$ - δ - β - 3' (Fig. 1).

Additionally, the pseudogene (which could not be identified with known polypeptides), $\psi\beta_1$, is located between the $A\gamma$ and δ genes.

By linkage disequilibrium analysis of family data on 15 restriction site polymorphisms (RSPs), a "hotspot" for meiotic recombination at the 5' end of the β gene was seen²². Recombinant leftward (in the 5' direction) from a point called "chi" near the end of the beta-globin gene is 3 to 30 times the expected rate. By a computer search of DNA sequence of the β -cluster, a "chi" sequence (5'-GCTGGTG-3') was located at the 5' end of the second intervening sequence of the β -gene²² in a large Amish pedigree. Gerhard et al.⁶⁵ observed an apparent crossover within the beta-globin gene cluster in the region of the recombinational "hot spot" postulated by Chakraborti et al.²². It was also possible to identify the orientation of the beta-globin cluster *vis-à-vis* the centromere: centromere - 5' - ϵ - β - 3'-pter.

Initial advances in globin gene mapping and isolation were made possible by the development of procedures for synthesizing and cloning double stranded DNA copies of poly(A) containing mRNAs. Sequences of human β -globin mRNA were determined by analysis of cDNA (88). Later cDNA plasmids carrying human α , β and γ -globin mRNA sequences were cloned and characterized^{94,137}.

Restriction sites within and surrounding the δ and β genes were mapped using the genomic blotting procedure⁵⁴. The existence of a large intron within each gene was demonstrated by comparing the restriction endonuclease cleavage map of the β -globin gene in genomic DNA with the map of a β -globin cDNA plasmid^{54,90}. These conclusions were also independently reached by analysis of bacteriophage recombinants that carry both the

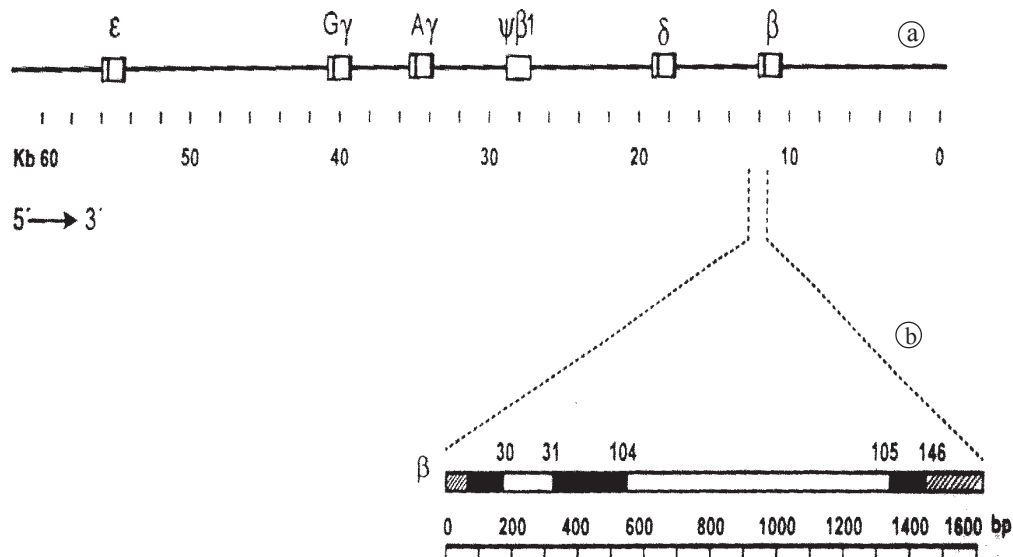


Fig. 1. a) Linkage arrangement of the human β -like globin gene cluster. b) Structure of human β -globin gene. Solid and open boxes represent coding (exon) and non-coding (intron) sequences, respectively. Introns are located between codons 30 and 31, and 104 and 105.

δ and β genes⁷⁶. The identification of the two genes and the precise location of the large intron within each gene were established by DNA sequence analysis. In addition, a second smaller intron was identified by comparing the fine structure maps of a human β -globin cDNA plasmid and of chromosomal gene⁷⁶.

All five of the known β -like globin genes were obtained from recombinant clones of genomic DNA : β and δ ⁷⁶; $G\gamma$ and $A\gamma$ ^{56,110,117} and ϵ ^{56,108}. Identification of the ϵ gene was made by determination of the partial amino acid sequence of the ϵ gene was made by determination of the partial amino acid sequence of ϵ globin polypeptide⁵³. All five β like genes have the small overall exonic structure interrupted by two introns at identical locations. The first one, 125-130 bp in length, is located between codons 30 and 31, and the second one, 700-900 bp, is between codons 104 and 105 (Fig. 1). The introns of human β -globin genes are transcribed as part of a nuclear mRNA precursor and are removed in steps by splicing to produce the mature globin mRNA^{73,89}. β -globin nuclear mRNA precursors are coterminal with their respective cytoplasmic mRNAs. The complete nucleotide sequences of the five human β -like globin mRNAs have been determined^{77,116,118}. The sequence comparison among β -like globin genes

revealed interesting sequence homologies in regions that are potentially involved in globin gene transcription and splicing⁵¹. Sequence 5' to the human β -like globin genes provides an example of these homologies. The first homology block (designated "ATA" box) is found 29-30 bp 5' to each gene and the second block (designated the "CCAAT" box) is located 70-78 bp 5' to each gene.

Variations of Beta Globin Gene Associated with Human Disease

The term "haemoglobinopathies" is given to the inherited disorders of the structure and synthesis of the globin part of the molecule, which fall into several overlapping groups.

Structural Variants

HbS Sickle Cell Disease

Herrick⁶⁶ reported sickling of red cells in the blood in a West Indian patient from United States. Pauling et al.¹⁰⁷ described "sickle cell anaemia as a molecular disease" and showed that it had different electrophoretic properties from normal human haemoglobin. Moreover, total haemoglobin of individuals with sickle cell disease was abnormal, but in symptomless

carriers it was a mixture of both normal and abnormal types called HbA and HbS, respectively controlled by paired genes with HbS being a mutant form.

Subsequently, this change in electrophoretic mobility was found to be due to the substitution of the glutamic acid residue at position 6 by valine in the beta chain of the haemoglobin molecule (β 6 Glu \rightarrow Val)⁶⁹. This was primarily observed by chromatographic analysis of tryptic digest of peptide followed by Edman's stepwise degradation of peptide method. Deoxy-HbS undergoes intracellular polymerization at physiologically relevant haemoglobin concentrations (MCHC = 32-34 gm/dl) with marked reduction in solubility. It was shown that β 6-Valine residue of HbS interacted stereospecifically with a β 88-Leucine and β 85-Phenylalanine is largely hydrophobic in nature and cause stacking of Hb tetramers helically first to form microfilaments and then aggregation laterally to form microfibrils^{96, 97, 98}, 14 stranded of 210 Å diameter which align to paracrystalline gels⁵⁰ accompanied by a change in the shape of the red cell from a biconcave disc to a sickle form.

Beale and Lehmann¹¹ proposed that abnormal structural haemoglobin variants could be explained on the basis of single base changes in the coding DNA of the structural genes. Kan and Dozy⁷² by linkage analysis using Hpa I restriction endonuclease polymorphism were able to perform the prenatal diagnosis of cases of sickle cell anaemia.

The recognition site of Dde I and Mst II was altered by β 6 Glu \rightarrow Val mutation, the 5th, 6th, 7th codon of Hb A being CCT-GAG-GAG; in Hb S it was CCT-GTG-GAG. This helped in the diagnosis of HbS mutation by RFLP analysis. HbS is relatively easy to detect due to its different electrophoretic mobility and technique like, sickle cell test, Murayama test and sickle solubility test⁷¹. The advent of molecular methods like direct detection by restriction enzyme analysis, oligonucleotide hybridization and PCR technology has improved the detection of HbS mutation¹⁰⁵.

The highest gene frequencies are found in equatorial Africa where it exceeds 20% in the Cameroon, Guinea, Zaire, Uganda and Kenya. In Quatif Ouses of Saudi Arabia and parts of India HbS is known to occur at frequencies of upto 20%. Modiano et al.⁹² reported HbS in Nepal

with a gene frequency of 5%. Around the Mediterranean (the coast of North Africa, Turkey, Lebanon, Syria, Greece and Portugal), in the Middle East and in Iran it occurs generally at frequencies of less than 5%. Frequencies of HbS vary substantially from one group to another in these regions (14% in Eti-Turks of Turkey, 25% in Khazramahs of Syria).

The first cases of sickle cell anaemia in an Indian of 22 years age, born of Indian parents in Durban was reported by Berk and Bull¹² from Cape Town. Sickle cell trait in South India was first described by Lehmann and Cutbush⁸⁰ among the aboriginal tribes of Nilgiri Hills. In the same year Dunlop and Mazumder⁴⁹ reported five cases of sickle cell trait and three presumptive cases of sickle cell anaemia among the tea garden labourers of Upper Assam, originating from the tribal population of Orissa and Bihar. HbS gene is widespread among many tribal and non tribal populations of India. (Table 1).

Haemoglobin C

Itano⁷¹ recognized a second abnormal haemoglobin which differed from HbA by the substitution of lysine for glutamic acid at position 6 in the β -chain (β 6 Glu \rightarrow Lys) and named this Haemoglobin C (HbC). Ranney¹¹ reported HbC to be an allele of HbS, manifested by decreased solubility, increased intraerythrocytic molecular aggregation and paracrystal formation, culminating in global red cell dehydration and target cell formation. Under physiological conditions HbC is less soluble than HbA and hence undergoes intracellular crystal formation which are less appreciable under light microscopy but clearly detectable by electron microscopy and 31p-NMR. X-ray crystallography and fourier diffraction analysis revealed the reason of decreased solubility of HbC which was stabilization of inter-molecular contacts by β 6-lysines of deoxy HbC forms. Intracellular crystallization of HbC occurs however only on the arteriolar or oxygenated side of microcirculation, melting out in the capillary or venous circulation, producing apparent lack of vasoconstriction similar to sickle cell disease. Because of the altered cellular properties HbC bearing red cells exhibit a marked decrease in deformability with an increased propensity for

Table 1: Incidence of HbS in different states of India

States	Sickle cell gene frequency					
	Highest frequency			Lowest frequency (not zero)		
	%	Population	References	%	Population	References
Andhra Pradesh	18.3	Pradhan	Blake et al. (1981)	0.4	Chenchu	Ramesh et al. (1980)
Assam	19.3	Karmakar (Tea Garden Labourers)	Balgir (1996)	4.5	Gowala	Balgir (1996)
Bihar	0.4	Oraon	Saha et al. (1988)	0.3	Ho, Desi, Bhumij	Balgir (1996)
Gujarat	15.7	Gamit	Vyas et al. (1962)	0.9	Vasava	Bhasin et al. (1985)
Karnataka	4.0	Jenu Kuruba	Banerjee et al. (1988)	0.5	Vokkaliga	Banerjee et al. (1988)
Kerala	14.3	Irula	Saha et al. (1976)	0.5	Kadar	Saha et al. (1974)
Madhya Pradesh	20.0	Mahar	Negi (1963)	0.4	Kawar	ICMR report (1986)
Maharashtra	15.4	Pradhan	Banker et al. (1984)	0.1	Koli	Iswad et al. (1984)
Orissa	22.2	Lohar	Balgir (1996)	0.2	Kissan	Balgir (1996)
Rajasthan	5.0	Mochi (S.C.)	Choubisa et al. (1986)	0.3	Gamet, Khatik	Choubisa et al. (1986)
Tamil Nadu	17.7	Irula	Undevia et al. (1981)	0.4	Pallar	Büchi (1955)
Tripura	0.2	Tribals	Chakraborty et al. (1996)	-	-	-
Uttar Pradesh	9.2	Gond	Balgir (1996)	4.8	Dhanukh	Bhatia et al. (1955)
West Bengal	0.6	Santal	Choudhuri et al. (1967)	0.3	Kaora	Das et al. (1974)

entrapment within the reticuloendothelial system, especially the spleen. Decreased red cell survival related to the poor deformability is imparted by red cell dehydration rather than intracellular crystal formation. Electrostatic interaction between the positively charged HbC and negatively charged protein on the inner membrane surface of RBC leads to enhanced K^+ efflux causing cellular dehydration by marked reduction in erythrocyte water content.

HbC mutation in codon 6 of beta-globin gene (codon 6 GAG→AAG) does not abolish or create any known restriction endonuclease site. Fischel-Ghodsian et al.⁵² described a AS-PCR amplification technique for rapid detection of HbC.

HbC occurs mainly in the autochthonous inhabitants (Negros) of West Africa. It is observable at a frequency up to 50% in the Ivory Coast, with decreasing frequency away from this epicenter. It is also noticed at a frequency of 3-7% in a culturally and geographically separate population living on the North African coast in the Maghreb⁸⁵. HbC has not yet not yet been reported in Indians.

Haemoglobin D Disease

Itano⁷¹ described the term haemoglobin D to described a haemoglobin variant with same electrophoretic mobility as HbS but not

associated with sickling of the red cells, the commonest being haemoglobin D Punjab (β 121 Glu→Gln). Heterozygotes for HbD are essentially normal except for the presence of a few target cells in some cases. Homozygous individuals have not significant clinical symptoms through there may be mild haemolysis with microcytic-hypochromic RBC.

Bird et al.¹⁶ recorded the first Indian case of HbD in a 19 years old Sikh Soldiers from the Hoshirpur district of East Punjab. It is however common worldwide, with incidence of 2-3% in the Sikhs of Punjab. It is also the most frequent abnormal haemoglobin in Xinjiang Uygur of China^{81,130}. Zeng et al.¹⁴⁰ used PCR methods for population studies of this variant. The codon 121 (G→C) mutation is the molecular location of the mutant.

Haemoglobin E

Itano et al.⁷¹ discovered a fourth abnormal haemoglobin with electrophoretic mobility like HbC and named it HbE. The mobility of this new variant (HbE) was however different from HbC when moving boundary electrophoresis was set up. The first patient described was double heterozygote possessing genes for HbE and β -thalassaemia, HbE being inherited from the father, who was a Guatemalan origin with Spanish and Hindu ancestry¹¹⁹.

Protein fingerprinting pattern by chromatographic analysis of trypsin digested globin revealed the substitution of normal glutamine residue by lysine in the 26th amino acid of β chain (β 26 Glu→Lys).

No difference was detected between the electron microscopic appearance of HbA and HbE or of different biophysical parameters including sedimentation coefficient^{40, 60, 91}.

Except some sporadic appearance HbE is confined exclusively in South Eastern Asia including India. HbE was first recorded in India in Bengalees by Chatterjee et al. in 1957.

Subsequently very high incidence has been reported in Eastern India including West Bengal, Tripura and Assam. In certain tribes inbreeding has produced an incidence as high as 50%^{3, 34, 35, 37, 38} (Table 2).

Orkin et al.¹⁰⁵ reported the complete nucleotide sequence of β^E globin gene with a GAG to AAG change in codon 26 as the only abnormality (codon 26 GAG→AAG). Expression of the β^E gene was tested by introducing it into HeLa cells. Two abnormalities of RNA processing were shown; slow excision of IVS-1 and alternative splicing into exon-1 at a cryptic donor sequence within which the Codon 26 (G→A) mutation resides producing mild β^+ mutation^{21, 122}. Thein et al.¹²³

demonstrated that the GAG to AAG change could be recognized by the restriction enzyme Mnl I which cleaves DNA at thesequence 3'-GAAG-5'.

Molecular Basis of Structural Haemoglobin Variants

Ever since the discovery of HbS in 1949, the number of haemoglobin variants is increasing and more than 600 are known today. Apart from common electrophoretic variants, many rare abnormalities have also been detected which are either silent (i.e. difficult to detect by electrophoresis) or associated with severe haemolytic disease. Characterization of silent variants needs advanced methodologies like isoelectric focusing, O₂ affinity measurement, HPLC, and sequence analysis of DNA and globin polypeptides.

Thalassaemia Syndromes

Thalassaemia syndromes are genetic disorders characterized by a reduced rate of production of one or more of the globin chains of haemoglobin. About 3% of the world's population are carriers for β -thalassaemia gene^{130, 134}. In the Indian subcontinent the first

Table 2: Incidence of HbE in different states of India

States	gene frequency					
	Highest frequency			Lower frequency (nolzenic)		
	%	Population	References	%	Population	References
Andhra Pradesh	1.0	Unspecified (from Hyderabad)	Reddi et al. (1978)	-	-	-
Arunachal Pradesh	18.1	Adis (Gallongs)	Balgir (1996)	5.2	Apatanis	Balgir (1996)
Assam	64.5	Boro Kachari	Deka et al. (1988)	0.7	Rengma-Naga	Balgir (1996)
Kerala	0.31	Malayali	Saha and Banerjee (1971)	-	-	-
Madhya Pradesh	0.34	Unspecified (from Raipur)	Sepha et al. (1979)	-	-	-
Maharashtra	-	Unspecified (from Bombay)	Sukumaran et al. (1976)	-	-	-
Meghalaya	37.5	Mixed Bodo	Balgir (1996)	21.9	Khasi	Das et al. (1971)
Manipur	10.1	Meitei	Singh et al. (1986)	2.8	Brahmin	Singh et al. (1986)
Nagaland	3.5	Mixed Naga	Balgir (1996)	-	-	-
Sikkim	4.7	Lepcha (South Sikkim)	Bhasin et al. (1986)	1.0	Bhutia (South Sikkim)	Bhasin et al. (1986)
Tripura	36.34	Tribals (Tibeto-Burman)	De et al. (1997)	-	-	-
Uttar Pradesh	0.07	Unspecified	Mehrotra et al. (1968)	-	-	-
West Bengal	61.2	Malda	Das et al. (1991)	0.4	Vaidya	Choudhuri et al. (1969)

case of uncharacterized haemoglobinopathy as Cooley's anaemia was reported in a 2.5 year old Bengalee boy (94). The carrier rate in different regions varies between 1% and 17% with a mean of 3.3%^{2, 3, 7, 19, 37, 127, 129, 131}. Based on this mean carrier frequency and the demographic profile of India, estimated number of β -thalassaemia carriers is about 29.7 million, while the birth of β -thalassaemics are 7000 per year¹³⁰.

Beta thalassaemias are a heterogeneous group of conditions and may be broadly subdivided into β^0 and β^+ thalassaemia. β^+ thalassaemia is characterised by reduced level of β globin production while in β^0 thalassaemia there is no detectable β globin synthesis¹³³. β^0 thalassaemia is fairly homogeneous at clinical and haematological levels while β^+ thalassaemia shows considerable heterogeneity.

In an Indian β^0 -thalassaemia patients Orkin et al.¹⁰⁵ and Flavell et al.⁵³ found a partial deletion of about 600 bp nucleotides in β globin gene.

Over 150 common different mutations that cause β thalassaemia have subsequently been reported from different parts of the world. A large number of mutants are being also reported regularly. β thalassaemia mutations can be classified as:

- 1) **TRANSCRIPTIONAL MUTANTS**
mutations in the promoter regions 5' to the β globin gene that effect transcription.
- 2) **RNA PROCESSING MUTANTS -**
 - a) **SPLICE JUNCTION -**
Substitution in the invariant GT and AG dinucleotides destroy the sequence that is essential for splicing.
 - b) **CONSENSUS SEQUENCE -**
Substitution around splice junctions destroy consensus sequences that are important but not essential for splicing.
 - c) **CRYPTIC SPLICE SITES IN INTRONS -**
in introns nucleotide substitutions may creat new donar or acceptor splice sites.
 - d) **CRYPTIC SPLICE SITES IN EXONS**
- 3) **NONFUNCTIONAL mRNA -**
 - a) **NONSENSE MUTANTS -**
polypeptide chain termination mutation.
 - b) **FRAMESHIFT MUTANTS -**
Change in reading frame cause premature termination of translation.

- c) **INITIATION CODON MUTATION -**
Initioation of translation mutation.
- 4) **RNA CLEAVAGE AND POLYADENYLATION MUTANTS.**
- 5) **CAP SITE MUTANTS.**
- 6) **FUSION GENES -**
Hb lepore (δ - β) and Hb anti Lepore (β - δ).
- 7) **PARENTIAL DELETION OF β GLOBIN GENE -**

β thalassaemia mutations are normally recessive in nature but a spectrum of dominantly inherited forms of beta thalassaemia mutation has been identified. At the molecular level, the mutations fall into four groups resulting in a highly unstable beta globin variant:

- 1) Single base substitution.
- 2) Deletion of intact codons- destabilization.
- 3) Premature termination- truncated beta globin.
- 4) Frameshift mutations- elongated beta globin.

Population studies indicate that only 20 β thalassaemia alleles account for > 80% of the β thalassaemia mutation in the whole world due to phenomenon of geographical clustering where each population has a few common mutations together with a varying number of rare ones. In India with about 4635 ethnic communities, only five common and 12 rare mutations have been reported (Table 3).

Uncommon Beta Globin Variants

Different uncommon beta globin variants have been reported from different parts of India, like, HbD Iran (β 22 Glu \rightarrow Gln), Hb Hofu (β 126 val \rightarrow Glu) etc. But in absence of peptide mapping, actual nature of different Hb variants like Hb J, Hb K, Hb M remains unspecified^{19, 112, 137}.

Compound Disorders of Haemoglobin Related to β Globin

Compound disorders of haemoglobin i.e. heterozygosity for two different globin gene mutations were also recorded from different parts of India and abroad e.g., Hb SD, Hb SE, Hb DK, Hb SC^{7, 19, 112}.

More common ones are S β , D β and E β thalassaemia E β thalassaemia has been reported from many parts of South Asia and Indian

Table-3: β -thalassaemia mutation from different parts of India

Population(n)	Reference	No. of mutant alleles																Others
		B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	
Gujarat(n=197)	Varawalla et al. (1991)	81	8	51	33	17	3	4	-	-	-	-	-	-	-	-	-	-
Punjab(n=142)	Varawalla et al. (1991)	54	19	22	15	20	1	1	-	4	-	2	-	1	1	-	-	2-?
Tamilnadu(n=59)	Varawalla et al. (1991)	48	2	1	-	6	-	-	-	-	-	-	-	-	-	-	-	2-?
Maharashtra(n=13)	Varawalla et al. (1991)	7	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	4-?
West Bengal [& Bangladesh](n=10)	Varawalla et al. (1991)	6	1	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-
Asian Indian(n=419)	Varawalla et al. (1991)	148	92	72	36	35	11	-	6	6	3	2	2	1	1	1	-	-
Gujarat(n=116)	Varawalla et al. (1991)	53	7	29	16	11	-	-	-	-	-	-	-	-	-	-	-	-
Punjab(n=76)	Varawalla et al. (1991)	33	15	11	7	10	-	-	-	-	-	-	-	-	-	-	-	-
Tamilnadu(n=294)	Venkatesan et al. (1992)	128	04	01	-	13	-	-	-	-	-	-	-	-	-	-	-	148 Normal allele
West Bengal(n=16)	Banerjee et al. (1992)	10	04	02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Uttar Pradesh(n=55)	Agarwal et al. (1994)	33	07	13	02	06	-	-	-	-	-	-	-	-	-	-	-	18-?
West Bengal (n=80)	Ghosh Dostidar et al. (1994)	54	2	3	-	7	3	-	-	-	-	1	-	-	1	-	-	9-?
West Bengal(n=244)	De et al. (1997)	71	-	3	-	3	-	-	-	-	-	-	-	-	-	-	-	39HbE, 40-? & 68 Normal allele
West Bengal(n=125)	Verma et al. (1997)	95	4	1	-	12	-	1	-	-	-	-	-	-	-	-	-	Normal allele
Punjab (n=80)	Verma et al. (1997)	38	9	10	6	9	-	-	-	2	-	3	-	-	3	-	-	33 HbE & S, 12-?
Haryana(n=59)	Verma et al. (1997)	34	1	10	6	5	-	-	-	1	-	1	-	-	1	-	-	-
Uttar Pradesh(n=61)	Verma et al. (1997)	30	7	2	-	8	3	1	-	3	-	2	-	-	3	-	-	3-HbE & s, 2-?
Rajasthan(n=50)	Verma et al. (1997)	33	5	2	1	6	-	1	-	2	-	-	-	-	-	-	-	2-[codon 30(G→A)] & 2-?
Bihar(n=33)	Verma et al. (1997)	22	5	-	-	1	-	-	-	1	-	-	-	-	-	-	-	123-HbE & 177- Normal allele
Bengal & Eastern India(n=582)	Das et al. (1998)	204	07	02	-	08	02	10	-	-	-	-	-	-	-	-	-	17-?
Western India(n=328)	Vaz et al. (1995)	159	12	61	28	26	10	6	2	2	2	1	-	2	-	-	-	-

Note :
 B1 = IVS-1 nt5 (G→C)
 B2 = Codon 8/9 (+G)
 B3 = 619 bp deletion 3' b
 B4 = IVS-1 nt1 (G→T)
 B5 = Codon 41/42 (-TCTT)
 B6 = Codon 15 (G→A)
 B7 = Codon 30 (G→C)
 B8 = Codon 5 (-CT)
 B9 = IVS-1 nt1 (G→C)

B10 = Codon 16 (-C)
 B11 = IVS-2 nt 837 (T→G)
 B12 = Cap site +2 (A→C)
 B13 = IVS-1 nt 1 (G→A)
 B14 = IVS-2 nt 1 (G→A)
 B15 = -88 (C@T)
 B16 = Codon 88 (+T)
 ? = Unspecified

subcontinent and is the most common thalassaemia syndrome encountered in parts of India, Pakistan and Bangladesh^{1,2,4}. There is a remarkable variability in the clinical expression and complications of this disorder, ranging from clinical severity similar to homozygous β thalassaemia to cases of reproductively active adults less severely affected^{42,132}.

When HbE mutation is in double heterozygote state with β^0 variant of β thalassaemia, it produces E β^0 -thalassaemia which is the typical form of severe E β -thalassaemia, prevalent in South East Asia and India. However when β^+ thalassaemia mutation interacts with Hb E, a less severe disease is produced.

In Eastern India HbE mutation (Codon 26 GAG→AAG) interacts mainly with β^+ thalassaemia [IVS-1 nt G→C) to produce E β thalassaemia phenotype^{41,42}.

$\delta\beta$ -Thalassaemia and hereditary persistence of fetal haemoglobin (HPFH) is also reported from India³⁷.

β -Thalassaemia unlinked to β -globin gene has been reported.

Apart from *cis* acting factors (proximal promoters, enhancers, silencer and LCR) different trans acting factors play different roles in modulating gene expression of β -globin gene. Trans acting factors are encoded by autosomal and/or X-linked determinants, namely locus control region binding protein NF-E2 (on 12 q13)¹⁰³, X-linked regulator of F-cell number (on X p22.2-p22.3)^{23,48}, autosomal quantitative trait locus (QTL) for HbF/FC ratio (on 6q23 or 6q22.3-q23.1)^{31,61}.

β thalassaemia unlinked to the β globin gene cluster is an uncommon phenotype and only three families presenting such phenotype have been described.

Murru et al.⁹⁹ described an Italian family, but it was not clear whether the unusually mild phenotype was caused by additional thalassaemia defect not linked to the β cluster.

Thein et al.¹²² described one such phenotype from an English family. Lack of informative polymorphisms hindered reaching any conclusion.

Pacheco et al.¹⁰⁶ demonstrated a three generation Portuguese family in whom extensive DNA analysis of the β globin gene and flanking regions failed to reveal any genetic alteration. They concluded that this family

carried a novel β thalassaemic autosomal determinant unlinked to the β globin gene.

These observations reinforce the view that haemoglobinopathies are single gene disorders under polygenic regulation.

Pathogenesis of Disease

Thalassaemias and haemoglobinopathies are characterized by anaemia of various degrees, weakness, growth retardation, enlargement of abdomen, enlargement of spleen and liver and bone changes. The terms thalassaemia major, intermedia and minor are no longer in use and in areas where diagnosis is made early the typical child with severe anaemia, very large spleen and abdomen, short stature and other facial and skull changes may not longer be seen.

The causation or pathophysiology of different states is yet not clear and genotype-phenotype correlations not perfect. However, knowledge of the etiology of some signs has been fairly well established.

Anaemia

There are several causes of the anaemia produced by different abnormal haemoglobins. In general the anaemia is both dyserythropoietic as well as haemolytic. In some, like HbS the cause is apparent as lowering of oxygen tension leads to a process known as 'sickling' of the red cells making them more susceptible to destruction by the spleen.

Red cell indices are taken to be the differentiating factor in anaemias due to thalassaemias and from iron deficiency and are very necessary as both occur in the same areas. Relative increase in red cell count in thalassaemias gives reduced MCH and MCV values. The degree of microcytosis and type of mutation in β thalassaemia had shown wide variation between range of MCV in β^+ mutations while β^0 had virtually identical range of MCV.

The peripheral blood smears show microcytosis and hypochromia with some anisocytosis and poikilocytosis. The bone marrow shows erythroid hyperplasia. Plasma ferritin levels are elevated or normal and are a good index of differentiating iron deficiency anaemia from thalassaemias.

Aplastic anaemia may occur temporarily while megaloblastic anaemia with E β thalassaemia had been reported by Chatterjee²⁴.

Growth Retardation

Growth retardation has been recorded in early descriptions of the disease and is found in untreated cases of β thalassaemia as well as HbE disease and has a direct correlation to the Hb%²⁵. The retardation is noted early (before 1 year) and is most marked between 8-10 years of age. No evidence of growth hormone deficiency has, however, been seen and growth hormone response to insulin, arginine or sleep was normal or elevated in thalassaemic children. Somatomedin deficiency was suggested to be the cause of growth retardation but not proved³⁰. In a multicentric study of 250 thalassaemic children from Italy, 37% of children between 10-25 years were below 2 SD for their normal height and weight¹⁸, two-thirds of males and one-third of females over 14 years were below 2 SD for normal levels. All patients in Greece below 10 years had normal patterns but in the group above 10 years increasing number were below 3rd percentile for weight and height and showed delay or absence of puberty. Kattamis and Kattamis⁷⁵ showed that girls continued to grow up to 20 years and boys of until 22-24 years.

Late impairment of growth in adequately treated thalassaemics is not clear²⁹. Earlier iron loading of the pituitary gland was said to cause defects in hormone synthesis. Subsequent adolescent growth spurt and development of secondary sex characters can also be found to be delayed in these cases and a picture alike to hypogonadotropic hypogonadism seen.

Deposition of iron in the endocrine glands (haemosiderosis) could be a cause of deficiency leading to developmental defects. Diabetes due to pancreatic insufficiency is the commonest endocrine defect. However excess chelation has also been considered responsible for growth retardation in children below 10 years age. Intensive chelation has been seen to restore physical and sexual characteristics. A relationship between ferritin levels and degree of growth retardation is seen⁵⁷. A low sperm count and/or motility has been seen in thalassaemic patients with low ferritin levels and

this has been attributed to desferrioxamine toxicity⁴⁷.

Circadian growth hormone secretory activity is preserved but amplitude is reduced in thalassaemias. Low levels of growth hormone and decreased response to GHRH is seen in thalassaemic children with retarded puberty⁴⁵. Treatment with biosynthetic growth hormones in prepubertal thalassaemic children with severe growth retardation and impaired response to GHRH has been shown to increase growth rate.

Skeletal Changes

One of the characteristic bone defects seen in untreated cases is a swelling of the facial bones due to maxillary overgrowth which also causes dental defects. Other defects are rarefaction of bones causing fractures and deformities. Abnormalities of the radiological appearance of the skull are also common. All these are due to increase in the bone marrow volume due to increased erythropoiesis.

Damage to long bones may cause defects which may need hip replacement.

Vascular Changes

Vascular changes are most marked in HbS disease and often cause painful crisis of bone, abdomen and lung. Skin ulcers are also known to occur.

HbA₂ has been found to be high in cases of ischaemic heart disease as a diffuse band about 3.8% of the normal. This disappears in 3-6 days of clinical improvement and may be a diagnostic problem in areas where high incidence of β thalassaemias are seen^{32,33}. Plasma lipids have been seen to be elevated in cases of thalassaemias.

Other Complications

Children with thalassaemias are very prone to infections leading to pericarditis, pneumonia and skin, ear and nose infections. HIV and Hepatitis B and C are also often found mainly due to the transfusion of unsafe blood^{50, 115}. Type A viral hepatitis and hepatitis due to cytomegalovirus have also been reported. Glasstones are common in these anaemias¹⁰⁸.

Molecular Studies of Haemoglobinopathy Patients and Correlation with Phenotypic Alterations

β thalassaemia, S β -thalassaemia, E β -thalassaemia, sickle cell anaemia, homozygous vary greatly in clinical course and severity.

Discordance of haemoglobin levels among patients who are sibs prevails, suggesting polygenic factor determinants. Researchers ruled out erythrocyte superoxide dismutase enzyme activity, reticulo-endothelial function and failure of erythropoiesis compensation as severity determining factors.

The clinical severity of β thalassaemia has been shown to be less where α thalassaemia^{132,133}, mild β thalassaemia mutation, hereditary persistence of fetal haemoglobin or β -thalassaemia haplotypes associated with a determinant that produces high fetal haemoglobin¹²¹ are inherited simultaneously.

In E β thalassaemia coinheritance of α thalassaemia can alleviate the severity of the disease¹³⁹. However, many patients without α thalassaemia also have high haemoglobin levels, suggesting additional factors responsible for the mildness of E β -thalassaemia¹³⁸.

Altay et al. in Turkey found that β -thalassaemia mutations trans to the HbS mutation do not exert any beneficial effect on the manifestation of the disease and patients with β^0 and β^+ types of HbS β -thalassaemia have same clinical course.

Presence of IVS - 1 nt 5(G \rightarrow C) a β^+ thalassaemia mutation in both severe and non-severe group of E β thalassaemia patients from Uttar Pradesh, India, indicated the presence of separate severity factors¹.

Nagel et al.^{100, 101} suggested that the level of HbF and relative proportion of gamma chains (G γ / A γ) in the fetal haemoglobin (HbF) are linked to specific haplotypes. The level of HbF in sickle cell disease associated with Senegalese (- + - + +) haplotype and Arab-India (+ + - + +) haplotype are generally increased and are associated with relatively milder form of disease.

Since high level of HbF appears to ameliorate the severity of disease, two types of determinants are proposed to be involved in the HbF response in haemoglobinopathies. One of the genetic determinants that is linked to β -haplotypes is - 158nt5' G γ (C \rightarrow T) mutation

creating Xmn I site polymorphism and (AT)_xT_y motif 5' to beta gene (spanning from -542 to -520) and the second, an X-linked determinant(s) not linked to β -globin cluster¹⁰¹.

The Xmn I - G γ has been shown to be associated with increased expression of G γ globin in sickle cell anaemia and β -thalassaemia and E β -thalassaemia who had (- + - + +) and (+ + - + +) haplotype and were associated with increased HbF level and mild form of disease manifestation^{121, 135, 136, 137}. -158(C \rightarrow T) 5' 5 γ mutation is linked to Senegal haplotype (haplotype IX) and haplotype III, and (AT)₉T₅ motif of -540 to -525 5' to β is linked to Arab-Indian haplotype of β^s allele of β globin gene. It was observed that -158nt5' G γ (C \rightarrow T) and (AT)₉T₅ motif 5' β interact to generate a particularly high expression of HbF under the erythropoietic stress of anaemia in β thalassaemia. It was concluded that (AT)₉T₅ and Xmn I could be critical regulatory motif and two regions of the β like globin are cluster interact, when endowed with the proper sequences to enhance expression of HbF secondary to anaemia and making the clinical course milder.

Recent studies of the reasons for the clinical or phenotypic diversity of β -thalassaemia and other β -haemoglobinopathies suggest that it is determined by layer upon layer of complexity. A wide variety of primary mutations at the β -globin gene; two well-defined secondary modifying loci (δ and α gene) and several less well characterized tertiary modifiers (Vitamin D receptor, Estrogen receptor, collagen, locus for hereditary haemochromatosis, UGT glucuronyl-transferase 1, HLA-DR, TNF α , ICAM1 etc.) interact with strong environmental component^{135, 136}.

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