Thalassemia intermedia phenotype resulting from rare combination of c.46delT [Codon15 (-T)] mutation of beta globin gene and HPFH3

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Key Clinical Message
The beta thalassemia intermedia phenotype has several genotypes. Hematological and molecular diagnostic approach and logical and sequential conduct of various investigations are necessary for the diagnosis of these disorders. Close observations of the genotype–phenotype correlation will provide a better insight for the development of molecular therapy.

Keywords
Genetics, hematology, paediatric disorders, thalassemia.

Introduction
Thalassemia is a genetic disorder characterized by a deficiency in the synthesis of globin chains. Mutations in the globin gene or the regions flanking the globin genes lead to impaired or complete absence of hemoglobin synthesis. Thalassemias are classified as (a, b, or d) depending upon the type of globin chain involved. Phenotypically β-thalassemias are classified as β-thalassemia major, β-thalassemia minor and β-thalassemia intermedia. Hereby, we would like to share our experience on thalassemia intermedia phenotype, with a rare combination of mutation involving the β-globin gene and the gamma (γ) globin gene. Most of the fetal hemoglobin (a2γ2) will be replaced by adult hemoglobin (a2b2) within 1 year of age. But mutations in the regulatory site of the γ globin gene lead to the continuous production of fetal hemoglobin throughout the adult life.

Case History
A three-year-old male presented to us with anemia and a history of occasional blood transfusions. On examinations, he had abnormal RBC indices (with no improvement after iron supplements), his father had normal RBC indices, and his mother had low Hb and low MCV values. During further screening by HPLC, the mother had an Hb A2 of 5.2% (indicative of β-thalassemia trait), the father had an Hbf of 35% (suggestive of heterozygous for HPFH) and the patient had an Hb F of 100%. This indicated the patient to be a compound heterozygote for β-thalassemia and HPFH (Table 1).

Molecular Study
Screening for eight common Indian β- thalassemia mutations
Reverse do blot (RDB) [1] was carried out to detect the eight common mutation [COD 8/9(+G), COD 15(G-A), IVS 1:1(G-T), IVS 1:5(G-C), COD 30(G-C), IVS 1:1(G-A), COD 41/42 (- TCTT) & (COD26(G-A))] in the β-globin gene. Results turned out to be negative in all the three for the eight common mutations. But interestingly in the patient, there is no evidence of normal as well as mutant probe bound to RDB membrane in the Codon 15 position. This gave a clue that a mutation in the patient lies

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somewhere in the region covering the Codon 15 of the \(\beta\)-globin gene (Fig. 1).

### Screening for HPFH-3 deletion

To detect the mutation causing elevated Hb F, primers flanking the break points of \(\psi\beta\), \(\delta\), \(\beta\) gene were used (HPFH-type 3 deletion) [2]. HPFH-type 3 deletion involves the deletion of 48.5 kb DNA including codon 15 of the \(\beta\)-globin gene. PCR flanking the break points was carried out, and the amplified products were analyzed in an agarose gel. Father and the patient were found to be heterozygous (+/-) for HPFH-type 3 deletion, whereas the mother was negative [3, 4] (Fig. 2).

### Sequencing for rare mutation screening

Since screening for the eight common mutations were negative, sequencing was carried out for rare mutation screening. Sequencing showed that the mother is having a deletion of (-T) at codon 15 of the \(\beta\)-globin in a heterozygous state (leads to stop codon in 18th position [5]), the father had a normal pattern, and the patient showed a homozygous pattern for c.46delT [codon 15 (-T)] deletion, since he has two different deletions in each allele in the same genetic region (Fig. 3).
Discussion

Based on the above findings, we would like to put forward the following points which will be helpful in early diagnosis and genetic counseling of thalassemia cases.

Significance of HPLC and family study

In this case, HPLC data and family study joined hands in solving the genetic basis of thalassemia intermedia phenotype. Suspecting a carrier status on the father with normal Hb levels and RBC indices is quite uncommon. But if we have a closer look in this case, a child of an asymptomatic father (normal indices) and a thalassemia trait mother, presenting as thalassemia intermedia raised a doubt on the carrier status of the father. So an HPLC was done on the father’s sample which revealed that he is heterozygous for HPFH. Hence, the child’s phenotypical behavior as thalassemia intermedia could be explained.

The key to this case is the affected child whose presentation as thalassemia intermedia phenotype (in spite of father’s normal Hb & RBC indices) provoked us to look for the cause of the presentation. But this may not be the scenario (family with an affected child) in all the cases with transfusion-dependent anemia. If a woman is expecting for the first time, and the carrier detection of thalassemia is only based on her Hb levels and RBC indices, then there are chances of missing out her carrier status. So we put forward to include Hb-HPLC as a mandatory test in antenatal screening. If an expecting woman is found to be carrier, then her partner also has to be screened for hemoglobinopathy irrespective of the RBC indices.

Exposure to the molecular basis of the disorder

Good exposure on the molecular basis of the disorder is essential for the accurate diagnosis. In this case, chances

Figure 3. Sequencing for the detection of rare mutations in the beta globin gene in the region from Promoter to IVS2. 1. Mother: Heterozygous for c.46delT [Cod15 (-T)], 2. Patient: Homozygous pattern for c.46delT [Cod15 (-T)], 3. Father: Normal.
of misinterpreting the child as β-thalassemia major was high, in view of 100% Hb F and the homozygous pattern for c.46delT [codon 15 (-T)]. But a thorough diagnosis at the molecular level revealed the compound heterozygous state of the patient for HPFH 3 deletion and c.46delT [Codon 15 (-T)], accounting for the β-thalassemia intermedia phenotype.

HPFH-type 3 mutation deletes 48.5 kb DNA (starting from the 5' end of the psi (Ψ) β-gene to a region 30 kb downstream of the β-globin gene). Hence, a heterozygote state of HPFH 3, has one allele normal for coding β-globin gene and the other allele deleted for β-globin gene. Both the father and the patient had this genotype. The nonbinding of probes in either of the alleles of codon 15 in the patient by RDB indicated the complete absence of codon 15 normal allele in the patient. Sequencing also confirmed the same where the patient showed homozygous pattern for c.46delT [codon 15 (-T)] deletion (false positive), for which the patient is actually heterozygous, because the mother showed a heterozygous pattern for c.46delT [codon 15 (-T)] and the father showed a normal pattern. Since the patient has inherited one mutant allele from the father (HPFH 3 deletion) in which there was a deletion of 48.5 kb DNA including the codon 15 region and one mutant allele from the mother c.46delT [(codon 15 (-T))], the patient is compound heterozygous for HPFH 3 and c.46delT [codon15 (-T)].

As a result, the patient’s both β-globin gene allele was defective (one with β- globin gene deleted and the other with c.46delT [codon15 (-T)] point mutation). So there is a complete absence of normal allele for codon 15 in the patient. These two mutations together contributed to the 100% Hb F and the homozygosity of the c.46delT [codon 15 (-T)] and thereby to the thalassemia intermedia phenotype in the patient. Previously, Fucharoen et al. [5], Winichagoon et al. [6], and Kazi Nadim Hasan et al. [7] have reported the mutation (c.46delT) in combination with other beta globin gene mutations and Shaji et al. [8] have reported it in the homozygous state.

Authorship

AM: worked on the acquisition of data, analysis and interpretation of data, drafting the manuscript. AK: was responsible for conception and design of the study, analysis and interpretation of data, revising the manuscript critically for important intellectual content and approving the final version.

Conflict of Interest

There are no conflicts of interest by the authors. The necessary consent was obtained from the patient’s family involved in the study.

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