

β -Thalassaemia Major Malay Patient with Compound Heterozygosity for IVS 1-5 (G>C) Mutation and IVS-1 25bp Deletion: Case Report

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Abstract

IVS-1 25bp deletion in the β -globin gene is rare entity in South East Asia region. We report the first case of β -thalassaemia major in a Malay girl from Malaysia with IVS-1 25bp deletion heterozygously compounded with IVS 1-5 (G>C) consensus mutation. Clinical presentation and molecular diagnostic procedures was described. Molecular diagnosis of rare and emerging thalassaemia alleles can be challenging. Sequence haplotype analysis using classical SNP markers for the family members suggest the IVS-1 25bp deletion probably was derived from Middle East.

Keywords: β -thalassaemia; Thalassaemia major; IVS-1 25bp deletion; IVS 1-5 mutation; Direct DNA sequencing

Introduction

β -thalassaemia syndromes are a heterogeneous group of hereditary blood disorders characterized by reduced (β^+) or absent (β^0) beta globin chain synthesis which affect the biosynthetic balance between α and non- α -globin chain production causing large scale ineffective erythropoiesis and hence anaemia [1]. To date, an astounding amount of more than 810 entries of β -thalassaemia alleles have been documented in the haemoglobin variants and thalassaemia mutations database, HbVar [2]. Vast majority of these are nucleotide substitutions, frameshifts or small deletions. In contrast to the α -thalassaemia, large deletions are rare in β -thalassaemia syndromes [3]. The most prevalent deletion begins in the second intervening sequence and extends beyond 3' end of β -globin gene. The common β -globin gene mutations in Malaysia are IVS 1-5 (G>C), Codon 41/42 (-TTCT) and IVS 1-1 (G>T). The first two are the predominant β -thalassaemia mutations found in Malay and Chinese that affect RNA processing and premature chain termination respectively [4-6]. Deletions represent an extremely scant proportion of all β -thalassaemia alleles in inhabitants of Malaysia. We report our first case of IVS-1 25bp deletion at the β -globin gene in an attempt to improve the diagnostic accuracy of β -thalassaemia alleles in Malaysia, to discuss the phenotypic expression when compounded with IVS 1-5 (G>C) and its epidemiological history.

Case Report

The proband, a six-year-old Malay girl from a hospital in the Northern state of Malaysia, was referred to Institute for Medical Research (IMR) with her parents' blood samples for molecular diagnosis work-up for β -thalassaemia.

Review of past medical records revealed the patient presented with generalised weakness and anorexia at the age of six to eight months

old. On clinical examination, the patient was pale with expanded abdominal girth. Initial complete blood count (CBC) showed marked hypochromic microcytic anaemia with few nucleated red cells on the peripheral blood smear. Haemoglobin typing using high-pressure liquid chromatography (HPLC) performed on index case showed predominance of HbF, normal amount of HbA₂ and virtual absence of HbA. There was no abnormal peak on the haemoglobin-chromatograph. In view of these findings, a provisional diagnosis of β -thalassaemia major was then made and the patient was put on regular blood transfusion. Subsequent CBC and haemoglobin typing analysis on the referred blood samples are shown in Table 1. The parents' blood counts showed characteristic elevation of red cell counts, and hypochromia and microcytosis. Paternal and maternal HPLC chromatogram showed the hallmark elevation of HbA₂ suggestive of heterozygous β -thalassaemia. The family pedigree is shown in Figure 1.

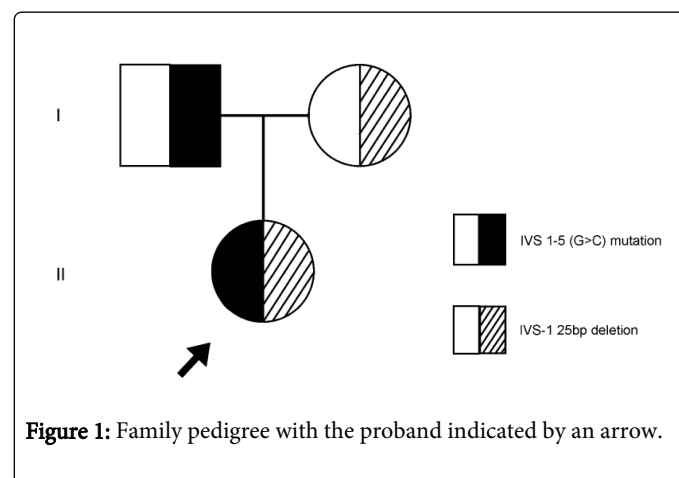


Figure 1: Family pedigree with the proband indicated by an arrow.

| Parameters | Patient | Mother | Father |
|---------------------------|---------------------|---------------------|---------------|
| Age (years) /sex | 6/F | 33/F | 38/M |
| Hb (g/dL) | 5.5 | 10.6 | 12.8 |
| RBC (106/ μ L) | 2.6 | 6.1 | 6.5 |
| Hct (%) | 18.3 | 35.0 | 41.2 |
| MCV (fL) | 70.7 | 57.1 | 63.4 |
| MCH (pg) | 21.2 | 17.3 | 19.7 |
| MCHC (g/dL) | 30.1 | 30.3 | 31.1 |
| RDW-CV (%) | 33.6 | 18.9 | 18.9 |
| HbA2 (%) | 2.8 | 5.6 | 6.6 |
| HbF (%) | 89.5 | 0.8 | 0.8 |
| β Filipino deletion | ND | ND | ND |
| β MARMS | IVS I-5 (G>C) | ND | IVS I-5 (G>C) |
| HBB gene sequencing | IVS-1 25bp deletion | IVS-1 25bp deletion | NT |

Hb: Hemoglobin; RBC: Red Blood Cell; Hct: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW-CV: Red Cell Distribution Cell-Coefficient Of Variation; ND: Not Detected; NT: Not Tested

Table 1: Haematological findings from the referred hospital and molecular analysis of the family.

Genomic DNA from both the parents and proband was extracted from whole blood samples using the protocol of QIAamp DNA Blood Midi Kit (Qiagen GmbH, Hilden, Germany). Genomic DNA from the trio was tested using polymerase chain reaction (PCR) based multiplex gap (M-Gap) and multiplex amplification refractory mutation system (MARMS) methods for the detection of Beta Filipino deletion and the following β -globin gene point mutations; -29 (A>G), -28 (A>G), -86(C>G), -88 (C>T), Cap+1 (A>C), Initiation Codon (ATG>AGG), Codon 8/9 (+G), Codon 15 (G>A), Codon 16 (-C), Codon 17 (A>T), Codon 19 (A>G) Malay, Codon 26 (G>A) HbE, IVS 1-1 (G>T), IVS 1-1 (G>A), IVS 1-5 (G>C), Codon 41/42 (-TTCT) Codon 43 (G>T), Codon 71/72 (+A), IVS 2-654 (C>T) and Poly A (A>G) [5,7].

PCR amplified of the entire β -globin gene was done to generate an amplicon size of 2020 basepairs for direct DNA sequencing. The amplicon was checked using gel electrophoresis (1.2% w/v agarose gel) for 60 mins at 70 volts. Cycle sequencing of the purified PCR products were done using the BigDye[®] Terminator v3.1 cycle sequencing kit and the sequences were studied in an ABI 3730XL DNA Analyser (Applied Biosystems, Foster City, CA, USA). The sequencing data were analysed using CLC Main Workbench (CLC Bio, Aarhus, Denmark). Sequence analysis was done using the accession NG_000007.3 from the National Center of Biotechnology Information (NCBI) as the reference sequence.

The initial molecular workup using polymerase chain reaction (PCR) based multiplex gap (M-Gap) and multiplex amplification refractory mutation system (MARMS) methods on mother's DNA was unrevealing for any β -thalassaemia alleles [5,7]. However, the index case and the father were heterozygous for IVS 1-5 (G>C) mutation. To further comb the β -globin gene for any other mutation or deletion not covered by the MARMS method, direct DNA sequencing was done on the index case and the mother. In both cases, the sequencing result

revealed a 25bp deletion involving the 3' end of IVS-1 extending to the invariant AG nucleotide of the acceptor splice site to two or three nucleotides in exon 2 (Figure 2).

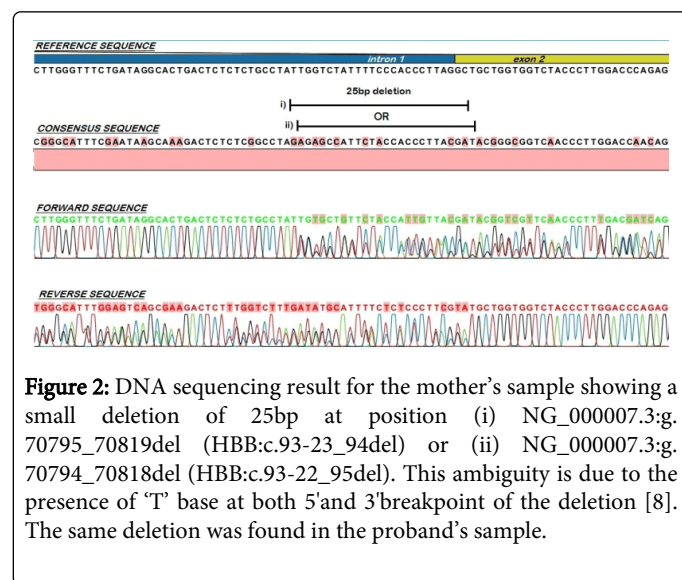


Figure 2: DNA sequencing result for the mother's sample showing a small deletion of 25bp at position (i) NG_000007.3:g.70795_70819del (HBB:c.93-23_94del) or (ii) NG_000007.3:g.70794_70818del (HBB:c.93-22_95del). This ambiguity is due to the presence of 'T' base at both 5' and 3'breakpoint of the deletion [8]. The same deletion was found in the proband's sample.

Direct DNA sequencing on the proband's sample also reconfirmed the IVS 1-5 (G>C) demonstrated by the MARMS method. The PCR analysis and DNA sequencing findings led to the molecular confirmation of $\beta^{IVS-1\ 25bp}/\beta^{IVS\ 1-5(G>C)}$ compound heterozygous β -thalassaemia major in the index case, and heterozygous β -thalassaemia in the mother and father with alleles IVS-1 25bp deletion and IVS 1-5 (G>C) mutation respectively.

Discussion

β -thalassaemia arising from deletions are infrequent and to the best of our knowledge, this is the first case of IVS-1 25bp deletion reported from Malaysia. The 25-nucleotide deletion involving the IVS-1 acceptor splice site in β -globin gene is characterized by removal of the invariant AG dinucleotide [8]. The RNA derived from this gene is useless as a messenger for β -globin gene synthesis as it completely abolishes the normal splicing at the IVS-1 site. Consequently, the allele gives rise to β^0 thalassaemia phenotype [2]. Although 25bp deletion was first described in an Asian Indian [8], Varawalla et al. reported that the deletion is a rare allele in Asian Indians accounting to 0.4% [9]. The high incidence of IVS-1-3' end 25bp deletion has a more localized distribution across the littoral populations and ethnic groups bordering Persian Gulf with highest being reported in Bahrain accounting for 36% of its β -thalassaemia chromosomes studied [10]. Within the region, high prevalence was also observed in Saudi Arabia, UAE, Kuwait and Fars of Iran.

IVS 1-5 (G>C) mutation, on the other hand, is globally a ubiquitous β -thalassaemia allele common in Malaysia and regional countries [4-6]. This consensus sequence mutation reduces the efficiency of 5' normal splicing without completely abrogating it. Hence, the allele gives rise to β^+ thalassaemia phenotype [2]. Consequently, compound heterozygous state for IVS-1 25bp deletion and IVS 1-5 (G>C) mutation in the index case, lead to severe transfusion dependent clinical phenotype with a genotype of β^0/β^+ .

The 25bp deletion in Bahrain tends to exist on two different haplotypic backgrounds. At significantly different frequencies; haplotype IX is more frequently in linkage disequilibrium with the deletion than haplotype I, as designated by Orkin and Kazazian et al. [11]. However, their existence on the same framework thus indicates a single common and recent Mediterranean origin. Furthermore, it was postulated that this deletion originally occurred on the chromosome of haplotype IX and later spread to the haplotype I chromosome.

To determine the haplotype associated with IVS-1-3' end 25bp deletion in the Malaysian, father-mother-child pedigree approach was used using single nucleotide polymorphic (SNP) markers (rs3759070, rs113425530, rs28440105, rs10128556, rs968857, rs10768683, rs10837631) along the length of β -globin gene cluster. The deletion in the Malaysian trio tends to be in linkage disequilibrium with sequence-haplotype (G G A T A G T) which has a close resemblance to RFLP-haplotype IX [11]. Since, haplotype IX reported bearing the deletion has already been reported in Bahrain, Saudi Arabia and Kuwait, and its strong concordance with sequence haplotype observed in the index case, this deletion in the family probably has a Middle East origin.

This above observation is consistent to the subtle globalisation with increased number of immigrants from endemic areas settling as non-citizens of Malaysia; especially in the forms of 'wives of Malaysians' and 'husbands of Malaysians'. Intuitively, miscegenational marriages and racial heterogeneity of the immigrant population in an endemic country such as Malaysia changes and increases the epidemiological spectrum of β -thalassaemia alleles within different ethnic groups making the provision of molecular diagnosis more demanding and challenging.

Expanding demographic boundaries of thalassaemia genes due to bi-directional population migration introduces of new alleles to communities that never had it before. This results in disrupting the distinctive thalassaemia allele heritages within defined ethnic groups

and further complicating the laboratory diagnostics. Although it is not justifiable to include these newfound rare thalassaemia alleles in routine β -thalassaemia screening panels for any given ethnic group, it is important to disseminate the knowledge of contemporary gene movement patterns amongst the relevant clinicians for proper management and for effective counselling towards reproductive planning especially for immediate families.

Since it is not possible to widely use DNA sequencing for molecular diagnosis of β -thalassaemia, sensitive techniques such as high-pressure liquid chromatography (HPLC), capillary electrophoresis (CE) or isoelectric focusing (IEF) should be done available as first-line tools for thalassaemia screening to discover cases similar to this. Wherever possible, single-plex gap PCR can be optimised to detect the 25bp deletion by amplification of the region between exon 1 and exon 2 of β -globin gene, which results in the additional smaller band with missing of 25bp nucleotides when run on gel electrophoresis.

The dynamic of emerging allele of simple Mendelian genetic disorder typically mean that they impose relatively low selective cost on the ethnic group and population at large; and could be expunged in a population with less genetic drift. As intuition would suggest, the fixation probability of this allele in a given ethnic group in the absence of selection would be extremely low. However, if this allele was introduced into a small effective population size, the probability of reaching conspicuous frequency through genetic drift would be high. As it was observed in Bahrain and neighbouring countries, low diversity of 25bp deletion accompanied by relatively high frequency of the gene is highly suggestive of recent positive selection.

Conclusions

We reported the first case of IVS-1 25bp deletion at the β -globin gene that occurs among Malaysian. The physical presentation of the index patient and the parents has described. We believed this rare mutation came from the Middle East region based on the haplotype analysis findings and the high frequency for the mutation in Bahrain. The occurrence of this rare mutation in Malaysia may result from the intermarriage between Malaysian and people from other different region in the world. Therefore, the molecular diagnosis of rare and emerging thalassaemia alleles in Malaysia can be challenging in the future.

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