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β-Thalassemia Caused by *De novo* Mutation in the *β-globin* Gene Identified in Two Bangladeshi Families

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Abstract

De novo mutations represent a unique example of how rare mode of inheritance of genetic variations can influence the onset of genetic diseases. Most of the mutations causing β- thalassemia in Bangladeshi population were found to be inherited germ line. The study aimed to present two cases of *De novo* mutations of the *β-globin* gene causing β thalassemia. Out of one hundred Bangladeshi β-thalassemia carrier families who were advised for both pre and postnatal molecular characterization of the *β-globin* gene to detect the mutation, two cases of *De novo* mutations were identified in two Bangladeshi families, which is the first of this type of mutation inheritance in Bangladesh. Mutations were determined by sequencing of the entire *β-globin* gene by Sanger method. In one family as case A, the foetus proband in the amniotic fluid was found homozygous for the mutation in the codon 26 (G>A) (G>A) [HBB: c.79G>A] and was diagnosed as the β-thalassemia major. The paternal allele was normal and only the mother carried one mutant allele. In another family as case B, the proband in the blood of the affected child was found homozygous for the mutation IVS-I-5 (G>C) [HBB: c.92+G>C]. The father did not carry any mutation while the mother was heterozygous for the mutation. In both cases, the possibility of non-paternity was excluded by using STR-based parentages testing, indicating that the acquired mutation in the probands was the result of a *De novo* event. The study demonstrates the significance of the prenatal diagnosis of β-thalassemia by DNA sequencing to detect novel, rare or *De novo* mutation that cannot be identified during screening by haemoglobin electrophoresis.

Keywords: *De novo* mutation; β-thalassemia; DNA sequencing; Proband; Haemoglobin electrophoresis

Introduction

β-thalassemia is one of the most common single-gene autosomal recessive disorders. The epidemiological data suggest a high frequency of β-thalassemia in Mediterranean and South-east Asian population [1]. Mutations in the coding or regulatory region of the *β-globin* gene result in reduced or complete loss of *β-globin* synthesis causing β-thalassemia. The phenotypic severity of the disease may vary depending on the particular type of genetic mutations the patient harbours. Three different clinical and haematological conditions categorize thalassemia: β-thalassemia minor, β-thalassemia major, and the β-thalassemia intermedia. β-thalassemia minor individuals are asymptomatic and apparently lead a healthy life. Individuals with β-thalassemia major, on the other hand, develop severe anaemia and require regular blood transfusions associated with chelating therapy to treat iron overload. Individuals in whom the anaemia is not so severe as to necessitate regular transfusions are said to have β-thalassemia intermedia [2,3]. There are more than 200 documented mutations involving *β-globin* gene [4]. In Bangladesh, about 4% of the populations, are carriers of β thalassemia and consistently 5 to 7 mutations of β-thalassemia have been reported so far [5]. Since β-thalassemia is an autosomal recessive disorder; both alleles of the *β-globin* gene must be mutated. Usually, a child with β-thalassemia major inherits mutated alleles from both the parents and is homozygous for the respective mutation. However, in the event of a *De novo* mutation, the affected child acquires a homozygous mutation in his/her *β-globin* gene despite the maternal and paternal alleles being either normal or heterozygous for the mutation. β thalassemia cases caused by *De novo* mutation have been reported in some studies [6-10]. Prenatal diagnosis of β-thalassemia is essential to identify mutations responsible for the disease and carrier persons as the strategic approach for the management of the disease and prevention of emergence of new cases in the Bangladeshi population. However, accurate and reliable identification of mutations might not be accomplished if advanced molecular methods are not applied. Moreover, Rare, compound heterozygous or *De novo* mutations might remain undetected that lead to β-thalassemia. Most of the *β-globin* gene mutations causing β thalassemia so far have been identified in Bangladesh were documented as inherited germ line. Compound heterozygosis for the rare *β-globin* gene mutation, in a Bangladeshi patient has also been detected [11]. However, there was no report of *De novo* mutation in Bangladesh causing β-thalassemia. This study describes two cases of *De novo* mutations that caused β thalassemia in two individuals, identified first time in Bangladesh.

Methodology

One hundred Bangladeshi families, where any or both of the parent were diagnosed as the carrier of β-thalassemia and in many families having affected child, were referred to the DNA Solution Limited (Molecular Diagnostic Institute), by the specialist physician for the molecular characterization of *β-globin* gene to detect mutations both pre and postnatal. All procedures followed in the present study were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the individual and the institutional ethical review committee approved the study protocol. Peripheral blood samples were collected from the parents and affected child. Haemoglobin electrophoresis was performed using both isoelectric focusing and high performance liquid chromatography with similar results. The amniotic fluid was collected from the women trans-abdominally using recommended protocol. Genomic DNA was extracted from the biological samples using the Qiagen genomic DNA extraction kit (Qiagen, Germany). Molecular analysis was performed by sequencing of the entire coding region (Exon I, II and II), part of intron I and 2, promoter region and splice junction site of the beta globin gene. Firstly, the genomic DNA extracted from the peripheral blood and the amniotic fluid was amplified by using the polymerase chain reaction (PCR) method. The PCR products were run in 2% agarose gel, and the bands were visualized using the Bio View Trans illuminator from Bio Step. Sequencing reaction was carried out by Big Dye terminator cycle sequencing kit (Applied Bio systems, USA). Analysis was performed by automated capillary electrophoresis in the 3500 genetic analyzer (Applied Bio systems, USA). The sequences obtained from the capillary electrophoresis were then aligned using the Reference Sequence of the *β-globin* gene [NCBI Ref Seq entry of HBB (NG_000007.3)] in the Seqscape sequence alignment software version 2.5 (Applied Bio systems, USA). The observed genetic variants were then analyzed for their clinical significance in the HbVar database of haemoglobin variants and thalassemia mutations [12]. To explore the possibility of non-paternity of the probands with their alleged parents were tested using classical STR based DNA typing method. Briefly, the DNA test was performed using PowerPlex® 16 HS STR based PCR amplification kit (Promega Inc., USA). A total of 15 autosomal STR loci plus one sex typing marker, amelogenin, were amplified by PCR. The PCR products were separated on ABI Prism® 3500 Genetic Analyzer (Applied Bio systems, USA). Data was analyzed using Gene Mapper®IDsoftware (Applied Bio systems, USA). The probability of paternity was calculated by using Geno Proof® 3 Software (Qualitype GmbH, Dresden, Germany).

Results

In one case (Case A), the proband was found to carry the homozygous mutation in the HbE codon 26 while only the maternal allele was heterozygous for the HbE codon 26. In the second case (Case B), the proband was found to carry a homozygous IVS1-5 mutation while the paternal allele was normal and the maternal allele was heterozygous for the mutation.

Case A

A 22 years old woman was diagnosed as HbE trait during her routine check-up. The haemoglobin electrophoresis showed that her HbA was 71.3%, HbA_2 3.45% and HbE 25.3% respectively. Her husband, a 30 years old man had a normal phenotype. Both the parents were clinically healthy. There was no history of unexplained anaemia or blood transfusion in the family. Few months later, the woman conceived and was recommended for prenatal diagnosis of beta-thalassemia at the 16 weeks of gestation. The sequencing of beta globin gene showed that the mother was heterozygous for HbE codon 26 (G>A) mutation. Though the father did not carry any pathogenic mutation the, foetus showed homozygous G>A mutation in the HbE codon 26 which is indicative of HbE disease (Figure 1). The relationship test proved that the couple was the real biological parent of the foetus thereby confirming the *De novo* nature of the mutation in the foetus. The probability of paternity score was 99.99999% (Table 1).

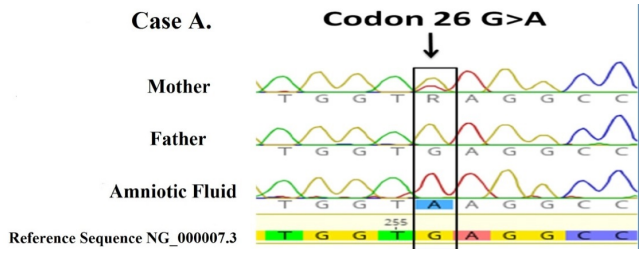

Figure 1: *De novo* mutation in the HbE codon 26. The genotype status of codon 26 in mother (heterozygous), Father (non-pathogenic) and amniotic fluid (foetus, homozygous) are shown in vertical box indicated by the arrow.

Genetic loci tested	Putative Father		Mother		Affected Foetus	
D3S1358	15	16	15	18	15	15
TH01	9	9.3	8	9.3	9	9.3
D21S11	29	30	30	32.2	30	32.2
D18S51	13	16	14	17	13	14
Penta E	12	15	7	7	7	15
D5S818	11	12	10	12	11	12
D13S317	8	12	11	13	12	13

D7S820	11	12	11	12	11	12
D16S539	11	12	10	12	12	12
CSF1PO	12	13	10	10	10	13
Penta D	9	10	12	12	10	12
vWA	16	18	16	19	18	19
D8S1179	12	13	13	14	13	13
TPOX	8	8	10	11	8	10
FGA	19	20	22	23	20	23
AMEL	X	Y	X	X	X	X
Probability of Paternity (W): 99.9999800184%						

Table 1: Comparison of DNA profiles of the foetus and the respective parents.

Case B

A two years old child was diagnosed with beta thalassemia based on haematological parameters. Her mother was suggestive of having β-thalassemia trait but the father was healthy based on haemoglobin electrophoresis result. The couple already had an affected child and was expecting another baby. Haemoglobin electrophoresis report showed that the proband has abnormal electropherogram, suggestive of β-thalassemia. The father's 139 electrophoresis result was normal and the mother's haemoglobin electrophoresis showed 5.6% 140 of HbA_2, indicative of β-thalassemia trait. The sequencing result showed that the mother was heterozygous for IVS-I-5G>C mutation. The father did not carry any pathogenic mutation. The foetus showed heterozygous IVS-I-5G>C mutation and the affected child showed homozygous IVS-I-5G>C mutation, which is indicative of β-thalassemia (Figure 2). The relationship test proved that the couple is the actual biological parent of the proband, thereby confirming the *De novo* nature of the mutation in the affected child. The probability of paternity score was also 99.99999% (Table 2).

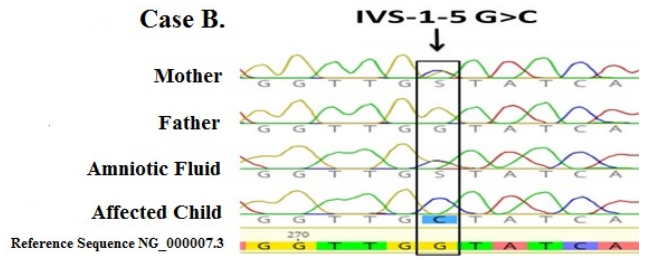

Figure 2: *De novo* mutation in IVS-I-5. The genotype status of codon 26 in mother (heterozygous), Father (non-pathogenic) and amniotic fluid (foetus, homozygous) are shown in vertical box

Genetic loci tested	Putative Father		Mother		Affected child	
D3S1358	15	15	15	16	15	16
TH01	9.3	9.3	8	9	8	9.3
D21S11	29	32.2	32.2	33.2	29	32.2
D18S51	15	17	14	16	15	16
Penta E	5	11	11	12	11	11
D5S818	11	11	13	13	11	13
D13S317	9	12	11	12	9	12
D7S820	8	8	11	12	8	12
D16S539	9	12	11	12	9	12
CSF1PO	11	13	10	12	11	12

Penta D	9	13	10	10	10	13
vWA	16	19.2	14	17	14	16
D8S1179	14	17	13	16	13	17
TPOX	8	9	9	11	8	11
FGA	20	21	21	24	20	24
AMEL	X	Y	X	X	X	Y
Probability of Paternity (W): 99.9999994473%						

Table 2: Comparison of DNA profiles of the affected child with the respective parents.

Discussion

β-thalassemia is a genetic condition results from decreased or absence of production in the β chain of haemoglobin. Mutations in the *β-globin* gene have been shown to regulate the production of *β-globin* chain. So far, 1265 haemoglobin variants are reported in the updated HbVar database [12]. These mutations can occur in any of the three exons or the regulator region of the *β-globin* gene located in the chromosome 11.

The β-thalassemia is predominantly an inherited autosomal recessive disorder with mutated alleles from both parents contributing to the homozygous diseased status of the affected child. However, in some populations it is reported that *De novo* mutations can also cause β-thalassemia.

This study identified *De novo* mutations in the *β-globin* gene in two cases of two Bangladeshi that caused β-thalassemia. *De novo* mutations in the *β-globin* gene were reported to be the cause of β-thalassemia in several other studies [6-10]. However, this type of mutation causing β-thalassemia was identified for the first time in Bangladesh. In the first case, the proband was a foetus that carried a homozygous mutation in the HbE codon 26.

The mother was heterozygous for the same mutation and the father did not carry any pathogenic allele as found from the direct sequencing of the *β-globin* gene. This *De novo* mutation is well documented to be causal agent for HbE disease. HbE is one of the most frequently studied variants. The GAG>AAG mutation at codon 26 is associated with a β-thalassemia phenotype; the βE synthesis is less than expected because the formation of functional βE-mRNA is decreased due to abnormal alternative splicing of precursor β-mRNA at a site 5/ to the IVS-I.

In another case, we found that although only the mother was heterozygous for the IVS-I-5 mutation; the child carried homozygous mutation at the same locus suggesting that the other allele is mutated *De novo*. The individual positive for both copies of the IVS-I-5 (G>C) mutation is indicative of severe β-thalassemia. The mutation is a G>C change at position 5 of intron 1. It interferes with *β-globin* mRNA splicing and dramatically reduces the synthesis of the β chain. The IVSI-5 (G>C) mutation is associated with severe β+thalassemia. The affected child in this study was transfusion dependent.

The second expected baby of the same parent is a carrier of β-thalassemia as the foetus was found to be heterozygous for this mutation. In both cases, the *De novo* nature of the somatic mutation was proved by excluding the possibility of non-paternity among the studied individuals. In conclusion, *De novo* mutations in *β-globin* gene represent another mechanism through which β-thalassemia can occur in the Bangladeshi population and should be monitored carefully.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

1. Origa R (2017) β-Thalassemia. Genet Med 19: 609-619.
2. Danjou F, Anni F, Galanello R (2011) Beta thalassemia: From genotype to phenotype. Haematologica 96: 1573 -1575.
3. Pignatti BC, Rugolotto S, Stefano DP, Zhao H, Cappellini MD, et al. (2004) Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 89: 1189-1193.
4. Baysel E, Carver MFH (1995) The beta- and delta-thalassemia repository (Eighth Edition). Hemoglobin 19: 213-236.
5. Waqar K, Banu B, Amin SK, Selimuzzaman M, RahmanM (2005) Prevalence of β thalassemia trait and Hb E trait in Bangladeshi school children and health burden of thalassemia in our Population. Dhaka (Shishu) Hosp J 21: 1-7.
6. Beris P, Darbellay R, Speiser D, Kirchner V, Miescher PA (1993) De novo initiation codon mutation (ATG→ACG) of the beta-globin gene causing beta-thalassemia in a 210 Swiss family. Am J Hematol 42: 248-253.
7. Croteau SE, Luo HY, Lehmann LE, Chui DH, Neufeld EJ (2013) Novel dominant beta thalassemia: Hb Boston-Kuwait [codon 139/140(+T)]. Pediatr Blood Cancer 60: E131-E134.
8. Kanathezhath B, Hazard FK, Guo H, Kidd J, Azimi M (2010) Hemoglobin Hakkari: An autosomal dominant form of beta thalassemia with inclusion bodies arising from De novo mutation in exon 2 of beta globin gene. Pediatr Blood Cancer 54: 332-335.

9. Waye JS, Eng B, Patterson M, Barr RD, Chui DH (1997) De novo mutation of the beta-globin gene initiation codon (ATG →AAG) in a Northern European boy. Am J Hematol 56: 179-182.
10. Rojnuckarin P, Settapiboon R, Vanichsetakul P, Sueblinvong T, Sutcharitchan P (2007) Severe β0 thalassemia/hemoglobin E disease caused by De novo 22-base pair duplication in the paternal allele of beta globin gene. Am J Hematol 82: 663-665.
11. Hasan KN, Khaleque MA, Majumder AK, Rahman MM, Akhteruzzaman S (2016) Identification of compound heterozygosity for rare beta-globin gene mutation, codon 15 (-T) with a common mutation IVS1-5 (G>C) by direct sequencing in a Bangladeshi patient. Biorescomm 2: 245-248.
12. Belinda G, Joseph B, Emmanouil V, Cristiana P, Kamran M, et al. (2014) Updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res 42: D1063-D1069.