CASE REPORT

Revisiting a Complex Rearrangement Involving a 619 Base Pairs Deletion, 6 Nucleotide Insertion Followed by a A > G Substitution Causing β^0 -Thalassemia

Samira DabbaghBagheri³ · Shirin Ghadami^{1,2} · Faeze Mollazadeh³ ·
Ameneh Saadat³ · Sirous Zeinali^{1,3}

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Abstract One of the prevalent inherited blood disorders is thalassemia syndrome that characterized by reduction (β^+) or absence (β^0) of β globin chain synthesis. The β globin (HBB) gene map in the short arm of chromosome 11 and most of the mutations in this gene are single nucleotide substitutions, insertions or deletions of nucleotides. Nucleotide sequence analysis of a partially deleted β -globin gene from an Iranian carrier of β -thalassemia displayed a complex rearrangement involving a 619 base pairs (bp) deletion. This rearrangement had originally been named as the 619 bp deletion and later on as the 619 bp deletion with a 7 bp insertion. In our study, using by single chain sequencing, we have shown that the actual rearrangement involves a 619 bp deletion, a 6 bp insertion followed by a G > A substitution deleting the exon 3 of the β -globin gene. This clarification has to be inserted into the relevant databases as some of them still site the original 619 bp deletion with wrong breakpoints.

Keywords β -thalassemia · HBB gene · 619 bp deletion · Mutation

Introduction

β -thalassemia is the most common autosomal recessive disease in the world, particularly in Middle Eastern countries [6, 7, 10]. It results due to deficiency (β^+) or loss of (β^0) synthesis of the β -globin chain [1, 4]. Mutations in this gene cause three clinical and hematological pathogenic states, β -thalassemia (β -thal) carrier, intermedia, and major. It is estimated that there are nearly 80–90 million carriers of β -thal worldwide [4]. The prevalence of carriers in Iran is estimated to be between two and three million with a broad spectrum of mutations [16].

β -thal are heterogeneous at the molecular level and can be caused by more than 200 different mutations in the β -globin gene, mostly as single nucleotide substitutions, deletions or insertions [1, 8]. In rarer cases it is caused by deletions or insertions, which some may be complex like Hb Jambol [2]. One such deletion is so called 619 bp deletion. This mutation is more common in Indian population [11, 21] expanding to Pakistan [5, 9, 20] and rarely seen in Iran [13, 17, 18]. Its frequency can reach up to 50 % in some Indian population [21]. This deletion was first reported in 1979 by restriction endonuclease mapping [14]. It was later characterized by sequencing as being a 619 bp-deletion with a heptanucleotide insertion (AAGTAGA) between the breakpoints [19]. We reanalyzed this mutation in an Iranian patient and confirmed that the start of the breakpoint is as claimed [15] but the deletion is only six nucleotides followed by a G > A substitution.

✉ Sirous Zeinali
Zeinalipasteur@yahoo.com

¹ Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Pasteur St., Tehran, Iran

² Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

³ Medical Genetics Lab, Kawsar Human Genetics Research Center, No. 41 Majlesi St., ValiAsr St., Tehran, Iran

Materials and Methods

Sample Preparation

A peripheral blood sample (2 ml) was drawn from a carrier of β^0 -thalassemia after completion of informed consent form. Genomic DNA was extracted by a standard salting out method [12].

Primer Design and PCR Amplification

First, three pairs of primers were designed according to a gap-PCR protocol to detect the 619 bp-deletion [3].

Primers were designed using Gene Runner v 5 (<http://www.generunner.net/>) and primer 3 (v. 0.4.0) softwares [21]. Each oligonucleotide primer was examined for specific amplification by nBLAST software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Primer sequences are listed in Table 1. PCR were performed according to the protocol given by Faa et al. [3]. The PCR product was electrophoresed and the smaller band was gel extracted using

KBC PCR purification kit (KBC, Tehran, Iran). Sequencing was performed using Big Dye terminator kit [Life Technologies, USA, LT). Sample was run on ABI 3130XL Genetic Analyzer [LT).

Sequences were analyzed using Gene Runner software.

MLPA

Multiplex ligation-dependent probe amplification (MLPA) was carried out as described previously [22, 23].

Result and Discussion

MLPA results indicated a deletion in the second intron of the β -globin gene. This deletion was identified as the 619 bp deletion by DNA sequencing, based on MLPA analysis by HBB ex3 11884-L12684 and HBB ex3 11885-L13080 probes which are in exon 3 HBB gene. We used the data from the HbVar (<http://globin.bx.psu.edu/hbvar/menu.html>), UCSC (<https://genome.ucsc.edu/>), lovd (<http://www.lovd.nl/3.0/>) databases and the sequence base position based on HbVar data, to design a gap-PCR primer set. After amplification we did not obtained the right band size. We then moved the reverse primer toward the 3' of the gene. Two different band sizes were observed. The shorter band was gel purified and was subjected to Sanger sequencing. Comparison of this sequence with reference DNA sequence from a normal β -globin gene showed a deletion of 619 nucleotides which extended position

Table 1 Primer sequences used in this study

Primers	Amplicon length (bp)
Forward primer of the normal sequence: GTACACATATTGACCAAATCAGG	796
Forward primer of the 619 deleted sequence: GTACACATATTGACCAAATCAGG	291
Common reverse primer: CCTCACCTTCTTTCATGGAG

Fig. 1 The sequence alignment of small part of β -globin sequences, between β^0 -thalassemic patient and normal sequence of β -globin gene

Fig. 2 The sequence chromatogram of the NG_000007.3:g.71609_72227del619 G > A variant

chr11:5225257-5225875 nucleotides bases (Genome assembly GRCh37/hg19: Feb. 2009) (Fig. 1).

We also noted that there was an insertion of a hexa nucleotide (AAGTAG) followed by a G > A substitution at the end of the sequence (Fig. 2). In the previous reports, a 619 bp deletion followed by an insertion of a heptanucleotide (AAGTAGA) had been given [15, 19]. Our finding clearly showed that the above report is more accurate than the original one [15] but still lacks clear definition of the molecular makeup of the mutation. Some of the major databases (i.e. Globin Gene Server or HbVar (<http://www.globin.cse.psu.edu>); UCSC genome browser; lovd, (<http://lovd.bx.psu.edu/>) and ensemble, (<http://www.ensembl.org/>) still provide wrong break point information for the 619 bp del. According to the results presented here, the exact domain of this β^0 -thal deletion is from hg19: chr11 5225257-5225875 GRCh37.p13) (NG_000007.3:g.71609_72227del619 as shown by the HbVar).

In this report, we clarified an error published in the previous studies regarding the exact molecular bases of the deletion and insertion related to the 619 bp del.

This clarification may be important when analyzing the β -thalassemia molecular results.

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