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ORIGINAL PAPER

A novel β-globin gene CAP site mutation in association with the 92+1G>A mutation was found in a thalassemia intermedia patient

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Abstract

This paper describes a novel β-thalassemia mutation 3 base pairs downstream of the CAP site of the β-globin gene, -48A>T. The proband, an 11-year-old Romanian girl, is a compound heterozygote for this mutation and the common 92+1G>A β(0) thalassemia mutation. She has a mild thalassemia intermedia phenotype and is transfusion independent. Her mother (N/92+1G>A) has total hemoglobin levels of 11.1 g/dl, while the proband's father (N/-48A>T) has normal hematological indices. These data indicate that this novel CAP site mutation may play a role in the phenotypic expression of the disease in this case.

Keywords: HBB GENE, THALASSEMIA, 5'-UTR MUTATION

Introduction

Although β-thalassemia is a monogenic disease, its underlying genetic basis is often complex, reflected by a large spectrum of phenotypes. In terms of severity of disease, thalassemia intermedia lies between asymptomatic carriers and fully symptomatic patients. A comprehensive review of the factors affecting disease severity for thalassemia intermedia is provided elsewhere (WEATHERALL [1]). As expected, the primary determinant of the mildness of the intermedia phenotype is the combination of contributing *β-globin* mutations. These are very heterogeneous and can range from silent mutations which are asymptomatic, even in the homozygous condition, to dominant mutations which are symptomatic even in heterozygous state (THEIN [2]).

The battery of thalassemia mutations has been extensively characterized and approximately 400 thalassemia mutations have been identified so far (R.C. HARDISON & al. [3]). Here we report a novel CAP site mutation co-inherited with 92+1G>A mutation in a case of β-thalassemia intermedia in a Romanian patient. The proband has HbA2 levels of 4.9%, total Hb levels of 8.5g/dl and MCV of 63.9fl. Molecular testing of the *β-globin* gene revealed an unknown Denaturing Gradient Gel Electrophoresis (DGGE) pattern and DNA sequencing identified a previously unreported -48A>T mutation. Subsequently, several members of the family were investigated and the proband's father was found to be a simple heterozygote for the -48A>T mutation. However, he appears to be hematologically normal and shows no evidence of a β-thalassemia carrier status.

The heterozygous state for this 5'UTR mutation, therefore, does not affect hematological indices (normal hemoglobin levels with minimal changes in red blood cell

indices and HbA2 levels). However, the situation becomes clinically important when β-chain production is additionally impaired due to the presence of a β(0)-thalassemia allele. In the compound heterozygote state (β^0/-48A>T) the phenotypic severity increases resulting in a mild thalassemia phenotype but this does not require clinical intervention for survival.

Methods

DGGE screening.

Genomic DNA was extracted from peripheral blood using the QiaAmp blood DNA minikit (Qiagen, Hilden, Germany). PCR amplification of the *β-globin* gene from genomic DNA was performed using the HotStarTaq Master Mix kit (Qiagen, Hilden, Germany) in a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems, CA, USA). The sequences of the primers employed are described by Kanavakis et al (E. KANAVAKIS & al. [4], R. TALMACI & al. [5]). The *β-globin* gene was screened in four separate fragments, which cover the entire gene including the 5' and 3' untranslated regions. The PCR products were analyzed by DGGE based on the protocol described by LOSEKOOT & al. [6]. DGGE was performed at 100V for 12h using the DGGE Mutation Detection System (Bio-Rad, Ca, USA).

β-globin gene sequencing.

Sequencing of the *β-globin* gene was carried out on two different instruments: the 3100 Avant Genetic Analyzer (Applied Biosystems, CA, USA) and the CEQTM 8000 XL (Beckman Coulter, CA, USA), according to the manufacturers instructions.

ARMS PCR.

In order to verify the result, as well as for subsequent screening of patients, mutation-specific primers for Amplification Refractory Mutation System PCR (ARMS-PCR) were designed: 5' AAG TCA GGG CAG AGC CAT CTA TTG CTT AGT 3' (forward primer 5188328 - 5188301) and 5' CAT GCC CAG TTT CTA TTG GTC TCC 3' (reverse primer 5188106 - 5188129). The sequence numbers of primers in parentheses are parted of GenBank (NT_009237.18 GI:224514737). The primers were designed starting from ***Primer 3*** program available online at http://frodo.wi.mit.edu/primer3. Subsequently, the mutation-specific ARMS primer was modified to include one strong mismatch (TT) at the 3' end which base pair with mutant but not with normal DNA. To further increase the effect of destabilization, an additional weak mismatch (GG) with the target sequence is introduced at the penultimate 3' nucleotide. The size of the PCR product resulting from the amplification of the mutant allele is 200bp. The control primers used amplify a region of 861bp from 3' end of the *β-globin* gene, and were previously reported by OLD [7].

Gap-PCR for deletions *α-globin* gene mutations.

Gap-PCR for the three most common deletions *α-globin* gene mutations in the Mediterranean area: α3.7, MED I and α20.5 was carried out as described by Y.T. LIU & al. [8].

Results

The proband presented at the age of 4 years with moderate anemia, increased HbA2 levels (4.9%) with total Hb levels around 8,5 g/dl and slightly increased HbF levels (4%). Now she is 11 years old and has never been transfused.

The proband's hematological indices were consistent with a β-thalassemia intermedia phenotype. The proband's father, brother and paternal aunt were all clinically asymptomatic. The relevant hematological data are shown in Table 1. The proband's mother has a hematological profile consistent with β-thalassemia trait, while her father, brother and aunt

exhibit no hypochromia or microcytosis and have borderline-normal levels of HbA2 (3,2%; 2,2%; 3,2% respectively).

The proband's DGGE analysis showed the 92+1G>A mutation along with an unknown DGGE pattern. The proband's *β-globin* gene was subjected to sequencing from nucleotide -230 to nucleotide +1621 relative to the CAP site. This analysis confirmed that the propositus was heterozygous for the 92+1G>A mutation and an additionally identified novel mutation on the third nucleotide downstream of the CAP site, -48A>T. The presence of this molecular defect was confirmed by ARMS-PCR with an allele-specific primer complementary to this mutation (fig. 2).

In addition, the family was tested for the three most common *α-globin* gene mutations in the Mediterranean area: α3.7, MED I and α20.5 and were found to be negative.

Table 1. Hematological data of the proband and her family

Sample	**β-globin genotype**	**Detection methods**	**HbA (%)**	**HbF (%)**	**HbA2 (%)**	**RBC ($x10^{12}$/L)**	**Hb (g/dL)**	**MCV (fl)**	**MCH (pg)**
Proband	92+1G>A /-48A>T	DGGE, ARMS, sequencing	91.1	4	4.9	4.71	8.5	63.9	18
Father	N/-48A>T	DGGE, ARMS	96.8		3.2	4.91	14.4	93.7	29.3
Mother	N/92+1G>A	DGGE, ARMS	94.1		5.9	5.27	11.1	70.4	21.1
Brother	N/N	DGGE	97.8		2.2	4.87	14	86.2	28.7
Paternal aunt	N/N	DGGE	96.8		3.2	4.58	13.9	91.7	30.3

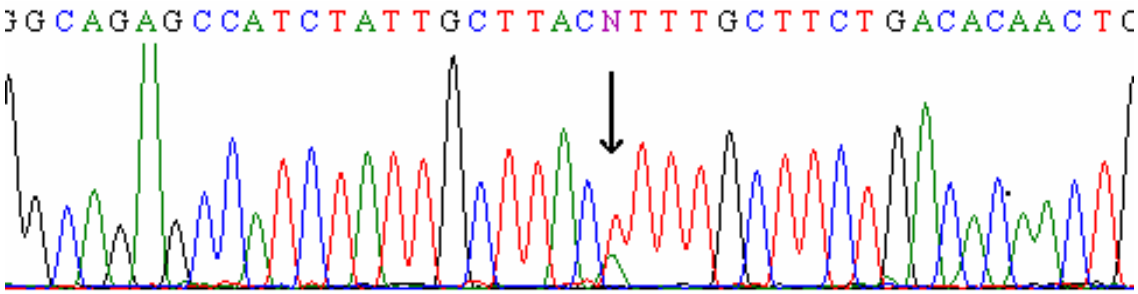
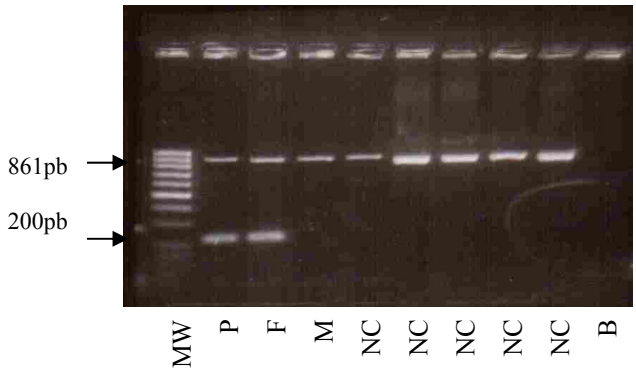


Fig. 1 Sequence results on proband genomic DNA showing the A to T mutation detected 3 base pairs downstream of the β-globin CAP site.

Este redundant 2 poze pentru acelasi rezultat



F Fig. 2 ARMS-PCR for the -48A>T(A-T) mutation. (MW – 100 bp molecular weight marker P – proband, F – father, M – mother, NC – negative control, B – blank)

Discussion

Here, a novel mutation located at CAP +3 (A-T) of the β-globin gene is reported in a patient affected by thalassemia intermedia. The patient has a hematological phenotype typical of a homozygous or compound heterozygous state for a mild β-thalassemia. Scanning methods revealed that the only abnormalities identified in the *β-globin* gene of the proband are the 92+1G>Amutation and the A-T transversion 3 nucleotides downstream of the CAP site. Secondary modification of the phenotype due to *α-globin* gene mutations was largely ruled out. The family is negative for the three most common *α-globin* gene mutations in the Mediterranean area.

A total of seven mutations in the *β-globin* gene 5'UTR region have been reported so far: c.-50A>C (C. WONG & al. [9]), -43(C>T) (S.K. MA & al. [10], N. van de WATER & al. [11]), -41delT (A ATHANASSIADOU & al. [12], P.J. HO & al. [13]), -29G>A (R. ONER & al. [14], S.P. CAI & al. [15]), -18C>G (P.J. HO & al. [16]) , -11_-7delAAAC (S.Z.HUANG & al. [17]) and -3G>C (M. de ANGIOLETTI & al. [18]). Generally, the 5'UTR region of *β-globin* gene contains silent β-thalassemia mutations which affect the level of mRNA expression. The mild degree of anemia in the proband is in accordance with this observation and it is most probable that the -48A>T mutation is the putative cause of the thalassemia intermedia phenotype. However, the transcriptional activity from the mutant *β-globin* mRNA has to be documented.

The nucleotide +3(A) from the CAP site is part of the initiator element (consensus sequence: Py-Py(C)-A+1-N-T/A-Py-Py) and an overlapping E-box (consensus sequence: CANNTG), both of which are core promoter elements (B.A. LEWIS & al. [19], B.A. LEWIS and S.H. ORKIN [20], K.M. LEACH & al. [21]) (fig. 3). There is strong evidence that the initiator element and the overlapping E-box motif along with the non-canonical TATA-like element contribute to the efficient assembly of preinitiation complex (PIC) on the *β-globin* gene (B.A. LEWIS & al. [19], K.M. LEACH & al. [21]). This mutation could further uphold the functionality of the *β-globin* initiator element.

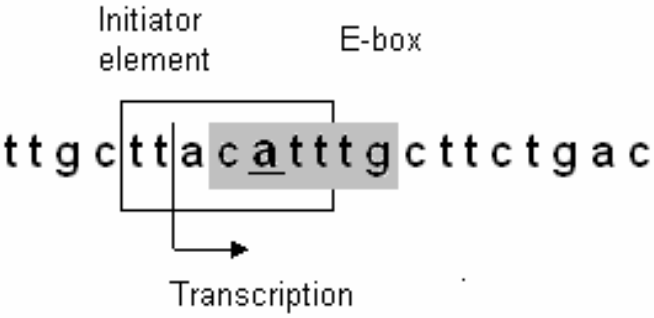


Fig. 3 Sequence of the human β-globin downstream promoter region. The open box indicates the position of initiator element (consensus sequence: Py-Py(C)-A+1-N-T/A-Py-Py). The shaded box indicates the E-box motif (consensus sequence: CANNTG)

Although in the homozygous state silent mutations result in a clinical picture of β-thalassemia trait, in combination with a severe thalassemia mutation silent mutations can lead to mild thalassemia intermedia phenotypes. However, this type of mutations occur very rarely, with the exception of the -151C>T mutation which is specific to the Mediteranean region (J.M. GONZALEZ-REDONDO & al. [22]). They still need to be properly diagnosed in order to allow specialists to offer correct genetic counseling and prenatal diagnoses.

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