Case Report

β-Thalassaemia Major in a Spanish Patient due to a Compound Heterozygosity for CD39 C → T/−28 A → C

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Received 12 October 2008; Revised 1 February 2009; Accepted 23 June 2009

Recommended by Giuseppe G. Saglio

A Spanish male patient with β-thalassaemia major was studied. Compound heterozygosity was found for one of the most common β-globin gene mutations in the Spanish population (codon 39 C → T) and for a mutation in the TATA box element of the β-globin gene promoter (−28 A → C mutation). To our knowledge this is the first report of a CD39 C → T and −28 A → C change association and the first report of the −28 A → C substitution in a Spanish patient.

1. Introduction

β-thalassaemia is a hereditary and heterogeneous group of disorders caused by mutations in the β-globin gene that result in the reduced or nonproduction of β-globin chains. The inheritance of one mutated allele is usually asymptomatic (β-thalassaemia trait), but the inheritance of two defective alleles (homozygote or compound heterozygote) would produce β-thalassaemia major (BTM) or intermedia (BTI) [1]. The severity of the symptoms of β-thalassaemia depends in part on the combination of β-globin gene mutations. However, predicting clinical phenotype based on the β-globin genotype is not always straightforward since there are other genetic factors than can ameliorate or worsen the β-thalassaemia phenotype [2–6]. Hence, clinical classification is based in the necessity to enroll a particular patient in a regular transfusion program (BTM) or not (BTI).

The mutations causing β-thalassaemia can be found in the β-globin gene exons, in the splice consensus sequences or in the transcription factor binding sites within the promoter region, resulting in a reduced efficiency of transcription initiation [1, 7]. Within each population, a small number of mutations are found. For example, in the Spanish population, a total of 86.6% of the alleles identified can be grouped into only five different mutations [8].

Here we describe a patient of Spanish descent with BTM (transfusion-dependent) who is compound heterozygous for one of the most common β-globin gene mutation in Spanish population (CD39 C → T) [8, 9] and an A to C substitution at position −28 relative to the transcription start site. This −28 mutation, first described in Kurdish Jew descendents, affects the TATA box element in the β-globin gene promoter reducing mRNA amounts [10, 11]. To our knowledge this is the first report of a CD39 C → T and −28 A → C mutations association and the first report of the −28 A → C substitution in a Spanish patient.

2. Materials and Patients

2.1. Case History. A Spanish male patient and his family were studied. The propositus is 21-year-old and was diagnosed to have β-thalassaemia major 20 years ago. He is transfusion-dependent each three weeks, to maintain a haemoglobin concentration ([Hb]) of 10 gr/dL. A blood count showed marked
Table 1: Oligonucleotide primers used in this work.

| Primers | 5’ → 3’ | Sequence (5’ → 3’ | Gene | Use |
|---------|---------|-----------------|------|-----|
| BF1     | Sense   | TCCAGGCAGAAACAGGTAGATG | β-Globin | Amplification and Sequencing |
| BF2     | Sense   | GAAGAGCCAAGGCAAGGTAC | β-Globin | Sequencing |
| BF3     | Sense   | TGGCTCACCCTGCAACCTCTC | β-Globin | Sequencing |
| BF4     | Sense   | TCAAGGCAAATGATGACAA | β-Globin | Sequencing |
| BR1     | Antisense | ATGAGCTGACCTCCCAATTTC | β-Globin | Sequencing |
| BR2     | Antisense | CCGCGGTATCCCAACCATAAA | β-Globin | Sequencing |
| BR3     | Antisense | TCACAGTGCAGCTACTCAGT | β-Globin | Sequencing |
| BR4     | Antisense | CAACCCGACAGTTACAAC | β-Globin | Amplification and Sequencing |

2.2. DNA Isolation. DNA was extracted from peripheral EDTA anticoagulated whole blood using the MagNa Pure LC automated system (Roche Applied Science, Manheim, Germany) following the manufacturer’s instructions.

2.3. β-Globin Gene Analysis. The propositus and his parents were studied for the most frequent β-thalassemia mutations in the Mediterranean area by procedures based upon real-time PCR and specific fluorescent labeled hybridization probes including: IVSI-II-745 C → G, CD5 (-CT), CD6 (-A), CD8 (-AA), CD39 C → T, CD37 G → A, IVSI-I G → A, IVSI-6 T → C, and IVSI-110 G → A [12, 13]. The full coding, the 5’ UTR, and the 3’ UTR sequences of β-globin gene (GenBank accession no. U01317) were amplified and sequenced to discard other mutations. Primers sequences are displayed in Table 1. The amplifications were performed in a 25 μL volume, containing 25 mM Cl2Mg, 200 μM of each dNTP (Promega, Madrid, Spain), 2.5 U Expand Long Template PCR System (Roche), 0.6 μM each primer, and 100 ng DNA. Amplification was performed in a PTC-100 thermal cycler (MJ Research INC, Madrid, Spain) for one cycle of 10 minutes at 95 °C and then for 35 cycles of 20 seconds at 95 °C, 20 seconds at 62 °C, and 1 minute at 72 °C, followed by one final cycle similar to the previous one but with 10 minutes at 72 °C. The PCR products were analyzed by electrophoresis.

2.4. DNA Sequencing. PCR products were purified using ExoSAP-IT (GE Healthcare, Little Chalfont, UK). The PCR fragments were sequenced by the BigDye terminator V3.1 Cycle Sequencing ready reaction system (Applied Biosystems, CA, USA) according to the manufacturer’s instructions. Sequencing primers are displayed in Table 1. Sequence analysis was performed on an ABI Prism 3100-Avant DNA sequencer (Applied Biosystems).

2.5. Analysis of α-Globin Genes as Secondary Modifiers of the β-Thalassemia Clinical Phenotype. β-thalassemia clinical manifestations could be alter by concomitant α-thalassemia or by extra copies of the α-globin genes [6, 14]. In order to evaluate this fact, the most frequent Mediterranean α-thalassemia deletions (–αβ7, –αβ12, –αMED) and the presence of extra copies of the α-globin gene were determined in the proband and in his parents by PCR-GAP [15].

3. Results and Discussion

We have studied a Spanish patient with a BTM phenotype due to a compound heterozygous genotype never published before, a β-globin exon gene mutation (C → T transition at CD39 position) and a β-globin TATA box mutation (A → C mutation in the –28 position). The first nucleotide substitution was inherited paternally while the TATA box mutation was inherited maternally. The CD39 C → T mutation is one of the most common mutations in Mediterranean countries causing up to 64% of all the Spanish β-thalassemias [9]. On the other hand, the –28 A → C mutation had been barely described in the scientific literature. The first report describing this allele was published in 1982 where two Kurdish Jews siblings with BTM were studied [10]. In a follow-up paper, the same group established that the patients were compound heterozygotes for this TATA box mutation [11]. In 1992, Basak et al. [16] studied the mutations in the β-globin gene in a group of Turkish patients exhibiting BTI and BTM. In this population the –28 A → C mutation was rare but the authors did not establish the genotype (homozygous or compound heterozygous) or the phenotype linked with the mutation. In 1996, Perea et al. [17] described a Mexican mestizo family with BTM due to a –28 A → C mutation and a CD11 –T frame shift compound heterozygosity. The TATA box mutation in this family was linked to the same haplotype as described previously for the Kurdish Jews siblings [11]. In 2005, two molecular epidemiological reports were published studying Middle East populations where the –28 A → C...
mutation appears to be a rare allele. Adekile et al. [14] described an Iraqi patient who was a compound heterozygote for the $-28$ A $\rightarrow$ C and the IVS-II-1 G $\rightarrow$ A mutations. This patient showed a BTI phenotype. The mild clinical manifestation was linked to a $-158$ C $\rightarrow$ T polymorphism of the $\gamma$-globin gene promoter with elevated Hb F together with $\alpha$-gene deletion. In the same year, Darwish et al. [18] report 1 patient harboring the $-28$ A $\rightarrow$ C mutation in homozygosis out of 148 Palestine $\beta$-thalassemia patients studied. Unfortunately, the phenotype of this patient was not mentioned. Turning to our patient, the BTM phenotype is consistent with the clinical manifestation observed in the other compound heterozygous patients described so far. Moreover, our patient and his family showed no $\alpha$-thalassemia deletions ($-\alpha^3$, $-\alpha^4$, $-\text{MED}$), extra copies of $\alpha$-globin genes, or HbF elevation. Thus, the propositus BTM phenotype could be related strictly to the $\beta$-globin mutations described here.

**Acknowledgments**

The authors thank Dr. Steven Cagas for English revision of the manuscript and an anonymous reviewer for helpful suggestions. This project was supported by Grants FIS 03/821 of the manuscript and an anonymous reviewer for helpful

**References**

[1] D. J. Weatherall and J. B. Clegg, *The Thalassaemia Syndromes*, Blackwell Science, Oxford, UK, 4th edition, 2001.

[2] I. Agouti, C. Badens, A. Abouyoub, et al., “Genotypic correlation between six common $\beta$-thalassemia mutations and the XmnI polymorphism in the moroccan population,” *Hemoglobin*, vol. 31, no. 2, pp. 141–149, 2007.

[3] C. Camaschella, A. C. Kattamis, D. Petroni, et al., “Different hematological phenotypes caused by the interaction of triplicated $\alpha$-globin genes and heterozygous $13$-thalassemia,” *American Journal of Hematology*, vol. 55, no. 2, pp. 83–88, 1997.

[4] R. Kukreti, D. Dash, K. E. Vineetha, et al., “Spectrum of $\beta$-thalassemia mutations and their association with allelic sequence polymorphisms at the $\beta$-globin gene cluster in an Eastern Indian population,” *American Journal of Hematology*, vol. 70, no. 4, pp. 269–277, 2002.

[5] T. Merghoub, B. Perichon, M. Maier-Redelsperger, et al., “Dissection of the association status of two polymorphisms in the $\beta$-globin gene cluster with variations in F-cell number in non-anemic individuals,” *American Journal of Hematology*, vol. 56, no. 4, pp. 239–243, 1997.

[6] I. Panigrahi and S. Agarwal, “Genetic determinants of phenotype in beta-thalassemia,” *Hematology*, vol. 13, no. 4, pp. 247–252, 2008.

[7] A. E. Kulozik, A. Bellan-Koch, S. Bail, E. Kohne, and E. Kleihauer, “Thalassemia intermedia: moderate reduction of beta globin gene transcriptional activity by a novel mutation of the proximal CACCC promoter element,” *Blood*, vol. 77, no. 9, pp. 2054–2058, 1991.

[8] A. Villegas, P. Rojero, F. A. Gonzalez, E. Anguita, and D. Espinós, “The thalassemia syndromes: molecular characterization in the spanish population,” *Hemoglobin*, vol. 25, no. 3, pp. 273–283, 2001.

[9] I. Moreno, G. P. Bolufer, and S. M. Perez, “The molecular changes in thalassemias in Spain. A review of existing studies,” *Medicina Clinica*, vol. 113, no. 20, pp. 789–794, 1999.

[10] M. Poncz, M. Ballantine, D. Solowiejczyk, I. Barak, E. Schwartz, and S. Surrey, “$\beta$-thalassemia in a Kurdish Jew. Single base changes in the T-A-T-A box,” *The Journal of Biological Chemistry*, vol. 257, no. 11, pp. 5994–5996, 1982.

[11] S. Surrey, K. Delgrosso, P. Malladi, and E. Schwartz, “Functional analysis of a $\beta$-globin gene containing a TATA box mutation from a Kurdish Jew with $\beta$ thalassemia,” *The Journal of Biological Chemistry*, vol. 260, no. 11, pp. 6507–6510, 1985.

[12] I. Moreno, P. Bolufer, M. I. Perez, E. Barragan, and M. A. Sanz, “Rapid detection of the major Mediterranean $\beta$-thalassemia mutations by real-time polymerase chain reaction using fluorophore-labelled hybridization probes,” *British Journal of Haematology*, vol. 119, no. 2, pp. 554–557, 2002.

[13] C. Vrettou, J. Traeger-Synodinos, M. Tzetis, G. Malamis, and E. Kanavakia, “Rapid screening of multiple $\beta$-globin gene mutations by real-time PCR on the LightCycler: application to carrier screening and prenatal diagnosis of thalassemia syndromes,” *Clinical Chemistry*, vol. 49, no. 5, pp. 769–776, 2003.

[14] A. Adekile, M. Haider, and F. Kutlar, “Mutations associated with beta-thalassemia intermedia in Kuwait,” *Medical Principles and Practice*, vol. 14, supplement 1, pp. 69–72, 2005.

[15] Y. T. Liu, J. M. Old, K. Miles, C. A. Fisher, D. J. Weatherall, and J. B. Clegg, “Rapid detection of $\alpha$-thalassemia deletions and $\alpha$-globin gene triplication by multiplex polymerase chain reactions,” *British Journal of Haematology*, vol. 108, no. 2, pp. 295–299, 2000.

[16] A. N. Basak, H. Ozcelik, A. Ozer, et al., “The molecular basis of $\beta$-thalassemia in Turkey,” *Human Genetics*, vol. 89, no. 3, pp. 315–318, 1992.

[17] F. J. Perea, M. A. Esparza, A. R. Villalobos-Arambula, B. Ibarra, and J. M. Old, “Haplotype analysis of the Mexican frameshift Cd 11 (-T) and $-28 A \rightarrow C$ $\beta$-thalassemia alleles,” *American Journal of Hematology*, vol. 51, no. 3, pp. 240–242, 1996.

[18] H. M. Darwish, F. F. El-Khatib, and S. Ayesh, “Spectrum of $\beta$-globin gene mutations among thalassemia patients in the West Bank Region of Palestine,” *Hemoglobin*, vol. 29, no. 2, pp. 119–132, 2005.