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SHORT COMMUNICATION

A Novel Mutation in the Promoter Region of the β-Globin Gene: *HBB*: c.-127G > C

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Abstract

Novel β-globin gene mutations are still occasionally being reported, especially when evaluating milder phenotypes. We report here a novel putative mutation in the promoter region of the β-globin gene and assess its clinical implications. A family, parents and four siblings, with hematological and clinical features suspected of being β-globin gene mutation(s), were involved in this study. In addition to hematological and clinical evaluations of the whole family, molecular analyses of the β-globin gene were performed by direct sequencing. Sequencing of the β-globin gene revealed a novel genomic alteration in the regulatory region of the gene. This novel genomic alteration was defined as *HBB*: c.-127G > C according to the Human Genome Variation Society (HGVS) nomenclature. Two siblings were found to be carriers of the *HBB*: c.-127G > C mutation, while the other two siblings were carriers of the codon 8 (−AA) (*HBB*: c.25_26delAA) deletion of the β-globin gene. The mother was a compound heterozygote for the codon 8 and *HBB*: c.-127G > C mutations. Based on hematological and clinical evaluations, we conclude that this novel β-globin gene promoter region change would be associated with a mild phenotype of β-thalassemia (β-thal).

Keywords

β-Globin, β-thalassemia (β-thal), promoter region

History

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β-Thalassemia (β-thal) is a hereditary blood disorder characterized by anomalies in the β chain synthesis of hemoglobin (Hb), and displays a high level of clinical heterogeneity (1). There are more than 850 different genomic alterations of the β-globin gene currently available in the HbVar database (2). Despite the fact that β-thal is the one of the oldest phenotypes in which the genetic background has been well characterized, novel mutations are still occasionally being described, especially when evaluating milder phenotypes. There are 32 different clinically important genomic changes in the promoter region of the β-globin gene described in the HbVar database (2). Almost all of them have been described as β^+ mutations and were associated with mild-to-moderate phenotypes. We here describe a novel genomic alteration located in the promoter region of the β-globin gene and discuss its potential clinical importance.

Cases

A 40-year-old woman, previously diagnosed as β-thal trait based on clinical and hematological findings at another clinic, was admitted to the Hemoglobinopathy Diagnostic Centre, Mediterranean Blood Diseases Foundation, Antalya, Turkey, because of her recently increased clinical symptoms. She had been complaining of pallor, weakness and fatigue. She had no transfusion history. On physical examination, she had subicterus and pallor but no hepatosplenomegaly. After hematological and clinical reevaluation, she was referred to the Antalya Genetic Diseases Diagnosis Centre, Antalya, Turkey for DNA analyses, to screen for β-globin gene mutations in order to explain the clinical findings. Because DNA analyses revealed a novel mutation on her β-globin gene, the study was later expanded to include her family in order to understand the clinical importance of this novel genomic alteration. It was also subsequently decided to screen for α-globin gene mutations. Hematological, clinical and genetic evaluations were performed after informed consent had been obtained from the family.

The hematological indices of the cases were obtained with an automated cell counter (Mythic 18; Orphée, Geneva, Switzerland). The Hb A_2 and Hb F levels were measured by high-performance liquid chromatography (HPLC) (TOSOH G8; Tosoh Bioscience Inc., San Francisco, CA, USA).

Following the isolation of genomic DNA with a commercial kit (AxyPrep Blood Genomic DNA Miniprep Kit, Axygen Biosciences Inc., Union City, NJ, USA), the genomic DNA was quantified by Nano Drop 1000 (Thermo Scientific,

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Wilmington, DE, USA). The β-globin gene was amplified as two fragments for mutation analyses by DNA sequencing. Thirty to 50 ng of genomic DNA was used in 25 μL reaction volumes for each fragment. The polymerase chain reaction (PCR) mixture contained 10 pmol of each primer. The PCR program consisted of 40 cycles of an initial denaturation at 93 °C for 150 seconds, 35 cycles of 30 seconds at 93 °C, 45 seconds at 58 °C, 60 seconds at 72 °C, and a final extension at 72 °C for 5 min. Then the PCR products were run on a 2.0% agarose gel containing ethidium bromide, and were visualized under UV light. The PCR products were purified by Purelink Genomic Mini Kit (Invitrogene, Carlsbad, CA, USA). The β-globin gene sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing kit and an ABI PRISM™ 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The α-globin genes were analyzed for common mutations by reverse dot-blot assay (α-Globin StripAssay; ViennaLab Diagnostics GmbH, Vienna, Austria).

DNA sequencing revealed a G > C substitution at position −77 of the β-globin gene (Figure 1) in combination with the well known codon 8 (−AA) (*HBB*: c.25_26delAA) in the mother. Literature screening of PubMed and related databases showed that the G > C change at position −77 was a novel variant of the β-globin gene. According to the Human Genome Variation Society (HGVS) nomenclature, this novel change was designated as *HBB*: c.-127G > C. Subsequent DNA analyses revealed that two of her sons were also carriers of the *HBB*: c.-127G > C mutation, while the other two were carriers of the *HBB*: c.25_26delAA deletion. Mutation analyses of the α-globin gene with the α-Globin StripAssay (ViennaLab Diagnostics GmbH) did not reveal any mutations. The hematological and molecular findings for all the cases are summarized in Table 1. Serum iron, total iron binding capacity and ferritin levels of all cases were within normal limits.

β-Thalassemia is a clinically heterogeneous disease including β-thal minor, β-thal intermedia (β-TI) and β-thal major (β-TM) clinical subgroups (1). β-Thalassemia intermedia patients are probably the most heterogeneous subgroup, displaying a diverse set of clinical features ranging from carrier state to transfusion-dependent β-TM. The proband in our study, a compound heterozygote for the *HBB*: c.25_26delAA deletion and a novel genomic alteration *HBB*: c.-127G > C, was categorized as carrying a mild type of β-TI. There are a number of genetic and nongenetic factors altering the clinical severity of the disease (3–6). Nevertheless, mutation analysis of the β-globin gene should be the first attempt followed by analysis of the α-globin mutation analysis. The factors contributing to the imbalance in α/β-globin peptide ratios should be analyzed for more realistic estimations (7). This makes it difficult to determine the clinical importance of a novel genomic alteration. Additionally, low incidences of newly-described mutations and the variability of accompanying mutations make it difficult to reach confident conclusions about the hematological and clinical effects of novel mutations. Here, we describe a novel putative mutation, *HBB*: c.-127G > C, located in the promoter region of the β-globin gene.

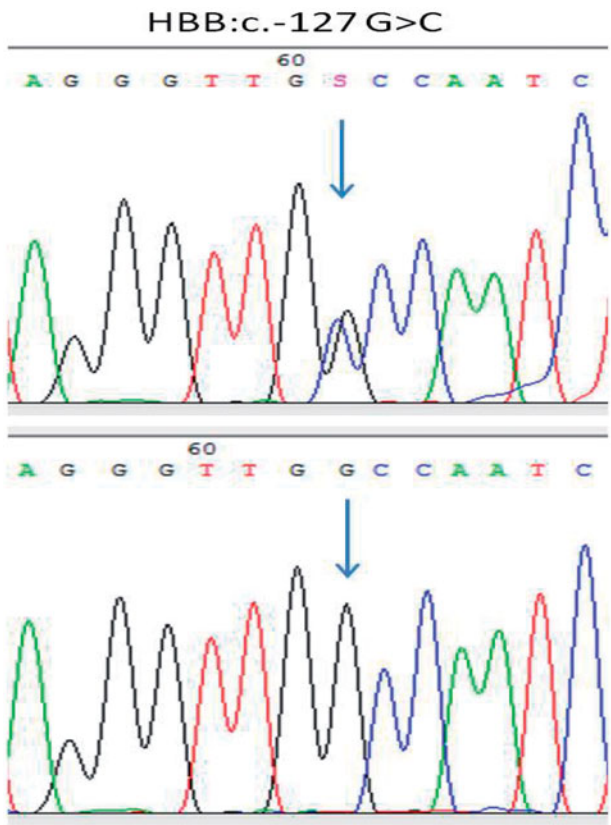

Figure 1. The G > C substitution detected at position −77 of the β-globin gene.

Table 1. The hematological and molecular data of the family involved in this study.

Parameters	Son 1	Son 2	Son 3	Son 4	Mother (proband)	Father
Sex-Age	M-1	M-10	M-15	M-18	F-40	M-42
β Genotype	$\beta^{-77(G>C)}/\beta^{A}$	$\beta^{codon\ 8(-AA)}/\beta^{A}$	$\beta^{codon\ 8(-AA)}/\beta^{A}$	$\beta^{-77(G>C)}/\beta^{A}$	$\beta^{-77(G>C)}/\beta^{codon\ 8(-AA)}$	β^{A}/β^{A}
Hb A (%)	79.60	75.90	78.90	83.50	76.00	97.10
Hb A_2 (%)[a]	3.20	6.60	5.90	3.20	5.80	2.30
Hb F (%)	3.90	4.50	2.50	0.60	4.50	0.60
Hb (g/dL)	10.60	11.50	10.80	14.60	9.10	12.80
MCV (fL)	69.90	59.00	59.00	88.00	54.00	83.00
MCHC (g/dL)	35.50	32.00	32.00	33.00	31.10	32.00
MCH (pg)	25.80	19.00	19.00	29.00	16.90	28.60
RBC (10^{12}/L)	4.30	6.10	5.80	5.00	5.39	4.81
RDW (%)	33.80	16.70	15.40	13.30	20.50	15.70

Hb: hemoglobin; MCV: mean corpuscular volume; MCHC: mean corpuscular Hb concentration; MCH: mean corpuscular Hb; RBC: red blood cell count; RDW: RBC distribution width.

[a]Normal Hb A_2 levels (between 1.5 and 3.5%) according to laboratory reference values.

Almost all the promoter region mutations of the β-globin gene have been associated with decreased β peptide synthesis. Promoter region mutations are most likely related to milder phenotypes in β-thal. The location of the *HBB*: c.-127G>C alteration is just next to the CAAT box transcription factor binding site. Mutations in almost every position in the proximal CACC box have been associated with β-thal. Therefore, we believe that this novel variant would have a clinical significance.

Today, postnatal diagnosis of β-thal is primarily based on molecular genetic testing of the β-globin gene following hematological and physical examinations. On the other hand, prenatal diagnosis is usually only based on detection of parental β-globin gene mutations in fetal samples. In the presence of a novel variant, it is difficult to have a comment on clinical importance and give correct genetic counseling to the family. Therefore, reporting of novel mutations with clinical importance allows better genetic counseling for the doctors and families involved in future cases. Moreover, the description of a novel β-globin gene variant and its clinical relevance can potentially improve our understanding of the heterogeneity of β-thal.

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. Trent RJ. Diagnosis of the haemoglobinopathies. Clin Biochem Rev. 2006:27(1):27–38.
2. Patrinos GP, Wajcman H. Recording human globin gene variation. Hemoglobin. 2004;28(2):v–vii.
3. Uda M, Galanello R, Sanna S, *et al*. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of β-thalassemia. Proc Natl Acad Sci USA. 2008;105(5):1620–1625.
4. Sollaino MC, Paglietti ME, Perseu L, *et al*. Association of α globin gene quadruplication and heterozygous β thalassemia in patients with thalassemia intermedia. Haematologica. 2009;94(10): 1445–1448.
5. Galanello R, Cao A. Relationship between genotype and phenotype. Thalassemia intermedia. Ann N Y Acad Sci. 1998;850: 325–333.
6. Rosatelli C, Leoni GB, Tuveri T, *et al*. Heterozygous β-thalassemia: Relationship between the hematological phenotype and the type of β-thalassemia mutation. Am J Hematol. 1992;39(1):1–4.
7. Danjou F, Anni F, Galanello R. β-Thalassemia: From genotype to phenotype. Haematologica. 2011;96(11):1573–1575.