"Silent" β-thalassemia mutation (promoter nt-101 C > T) with increased hemoglobin A_2

Deniz Aslan

Division of Pediatric Hematology, Department of Pediatrics, Gazi University, Faculty of Medicine, Ankara, Turkey.
E-mail: daslan@gazi.edu.tr, and drdagutf@ttmail.com
Received: 13 August 2015, Revised: 15 April 2016, Accepted: 29 April 2016

SUMMARY: Aslan D. "Silent" β-thalassemia mutation (promoter nt-101 C > T) with increased hemoglobin A_2. Turk J Pediatr 2016; 58: 305-308.

One of the most common silent β-thalassemia mutations is the C > T substitution at position -101 within the distal CACCC box, which leads to a mild reduction in the expression level of the β-globin gene. Carriers of this mutation have a normal hematologic picture without microcytosis and borderline hemoglobin A2 values, and may be missed during screening. Co-occurrence of this mutation with one of the classical β-thalassemia mutations leads to β-thalassemia intermedia, and this is important for Mediterranean populations where β-thalassemia is frequent. Awareness of this mutation, which may have a heterogeneous clinical presentation, is required. We herein present the unusual hematologic findings of a Turkish family carrying this mutation.

Key words: ***β-thalassemia mutation (promoter nt-101 C > T), increased hemoglobin A2, Turkish family.***

Thalassemias are diseases of hemoglobin (Hb) synthesis, with subtypes named after the specific globin chain involved[1,2]. β-thalassemia, with the defect affecting the β chain, is the clinically severe subtype with autosomal recessive inheritance. Patients with β-thalassemia can be either heterozygous (thalassemia minor) or homozygous (thalassemia major) for the defective globin chain. Some patients with β-chain mutations in homozygous state still have residual β-chain synthesis, resulting in an intermediate phenotype (thalassemia intermedia). While thalassemia major is manifested as severe transfusion-dependent anemia soon after birth and thalassemia intermedia usually presents with moderate anemia later in life, thalassemia minor is clinically silent. It may only cause mild microcytic anemia, which can be detected by laboratory tests. Initial laboratory suspicion for β-thalassemia minor depends on the presence of microcytosis (mean corpuscular volume [MCV] below 80 fl) on complete blood count (CBC) test[3-6]. In general, if a reduced MCV is detected, subsequent investigation for thalassemia is ordered. The patient's body iron status is studied and the hemoglobin (Hb) A2 concentration is quantified. In the presence of a normal iron status and an elevated HbA2 level, β-thalassemia minor is identified. Some carriers, however, have borderline or normal HbA2 levels. Carriers with normal CBC values have also been reported. Such carriers may be the individuals with silent β-thalassemia mutations[7-9]. Offspring of a carrier and an individual with a classical mutation may develop thalassemia intermedia. One of the silent β-thalassemia mutations with inconclusive HbA2 level and normal red cell indices is the promoter nt-101 C > T mutation, described predominantly in Mediterranean populations[7]. Clinicians' awareness of this mutation is necessary for proper carrier screening. The related data in the Turkish population in this region are very limited. In this report, a Turkish family with this mutation is presented.

Case Reports

The family reported herein includes three members: a mother and her two children. In 2011, a four-year-old boy was assessed in our department for microcytosis. He was the first child of a non-consanguineous young couple.

Prenatal, natal and postnatal history was unremarkable, and the physical examination was normal. The combination of slightly reduced red cell indices with low-normal body iron status and increased HbA2 level was consistent with β-thalassemia minor. Thereafter, a family study was ordered. CBC test in the mother and father (both biological parents) showed normal red cell indices. Health policy guidelines in our institution dictate that in cases suggestive of thalassemia minor, CBC, body iron status and HbA2 measurement are performed simultaneously. In this family, body iron status and HbA2 level of the father were found to be normal. HbA2 level in the mother, however, was elevated (4.5%, normal level <3.5%) despite a normal MCV value (88.3 fl) and a low-normal iron level. No accompanying vitamin B12 or folic acid deficiency, which might mask microcytosis or lead to HbA2 elevation, was present. There was no evidence of hemolysis. Other possible etiologies (e.g., medications, hemorrhage, hypothyroidism, liver disease, and bone marrow dysplasias) were excluded by clinical examination and the appropriate investigations. Re-testing of HbA2 in another laboratory revealed a similar result. A clinical and hematologic diagnosis of β-thalassemia minor was established. The family was informed that each child was at a 50% probability for heterozygous disease. The second sibling, born two years later, was investigated as she entered her second year and was also found to be a β-thalassemia minor, with slightly reduced red cell indices and an elevated HbA2 level, despite low-normal iron parameters.

In order to define the genotype of this unusual β-thalassemia in this family, molecular studies were carried out after obtaining informed consent. DNA was isolated from peripheral blood using standard laboratory methods. Their β-globin genes were amplified by polymerase chain reaction (PCR) separately, amplicons were purified, and nucleotide sequencing was done. The mother and her two children were found to be heterozygous for promoter nt-101 C > T silent β+-thalassemia mutation. Multiplex gap-PCR tests designed to detect single α-globin gene deletion of rightward ($-\alpha^{3.7}$) and leftward ($-\alpha^{4.2}$) types and also deletion of two α-globin genes in cis of the ($--^{MED}$) and $-(\alpha)^{20.5}$ types were carried out. None of the three family members had any of these α-globin gene deletions (Table I).

Discussion

Thalassemia is the world's most widespread

Table I. Erythrocyte Indices and Results of Hematologic Analyses and Molecular Genetic Testing of β- and α-Globin Genes in the Family

Clinical parameters	Patients		
	Mother	Son	Daughter
Age (years)	34	8	3
Hb (g/dl)	12.5	12.5	12.6
Hematocrit (%)	37.4	37.4	36.5
MCV (fl)	88.3	73.5	75.7
MCH (pg)	30	24.6	26.0
MCHC (g/dl)	29.5	33.5	34.4
RBC ($x10^6$/L)	4.2	5.1	4.8
RDW (%)	13.9	15.0	13.6
Serum iron (μg/dl)	36	41	83
UIBC (μg/dl)	404	315	369
Transferrin saturation (%)	8.9	13	22
Serum ferritin (ng/ml)	9.4	13.7	10
HbA_2 (%)	4.5	4.1	3.9
Hb F (%)	1.0	1.1	3.6
β-Globin genotype	Heterozygous for promoter nt-101 C>T "silent" β+-thalassemia mutation	Heterozygous for promoter nt-101 C>T "silent" β+-thalassemia mutation	Heterozygous for promoter nt-101 C>T "silent" β+-thalassemia mutation
α-Globin genotype	(α α / α α)	(α α / α α)	(α α / α α)

disease affecting particularly the populations of Mediterranean origin[1]. The medical and economic burden of thalassemia constitutes a serious public health problem. Prevention of homozygous β-thalassemia, the clinically severe subtype, is possible with prenatal diagnosis and simply by detecting carriers. Given that carriers of β-thalassemia are clinically asymptomatic, they can only be detected by laboratory tests. The initial laboratory test is CBC and the key index suggesting thalassemia is MCV[3, 4, 6]. Presence of microcytosis on CBC is the first indicator suggestive of thalassemia. Hb (normal/slightly decreased), mean corpuscular hemoglobin (MCH) (decreased), RBC (increased), and red cell distribution width (RDW) (normal) are also used for carrier screening[10]. A carrier with normal CBC values may be missed, since subsequent investigation for thalassemia is not ordered in such individuals. The possible contributing factors for normocytosis in a carrier, specifically for normal MCV value, might be either other accompanying clinical conditions or silent β-thalassemia mutations. In this situation, positive family history and the HbA2 level are important. In the presence of a family history, subsequent thalassemia investigation should always be performed and the HbA2 level determined. Even in the case of a borderline or normal HbA2 level, thalassemia should not be excluded. Molecular analysis should be carried out, since the most likely causes are silent β-thalassemia mutations [e.g.,Krüppel-like factor 1 (KLF1) gene mutation and β-promoter mutations (-101 C > T mutation and -101 C > G mutation)], which can only be detected by molecular analysis. Carriers of KLF1 mutations and also heterozygotes of β-promoter mutations present with borderline HbA2 and normal red cell indices[7,9,11]. An elevated HbA2 level in a heterozygote with normocytosis is most likely due to α-β compound heterozygosity[12]. Though rare, β-promoter mutations have also been demonstrated to cause elevated HbA2 level[13]. In the family presented here, positive family history and elevated HbA2 were present, and accompanying α-thalassemia was excluded. The results, when considered together, are consistent with a mutation in the β-globin gene promoter, and promoter nt-101 C > T mutation was confirmed by molecular analysis.

The silent β-thalassemia mutation, β^+ -101 C > T, first described[7] in 1989, is characterized by normal hematologic indices, normal or borderline HbA2 and hemoglobin F (HbF) level, and a slight imbalance in β-globin chain synthesis. Because of the inconclusive hematologic indices, it is difficult to detect the carriers of this mutation. They are usually identified at older ages as parents of thalassemia intermedia patients[13,14]. The original families in which this mutation was first described and most of the previously reported patients carrying this mutation are of Mediterranean origin (in particular Italian and Greek populations). Although it is the most common Mediterranean silent β mutation, a limited number of cases have been reported to date. The frequency and hematologic characteristics of this mutation in the Turkish population are very limited as well.

Despite its silent nature in heterozygous form, a study in a Greek population has revealed "non-silent" heterozygotes of this mutation[13]. In that study, less than half of the pure heterozygotes for the promoter β-101 C > T mutation had completely normal (silent) hematology; the remainder had either elevated HbA2 values (range: 3.7-5.1%) and/or low red cell indices and/or raised HbF levels. Among non-silent heterozygotes studied, after exclusion of cases with coexisting α-thalassemia, 8 were adult females, and 6 of the 8 presented elevated HbA2 with normal CBC, as seen in our mother. Six of the non-silent heterozygotes were children, and only one of them presented with elevated HbA2 plus slight microcytosis, although his iron status was not mentioned, as also observed in our children. No family with discordant CBC values among its members, as observed in our family, was determined. Since our patients had the same molecular pathology, the mother had no condition masking microcytosis, and our three patients had similar body iron status, which might lower red cell indices, we could not explain the red cell discordance in this family. The levels of HbF were slightly raised in our patients, as reported in the original study. In the same study, compound heterozygotes of this mutation have been reported to have moderate anemia with microcytosis, hypochromia, and elevated HbA2 and HbF levels (Hb 9.6 g/dl, MCV 62.4 fl, MCH 19.4 pg, HbA2 6.0 %, and HbF 11.8 %, respectively).

In conclusion, despite normal MCV, the possibility of thalassemia minor should not be

excluded. In the presence of a positive family history, subsequent thalassemia investigation should be performed and the HbA2 level determined. Even if the HbA2 level is not found to be elevated, molecular analysis should be studied to determine the causative mutation. Among these mutations, promoter β-101 C > T mutation, predominantly determined in Mediterranean populations, is of great importance. Clinical awareness of this mutation should facilitate carrier detection and genetic counseling, and contribute to the eradication of thalassemia.

Acknowledgement

We are grateful to the patient's family for their collaboration and patience, and to Drs. David H.K. Chui and Hong-yuan Luo (Boston University School of Medicine, Hemoglobin Diagnostic Reference Laboratory, Boston Medical Center) for performing the molecular genetic analysis of thalassemia.

REFERENCES

1. Higgs DR, Engel JD, Stamatoyannopoulos G. Thalassaemia. Lancet 2012; 379: 373-383.
2. Cao A, Galanello R. Beta-thalassemia. Genet Med 2010; 12: 61-76.
3. Van Vranken M. Evaluation of microcytosis. Am Fam Physician 2010; 82: 1117-1122.
4. DeLoughery TG. Microcytic anemia. N Engl J Med 2014; 371: 1324-1331.
5. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem 2000; 46: 1284-1290.
6. Ip HW, So CC. Diagnosis and prevention of thalassemia. Crit Rev Clin Lab Sci 2013; 50: 125-141.
7. Gonzalez-Redondo JM, Stoming TA, Kutlar A, et al. A C----T substitution at nt--101 in a conserved DNA sequence of the promotor region of the beta-globin gene is associated with "silent" beta-thalassemia. Blood 1989; 73: 1705-1711.
8. Galanello R, Barella S, Ideo A, et al. Genotype of subjects with borderline hemoglobin A2 levels: implication for beta-thalassemia carrier screening. Am J Hematol 1994; 46: 79-81.
9. Perseu L, Satta S, Moi P, et al. KLF1 gene mutations cause borderline HbA(2). Blood 2011; 118: 4454-4458.
10. Aslan D. Automated blood counts and identification of thalassemia carriers. J Postgrad Med 2008; 54: 242-243.
11. Moi P, Faà V, Marini MG, et al. A novel silent beta-thalassemia mutation in the distal CACCC box affects the binding and responsiveness to EKLF. Br J Haematol 2004; 126: 881-884.
12. Panigrahi I, Rafeeq PH, Choudhry VP, Saxena R. High frequency of deletional alpha-thalassemia in beta-thalassemia trait: implications for genetic counseling. Am J Hematol 2004; 76: 297-299.
13. Maragoudaki E, Kanavakis E, Traeger-Synodinos J, et al. Molecular, haematological and clinical studies of the -101 C --> T substitution of the beta-globin gene promoter in 25 beta-thalassaemia intermedia patients and 45 heterozygotes. Br J Haematol 1999; 107: 699-706.
14. Rund D, Oron-Karni V, Filon D, et al. Genetic analysis of beta-thalassemia intermedia in Israel: diversity of mechanisms and unpredictability of phenotype. Am J Hematol 1997; 54: 16-22.