

Investigation of the clinical and hematological significance of the first observed hemoglobin Ernzs variant [β 123(H1) Thr>Asn] in the Turkish population

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Aim: In this report, we aimed to investigate the clinical and hematological significance of the first observed hemoglobin Ernzs variant in the Turkish population.

Materials and methods: We identified the Hb Ernzs variant in 3 nonrelated females (Proband 1, 2, and 3). Proband 1's family was also included the study. Hematological data were obtained with an automated cell counter and routine methodology. The beta-globin gene was sequenced by automatic sequencing.

Results: Proband 1 was detected as a combination of Hb Ernzs/Hb S without any clinical symptoms. Her sister and brother had to be an Hb Ernzs/Hb S combination. Her mother and father only showed Hb Ernzs and Hb S, respectively. Proband 2 had the Hb Ernzs variant with IVS-I 5nt homozygous alpha 2 gene mutation. Proband 3 had a heterozygous Hb Ernzs variant. All subjects were clinically and hematologically normal but Proband 2 had low hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and high red blood cell distribution width levels.

Conclusion: In the present study, the Hb Ernzs variant is demonstrated for the first time in the Turkish population. Additionally, there is no published report in the world literature of Hb Ernzs in combination with IVS-I 5nt homozygote mutation in the alpha-globin gene or Hb S variant. The present report shows that the Hb Ernzs variant is not clinically or hematologically significant.

Key words: Rare hemoglobin variants, hemoglobin Ernzs, DNA sequence analysis

Introduction

Abnormal hemoglobins are the most common hemoglobinopathy group after beta thalassemia in the Turkish population (1,2). So far, at least 53 hemoglobin variants, most of them rare and without clinical symptoms, have been identified in Turkey (3–14). Hemoglobin (Hb) Ernzs, which does not cause

any pathology, was first described in the literature by Grof et al. in an Italian male, then by Fouladi et al. in an Iranian family and by Pietrapertosa et al. and Giambona et al., again in individuals of Italian origin (15–18). The rarely seen Hb Ernzs variant emerges as the result of C>A (ACC>AAC) conversion, leading to a substitution of Thr to Asn at codon 123

Received: 05.02.2012 – Accepted: 18.06.2012

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in the third exon of the β -globin gene. This variant cannot be identified with common electrophoretic techniques such as cellulose acetate electrophoresis, which is carried out in alkali and acidic pH and high-performance liquid chromatography (HPLC). The present study is the first to report the Hb Ern2 variant in the Turkish population as well as the Hb Ern2 variant in combination with an IVS-I 5nt homozygote mutation in the alpha-globin gene or Hb S variant in the world literature.

Materials and methods

A total of 7 individuals, including a 30-year-old female (Proband 1) who presented to the Mustafa Kemal University Medical Faculty, Department of Medical Biology and Genetics, for premarital screening of thalassemia mutations, her parents and 2 siblings, and 2 females aged 26 and 32 who had no familial relationship (Probands 2 and 3), were included in the study. Written informed consent was taken from the individuals. Hematological data from the subjects were obtained via an automated cell counter and routine methodology. Red cell lysates were analyzed by HPLC (Tosoh Bioscience Inc., San Francisco, CA, USA) and cellulose acetate electrophoresis at alkaline pH (Interlab, Milano, Italy). Genomic DNA was isolated from leukocytes using a standard salting out procedure, as described Miller et al. (19). PCR amplifications of the β -globin gene in 2 separate tubes were performed with forward

and reverse primers and amplified products were sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. The sequence reaction was analyzed using an automated fluorescence-based DNA sequence analyzer (ABI PRISM 3130, Applied Biosystems). The mutation was confirmed via sequencing of the antisense DNA strand, which was performed twice. Mutations of the alpha-globin gene were identified using a strip assay kit based on the reverse hybridization technique (ViennaLab, Austria).

Results

In the present study, Proband 1, her sister, and her brother were found to have a combined heterozygous genotype of Hb Ern2/Hb S. In addition, her mother was heterozygous in the Hb Ern2 and her father in the Hb S genotype. No clinical pathologic symptom was defined in the whole family. Hematologic data of the Hb Ern2 family are shown in the Table and the DNA sequence electropherogram of the Hb Ern2 genotype is shown in Figure 1. The Hb Ern2 variant could not be identified in cellulose acetate electrophoresis carried out at alkali pH (Figure 2).

In addition to being heterozygous in the Hb Ern2 genotype, Proband 2 carried an Hb Ern2 variant with IVS-I 5nt homozygous alpha 2 gene mutation. The Hb (8.7 g/dL), hematocrit (Hct) (27.3%),

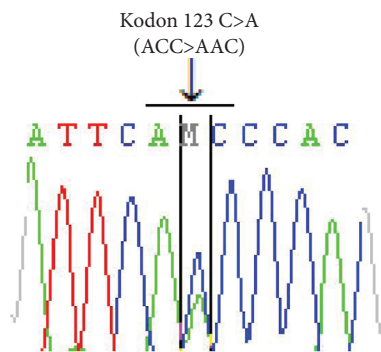


Figure 1. DNA sequence electropherogram of Hb Ern2 (β 123, ACC>AAC).

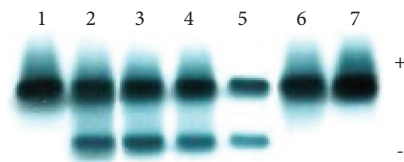


Figure 2. Hemoglobin electrophoresis analysis at alkaline pH of Proband 1 and her family members, Proband 2, and Proband 3. Lane 1, Proband 1's mother; lane 2, Proband 1's father; lane 3, Proband 1; lanes 4 and 5, Proband 1's sister and brother; lanes 6 and 7, Proband 2 and 3, respectively.

Table. Hemoglobin genotypes and hematologic values of Hb Ern2 family.

	Genotype	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg/dL)	MCHC (g/dL)	RDW (%)	Hb A1 (%)	Hb A2 (%)	Hb F (%)	Hb S (%)
Mother	Hb Ern2/Hb A	13.0	38.0	84.9	29.7	34.3	15.1	80.2	1.8	0.72	0.00
Father	Hb S/Hb A	11.9	34.8	76.4	28.0	34.8	16.8	44.3	3.9	1.41	39.6
Proband 1	Hb Ern2/Hb S	12.5	35.5	77.1	27.2	35.2	15.7	44.9	4.3	1.57	40.9
Sister	Hb Ern2/Hb S	12.1	35.3	76.2	28.1	33.6	14.3	39.3	3.8	1.81	41.3
Brother	Hb Ern2/Hb S	12.4	36.1	77.4	27.0	32.2	14.0	42.9	4.4	1.33	38.3

mean corpuscular volume (MCV) (59.2 fL), mean corpuscular hemoglobin (MCH) (18.9 pg), and mean corpuscular hemoglobin concentration (MCHC) (31.9 g/dL) values of Proband 2 were low and her red blood cell distribution width (RDW) value was high (33.8%), while the counts of erythrocytes, leukocytes, and platelets were in the normal range. In addition, Hb A1 was defined as 75.7%, Hb A2 as 1.7%, and Hb F as 1.9% in HPLC assay. Proband 3 had a heterozygous Hb Ern2 variant and her hematologic values were found to be completely in the normal range.

Discussion

Turkey is located where Asia, Africa, and Europe are closest to each other and in a geographic region in which Asia and Europe are connected. A wide range of Hb variants has been identified in Turkey since it has hosted many civilizations during its history. Abnormal Hb variants resulting from the change in amino acids on the globin chain, including those in the Hb molecule, are the most common hemoglobinopathy groups after β -thalassemia in Turkey. A recent study from Turkey suggested that the number of variant hemoglobins is higher than expected (1). Altay et al. reported 42 Hb variants found in the Turkish population as of 2002 (3). In the following 9 years that number reached 53 by the addition of new variants named Hb Setif (4), Hb Pyrogos (5), Hb Volga (6), Hb Tyne (7), Hb A2 Yialousa (8), Hb Bronovo (9), Hb J-Meerut (10), Hb Yaizu (11), Hb D-Ouled Rabah (12), Hb Tunis (13), and Hb Crete (14).

The Hb Ern2 variant is rarely seen and cannot be identified with classical electrophoretic techniques. It was identified by a DNA sequence analysis method in our patients. Reporting these cases is important, because it is the first time that the Hb Ern2 variant was reported in Turkey, and this variant was again confirmed not to cause any clinical pathology. Proband 1, her sister, and her brother had Hb Ern2/Hb S genotype with low levels of MCV and Hb A1, but high levels of Hb A2, whereas the mother, who was heterozygous for Hb Ern2, had levels of MCV, Hb A1, and Hb A2 in the normal range, demonstrating that elevated MCV and Hb A2 result from HbS and not from the Hb Ern2 variant. Levels of Hb, Hct, MCV, MCH, and MCHC are known to be usually within the

normal range, or slightly lower, in alpha thalassemia carriers (20). Therefore, Proband 2, who carried the Hb Ern2 variant with IVS-I 5nt homozygous alpha 2 gene mutation, had low values of Hb, Hct, MCV, MCH, and MCHC, which again resulted from having an alpha-globin gene mutation and not from the Hb Ern2 variant. For this reason, our findings indicate that Hb Ern2 is a silent Hb variant not causing any pathology.

Various Hb variants have been reported in Turkey, which contains many migration paths due to its geographic location. One reason for this diversity is that Turkey was a major transit point on the historical Silk Road that started in Italy and ran to China, passing through Iran. We detected the Hb Ern2 variant in Antakya (Antioch), which is located on the Mediterranean coast and known to have been a point on the Silk Road in the medieval era, supporting our hypothesis. However, the registration system with regard to Hb variants found in Turkey is inadequate, suggesting the number of variants is, in fact, greater than we are aware of. One of the reasons why the number of Hb variants in the Turkish population cannot be defined to a more accurate value is that some variants cannot be identified with screening tests such as HPLC or electrophoresis or by screening only the commonly seen mutations. Hb Ern2, which we found in Turkey for the first time, is one such variant, and it can only be detected by DNA sequence analysis. Therefore, we believe that DNA sequence analysis must be performed in patients suspected of hemoglobinopathy based on hematological tests and who could not be diagnosed with either biochemical tests, such as HPLC and electrophoresis, or with molecular systems screening a limited number of mutations.

Our study is important in terms of being the first to report the Hb Ern2 variant in the Turkish population as well as the Hb Ern2 variant in combination with IVS-I 5nt homozygote mutation in the alpha-globin gene or Hb S variant in the world literature. In summary, our results may be a guiding factor in the decision-making process in genetic counseling. Demonstrating the Hb Ern2 variant for the first time in Turkey may also be helpful in providing a contribution to the establishment of a national database of Hb variants.

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