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SHORT COMMUNICATION

A NEW HEMOGLOBIN VARIANT: Hb IZMIR [β86(F2)Ala→Val, G*C*C>G*T*C; HBB:c.260C>T]

Aydan Çelebiler[1], Derya Aksoy[2], Serkan Ocakcı[3], and Baysal Karaca[2]

[1]*Department of Clinical Biochemistry, Izmir University, Faculty of Medicine, Medical Park Hospital, Izmir, Turkey*

[2]*Department of Clinical Biochemistry, Izmir Education Hospital, Izmir, Turkey*

[3]*Department of Clinical Hematology, Izmir University, Faculty of Medicine, Medical Park Hospital, Izmir, Turkey*

□ *We report a new hemoglobin (Hb) variant [β86(F2)Ala→Val; HBB:c.260C>T] that we have named Hb Izmir. We have identified Hb Izmir in a Turkish woman by ion exchange high performance liquid chromatography (HPLC) during a premarital screening program in the Aegean region of Turkey. The mother and sister of the proband also carried the same variant. Using direct sequencing, we have characterized this variant as resulting from a GCC>GTC replacement at codon 86 of the β-globin chain, corresponding to an Ala→Val amino acid substitution. In the heterozygote, the level of Hb Izmir ranged from 41.38 to 45.6%. All heterozygotes had a Hb A_2 level of less than 3.5%. Total blood count values were normal and there were no other clinical findings. Although its clinical significance is thus far unclear, Hb Izmir may be important in hemoglobinopathy screening programs.*

Keywords Hemoglobin (Hb) variant, Hemoglobinopathy, Hb Izmir

Abnormal hemoglobins (Hbs) are the second most common hemoglobinopathies after β- thalassemia (β-thal) in Turkey. To date, more than 20 different Hb variants involving the β chain have been reported in the Turkish population (1). Some of these were originally described in Turkish laboratories, other in Europe or as a result of collaborative efforts (2–10). In

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Address correspondence to Aydan Çelebiler, M.D., Ph.D., Department of Clinical Biochemistry, Izmir University, Faculty of Medicine, Medical Park Hospital, Yeni Girne Bulvan 1825 sok No 12, Izmir, Turkey; Tel.: +902323995050; Fax: +903670559; E-mail: aydancelebiler@gmail.com

this study, we report a new silent Hb variant found in the Aegean region of Turkey. Detection technology and the hematological findings are presented.

The proband was detected during a premarital screening program. After informed consent was obtained, peripheral blood samples of the proband and three family members were collected in EDTA-containing vacutainers and in vacutainers without an anticoagulant. Background information such as age, sex, ethnic origin, reproductive history, clinical signs and symptoms were recorded.

Hematological data were obtained with automated cell counters (Advia 2120i; Siemens Healthcare Diagnostics, Munich, Germany), while other routine biochemical parameters were determined by standard methods (Dimension RxL Max; Siemens Healthcare Diagnostics). Peripheral blood examinations were done in all cases. Ion exchange high performance liquid chromatography (HPLC) was performed using the Primus Ultra^2 system (Primus Corporation, Kansas City, MO, USA) according to the manufacturer's instructions. DNA was isolated from whole blood samples in EDTA-containing vacutainers, using a commercially available DNA extraction kit (RTA Lab, Ltd., Sti, Turkey). Regions of the β-globin gene were sequenced bi-directionally using an ABI PRISM™ BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions, and the sequence reaction was analyzed using an ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems)

Ion exchange HPLC showed that the patient was a heterozygous carrier of an abnormal variant (Hb X) eluting very close to normal Hb A. The Hb variant produced a double peak at the Hb A window as shown in Figure 1. Two other members of the family were also carriers of the same Hb variant. Hb X represented between 41.38 and 45.60% of the total Hb concentration. Hematological and biochemical parameters of the family are listed in Table 1. The variant did not cause appreciable changes in red cell indices, the peripheral blood smears were not anomalous and all carriers were clinically asymptomatic.

The DNA analysis of the proband revealed heterozygosity for a C>T transition at codon 86 (G*C*C>G*T*C) in the second exon of the β-globin gene, inducing a valine→alanine neutral amino acid substitution (Figure 2). We have named the new variant Hb Izmir for the city where the case has been studied. According to the HUGO nomenclature, this amino acid exchange is identified as HBB:c.260C>T.

Currently, three mutations at codon 86 of the β chain are known: Hb Cardarelli (Ala→Pro), Hb Nebraska (Ala→Ile) and Hb Olomouc (Ala→Asp) (11–14). Hb Olomouc is associated with erythrocytosis; Hb Cardarelli and Hb Nebraska are high oxygen affinity variants. Hb Izmir is a variant with a normal clinical presentation in the heterozygous state. Although we cannot predict for sure, we presume that the homozygous form of Hb Izmir, or a combination

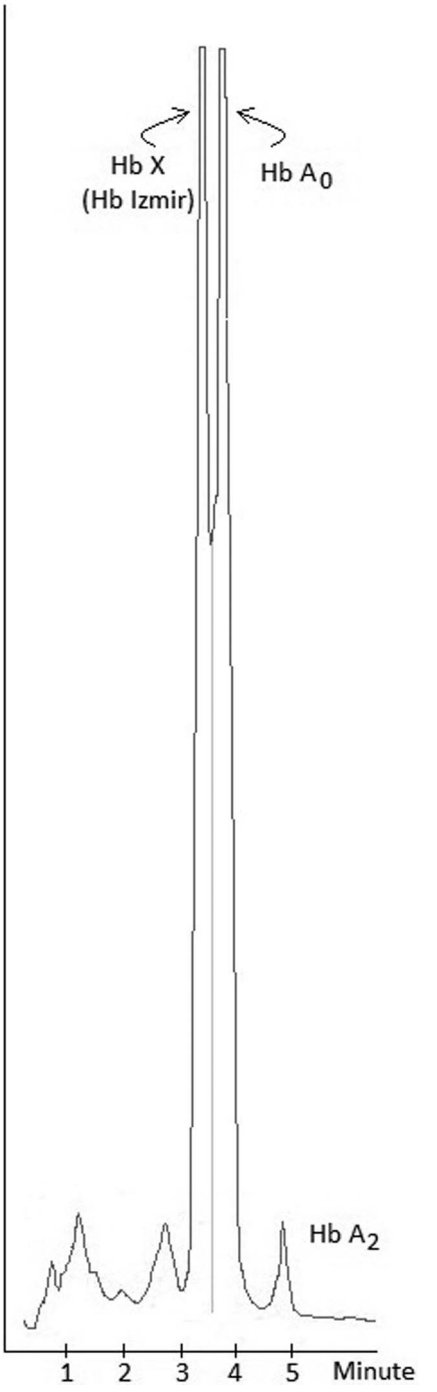

FIGURE 1 High performance liquid chromatography profile of the abnormal variant (Hb Izmir).

TABLE 1 Red Cell Indices, Hemoglobin Composition and Biochemical Data For Four Members of the Studied Family

Parameters	Proband	Mother	Father	Sister	Reference Values
Age	21	51	55	18	–
Place of birth	Mugla	Datca, Mugla	Datca, Mugla	Mugla	–
RBC (10^{12}/L)	4.86	4.91	5.02	4.67	Women: 4.0-5.0; Men: 4.5-5.5
Hb (g/dL)	12.8	13.7	14.1	12.3	Women: 12.0-16.0; Men: 14.0-17.0
PCV (L/L)	0.41	0.43	0.44	0.41	Women: 0.36-0.48; Men: 0.42-0.52
MCV (fL)	81.9	82.5	85.8	81.7	Women: 82.0-98.0; Men: 82.0-98.0
MCH (pg)	27.9	28.1	31.0	27.2	Women: 26.0-34.0; Men: 26.0-34.0
MCHC (g/dL)	32.0	32.7	35.3	31.9	Women: 32.0-36.0; Men: 32.0-36.0
Hb X (%)	45.60	41.38	–	44.53	–
Hb A_2 (%)	2.7	2.5	2.9	2.6	Women: <3.5; Men: <3.5
Haptoglobin (mg/dL)	87.0	110.0	174.0	156.0	Women: 30.0-200.0; Men: 30.0-200.0
Total bilirubin (mg/dL)[a]	0.6	0.8	0.7	0.3	Women: 0.2-1.0; Men: 0.2-1.0
Direct bilirubin (mg/dL)[a]	0.10	0.12	0.16	0.08	Women: 0.10-0.50; Men: 0.10-0.50
Iron (μg/L)[a]	64.0	51.0	12.8	72.0	Women: 50.0-170.0; Men: 65.0-175.0
Lactate dehydrogenase (U/L)[a]	124.0	101.0	127.0	180.0	Women: 100.0-243.0; Men: 100.0-243.0
Ferritin (μg/dL)[a]	24.2	36.4	54.1	29.5	Women: 5.0-148.0; Men: 28.0-365.0
Vitamin B12 (pg/mL)[a]	349.0	504.0	487.0	376.0	Women: 3.0-17.0; Men: 3.0-17.0
Vitamin folate (μg/dL)[a]	8.31	8.31	14.2	11.7	Women: 3.0-17.0; Men: 3.0-17.0

[a]Serum.

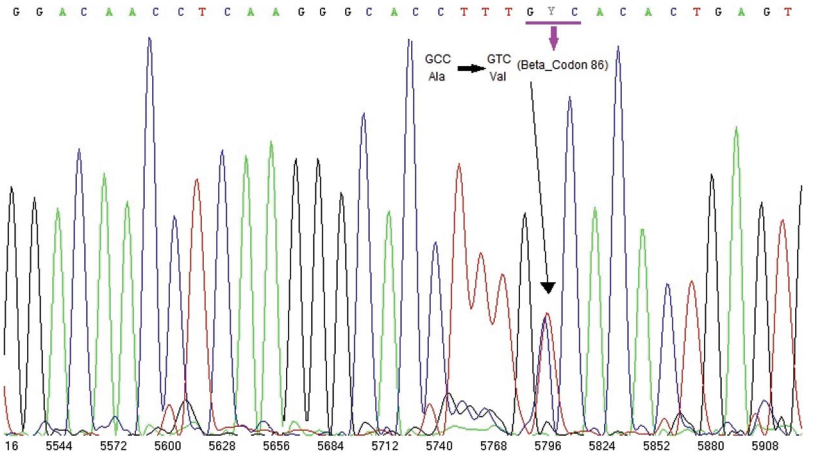

FIGURE 2 DNA sequencing of Hb Izmir [β86(F2)Ala→Val, G*C*C>G*T*C; HBB:c.260C>T].

of this variant with β-thal or Hb S [β6(A3)Glu→Val, G*A*G>G*T*G; HBB: c.20A>T], would not be associated with severe conditions. Both alanine and valine are neutral amino acids with non polar lateral chain. The number of alkyl groups influences the polarity. The more alkyl groups present, the more non polar the amino acid will be. This effect makes valine slightly more non polar than alanine, a physical characteristics that modifies the surface charge and eventually the elution speed in ion exchange chromatography. In this case, this modification was just sufficient to separate the variant on HPLC (Figure 1). However, the two peaks are very close to each other and in a more concentrated sample Hb Izmir could have been missed because of there being no gap between Hb A and Hb Izmir. In the end, once they are separated on HPLC, it is DNA sequencing that characterize abnormal Hbs. Nevertheless, some variants cannot be separated and are eventually identified by DNA sequencing if they reveal unexplained hematological anomalies. This is an important issue for an analytical decision to be taken when abnormal Hbs are suspected.

In this case, the new Hb variant was found by chance during a premarital screening program. Hemoglobinopathies are commonly present in populations of all Mediterranean countries, and are also common in Turkey. Therefore, the Ministry of Health initiated a control and prevention program in regions of Turkey where these anomalies are known to be prevalent. The Aegean region is located in the outer part of the west of Turkey and is one of the target areas for premarital screening. Rare, or new Hb variants, are regularly found during systematic programs of neonatal or premarital screening for the main hemoglobinopathies. Being difficult to separate from Hb A, we do not know how rare or how common Hb Izmir could be in the area as the variant could have been long overlooked using routine electrophoresis or other separation methods.

In conclusion, premarital or early pregnancy screening programs are essential for the identification of couples at-risk of bearing affected offspring. Prevention of severely affected children being born in countries with a high prevalence of hemoglobinopathies is a priority for countries with large, at-risk immigrant populations (15).

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