

# Rare Hemoglobin Variant Hb Yaizu Observed in Turkey

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## Key Words

Hemoglobin Yaizu · Abnormal hemoglobin · Laboratory diagnosis · High performance liquid chromatography · Premarital screening

## Abstract

**Objective:** To determine the characteristic features of the rare hemoglobin (Hb) variant Hb Yaizu to enable laboratory diagnosis of the hemoglobin variants during screening programs. **Materials and Methods:** Genomic DNA was obtained from the 4 members of a family living in Denizli province, an Aegean region of Turkey. Blood cell counts, hemoglobin composition, hemoglobin electrophoresis (both alkaline and acid), HPLC analysis, DNA sequencing and beta globin gene cluster haplotypes were done. **Results:** Hb Yaizu carriers were apparently healthy individuals. Hb Yaizu was slightly faster than Hb S at alkaline pH, but slower than Hb S at acidic pH in hemoglobin electrophoresis. An abnormal hemoglobin peak was observed with a retention time of 4.77 min in HPLC analysis attributed to Hb Yaizu. Two members of the family were heterozygous Hb Yaizu [beta 79(EF3) Asp>Asn] confirmed by DNA sequencing. The mutation was found to be linked with the Mediterranean haplotype I [+----++]. **Conclusion:** We have presented the details of Hb Yaizu, a rare hemoglobin variant that may be important to hemoglobinopathy screening programs, although its clinical significance is unclear.

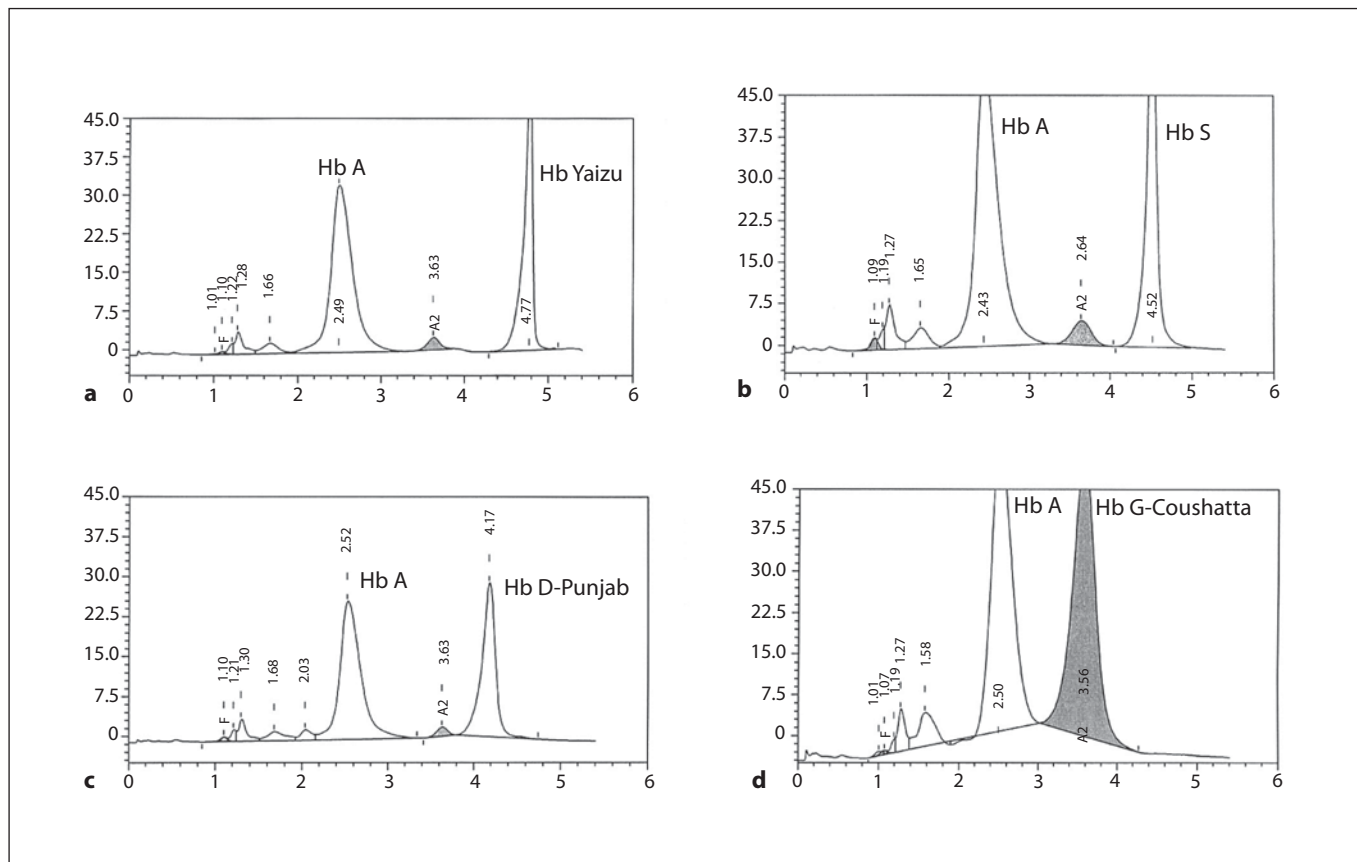
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## Introduction

Hb Yaizu [beta 79(EF3) Asp>Asn] is a hemoglobin (Hb) variant that has been reported in an apparently healthy Japanese woman [1]. This hemoglobin variant was identified by protein chemistry and DNA sequence analysis. Isoelectric focusing electrophoresis showed that Hb Yaizu is slow-moving compared to Hb A. However its detailed characteristic features are not yet known. We describe the molecular, genetic and hematological characteristics of Hb Yaizu in a Turkish family observed during premarital screening.

## Subjects and Methods

Four members of the family were included: proposita, sister, brother and mother. Blood samples were collected in EDTA vacutainers at Denizli Hemoglobinopathy Center, Ministry of Health. Written informed consent was obtained. Blood cell counts were done with a Beckman Coulter AcT10 instrument. High performance liquid chromatography (HPLC) was performed with the BioRad Variant II system for the chromatographic identification of the abnormal hemoglobins in the hemolysates of the patient's blood prepared according to the manufacturer's directions. DNA was isolated from the individuals by standard phenol-chloroform extraction method. Hemoglobin electrophoresis (alkaline and acid) and DNA sequencing were done as previously reported [2, 3]. Hb A<sub>2</sub> and Hb X (Hb Yaizu) quantifications were done by DE-52 microcolumn chromatography [4]. Beta globin gene cluster haplotype analyses were performed as previously reported [5].



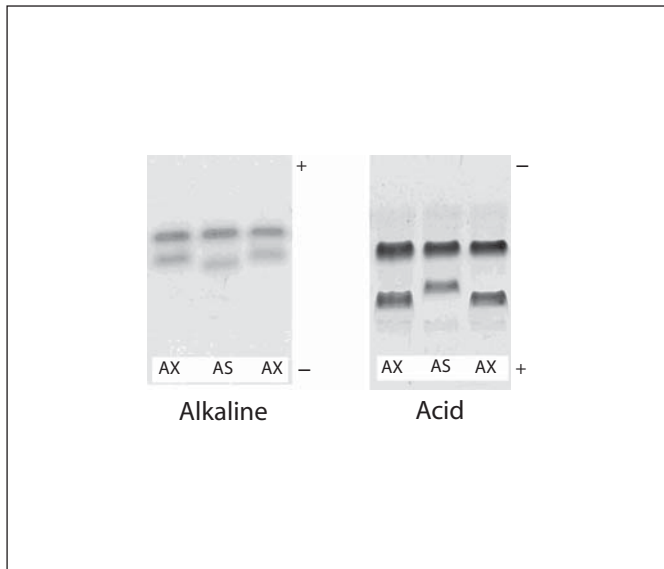
**Fig. 1.** Comparison of the HPLC profiles of Hb Yaizu and other abnormal hemoglobins. **a** Hb A/Hb Yaizu. **b** Hb A/Hb S. **c** Hb A/Hb D-Punjab. **d** Hb A/Hb G-Coushatta.

**Table 1.** Hematological data and hemoglobin composition of the Hb Yaizu carriers

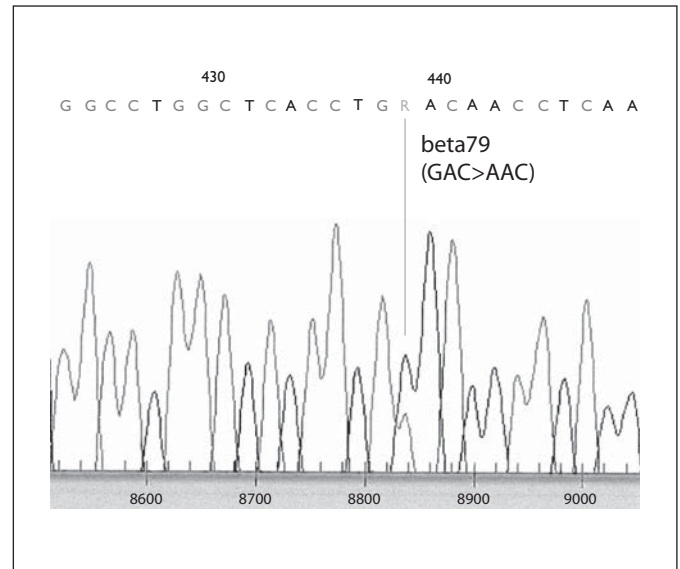
	Proposita	Sister
Age/sex	22/F	11/F
Hb type	A/Yaizu	A/Yaizu
RBC, $\times 10^{12}/l$	5.21	5.01
Hb, g/dl	15.3	13.9
Hct, %	45.6	42.4
MCV, fl	87.0	85.0
MCH, pg	29.3	27.7
MCHC, g/dl	33.6	32.7
RDW, %	12.7	13.0
Hb A <sub>2</sub> , %	2.6	2.9
Hb Yaizu, %	40.5	37.6

## Results

Hematological data and hemoglobin composition of the Hb Yaizu carriers are given in table 1. The HPLC result of Hb Yaizu is shown in figure 1. The proposita and her sister carry an abnormal hemoglobin peak with retention time of 4.77 min (fig. 1a). The HPLC profiles of Hb S, Hb D-Punjab (Los Angeles) and Hb G-Coushatta are also shown for comparison in figure 1. The retention times are 4.52, 4.17, and 3.56 min for the Hb S, Hb D-Punjab and Hb G-Coushatta, respectively. Hemoglobin electrophoresis done at alkaline and acidic pH values is shown in figure 2. Hb X is slightly faster than Hb S at alkaline pH, and slower than Hb S at acidic pH. DE-52 microcolumn chromatography shows that the elution for Hb X is slightly slower than that for Hb S with developer A-200, the same developer used also for Hb S (0.2 M glycine/0.003 M KCN/0.2 M NaCl). Nonradioactive DNA se-



**Fig. 2.** Hemoglobin electrophoresis of the Hb Yaizu in acid and alkaline pH.



**Fig. 3.** DNA sequencing of Hb Yaizu (beta 79, GAC>AAC).

**Table 2.** Beta globin gene cluster haplotypes of Hb Yaizu

	Hemoglobin	5'-ε <i>HincII</i>	Gγ <i>HindIII</i>	Aγ <i>HindIII</i>	5'-ψβ <i>HincII</i>	3'-ψβ <i>HincII</i>	5'-β <i>AvaII</i>	3'-β <i>HinfI</i>
Proposita	Hb A/Hb Yaizu	+/-	+/-	-/-	+/-	+/-	+/+	+/+
Mother	Hb A/Hb A	+/-	-/-	-/-	-/-	-/-	+/+	+/-
Sister	Hb A/Hb Yaizu	+/+	-/-	-/-	-/-	-/-	+/+	+/+
Brother	Hb A/Hb A	+/-	+/-	-/-	+/-	+/-	+/+	+/-
Deduced haplotype		+	-	-	-	-	+	+

quencing shows that the mutation at beta globin gene codon 79 (GAC>AAC) causes the abnormal Hb X (fig. 3). The beta globin gene cluster haplotype of Hb Yaizu is given in table 2. Beta globin gene cluster haplotypes of the family members show that the Hb Yaizu [beta 79(EF3) GAC>AAC] mutation is linked with the Mediterranean haplotype I [+-----++].

### Discussion

Hb Yaizu [beta 79(EF3) Asp>Asn] has been reported only once previously [1]. Heterozygotes are apparently healthy and their hematological data are within the normal range. In the present report, Hb Yaizu was identified

by protein chemistry and DNA sequence analysis, which confirmed the G-to-A mutation in codon 79. Isoelectric focusing shows that it is slow-moving compared to Hb A. Its HPLC profile is near the S window with a 4.77-min retention time on the Biorad Variant II system (fig. 1). The chromatographic pattern of Hb Yaizu is similar on both HPLC and DE-52 microcolumn, being slower than Hb S profiles due to the side chain of the asparagine residue at beta 79(EF3) exhibiting high affinity into the column matrix. Its beta globin gene cluster haplotype is reported here for the first time and it indicates a Mediterranean genetic origin. Since the beta globin gene cluster haplotype of the Japanese case was not determined, it is not possible to say that it shares the same genetic origin with the Turkish case.

## Conclusion

We have presented the details of Hb Yaizu, a rare hemoglobin variant that may be important to hemoglobinopathy screening programs, although its clinical significance is unclear.

## Acknowledgments

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