Molecular characteristics of three hemoglobin variants observed in a Chinese population: Hb Ube-1 [β98 (FG5) Val→Met], Hb Ube-2 [α68 (E17) Asn→Asp] and Hb Ube-4 [α116 (GH4) Glu→Ala]

YUE HUANG, MIN LIN, CHUN-PING LIN, JIAO-REN WU, LUO-HAN ZHENG and LI-YE YANG

Department of Central Laboratory, Chaozhou Central Hospital, Chaozhou, Guangdong 521021, P.R. China

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Abstract. In this study we report three cases of Hb Ube-1, one case of Hb Ube-2 and one case of Hb Ube-4 in mainland China. One case of Hb Ube-1 had the clinical manifestation of hemolytic anemia. Hb Ube-2 and Hb Ube-4 did not appear to be associated with clinical or hematological abnormalities. The variants were detected by Hb electrophoresis during a thalassemia screening. Genomic DNA was extracted from the peripheral blood leukocytes of Hb specimens. α1, α2 and β-globin genes were amplified by polymerase chain reaction (PCR). All variants were confirmed by DNA sequence analysis and the PCR-restriction fragment length polymorphism assay.

Introduction

A recent query of the Human Hemoglobin Variant database (HbVar) (http://globin.cse.psu.edu) identified 652 variants of the α-globin chain and 775 variants of the β-globin chain. The majority of these hemoglobin variants do not have any hematological or clinical effects and are often found incidentally, for example during newborn screening or health examination, and are finally identified by DNA sequencing.

Hb Ube-1 [β98 (FG5) Val→Met], also known as Hb Koln, was first observed in Cologne, Germany, by Pribilla in 1965. It is often found in Europe and America, and has occasionally been reported in Japan, South Korea and Taiwan (1-3). Hb Ube-1, in which the β98 (FG5) valine residue is replaced by methionine (G→A), is the most frequent unstable hemoglobin. However, it presents very few features that allow its distinction from other unstable hemoglobin variants. Mild anemia, jaundice and persistent excretion of dark urine characterize the clinical manifestations of Hb Ube-1. The relationship between the amino acid substitution (Val→Met) and the instability of this hemoglobin suggests that the valine residue is in contact with the heme in the normal β-chain. The substitution of valine for methionine changes the steric configuration of the β-globin, which either alters the enzymatic reduction of iron or results in an increased conversion of meta-hemoglobin into denatured hemoglobin. When Hb Ube-1 is in the oxygenated conformation, the molecule is saturated with heme groups and is stable. The transition to the deoxygenated state results in the loss of heme groups, the instability of Hb and the precipitation and formation of Heinz bodies, which are cytoplasmic degradation products attached to the cell membrane (4). Heinz bodies induce hemolysis and splenomegaly (5).

Hb Ube-2 [α68 (E17) Asn→Asp] was originally observed in a Japanese individual in 1967 (6). To our knowledge, it has only ever been observed in the Japanese and Taiwanese population (7). This Hb variant resulted from an AAC→GAC mutation at codon 68 of the α-globin gene. Hb Ube-2 carriers do not have clinical symptoms or hematological changes, and their oxygen affinity is normal.

Hb Ube-4 [α116 (GH4) Glu→Ala] is an abnormal hemoglobin, that was first observed in a Korean family residing in Japan (8). To the best of our knowledge, all Hb Ube-4 carriers have been found in Korea or Japan (9). This hemoglobin variant results from a GAG→GCG mutation at codon 116 of the α1-globin gene. Hb Ube-4 carriers also do not have clinical symptoms or hematological changes, and their oxygen affinity is normal.

Hb Ube-4 [α116 (GH4) Glu→Ala (α1)] is an abnormal hemoglobin, that was first observed in a Korean family residing in Japan (8). To the best of our knowledge, all Hb Ube-4 carriers have been found in Korea or Japan (9). This hemoglobin variant results from a GAG→GCG mutation at codon 116 of the α1-globin gene (10). Hb Ube-4 carriers also have no clinical symptoms or hematological changes.

According to previous reports, Hb Ube-1, Hb Ube-2 and Hb Ube-4 have not been reported in mainland China. In this study, we report the molecular and clinical features of these three rare variants in mainland China.

Materials and methods

Hematological analysis. Specimens were analyzed by cellulose acetate electrophoresis (pH 8.6) or agar gel electrophoresis (pH 8.6), and the percentage of Hb variants was measured by densitometry on alkaline electrophoresis or automated high-performance liquid chromatography (HPLC) (11,12). Tests for
Hb stability were carried out using the isopropanol and heat methods (13,14). Hb variants were dissociated by parachloro-mercuribenzoate (PCMB), which differentiates α- or β-globin mutation (15).

**DNA analysis.** Genomic DNA was extracted from the peripheral blood leukocytes of these specimens with the genomic DNA mini-preparation kit (Decipher Bioscience Shenzhen Ltd.) as previously described (16). α- and β-globin genes were amplified by polymerase chain reaction (PCR) in a MJ Mini thermal cycler (Bio-Rad Co.). The primer and product lengths were described previously (17-19) (Table I). The β-globin gene was sequenced using primers that flank the three exons of the β-globin gene, and DNA was amplified using Takara LA Taq. After initial denaturation at 97˚C for 7 min, 35 cycles of PCR (94˚C for 30 sec, 46˚C for 30 sec and 72˚C for 2 min) were carried out.

The amplification process of the α-globin gene was similar to that of the β-globin gene. The α1-globin gene was amplified in 50-µl PCR reaction mixtures containing 0.1 µg DNA, 15 pmol of primers, 200 µmol dNTPs and 2.5 units TaqDNA polymerase (Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., China) in 10 mmol/l Tris-HCl (pH 8.0), 50 mmol/l KCl and 3 mmol/l MgCl2. PCR conditions were as follows: 95˚C for 3 min to activate the DNA polymerase, followed by 35 cycles at 98˚C for 40 sec, 60˚C for 5 sec and 72˚C for 1 min. All amplified products were separated on 2% agar gel and detected with UV light after staining with ethidium bromide. The PCR products were analyzed by DNA sequencing with the ABI 3700 automated sequencer.

**Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).** The β-globin gene was amplified with the sense primer 5'-ACTCTTGGGTTTCTGATAGGCACT-3' and antisense primer 5'-AAAAGAAGGGGAAAGAAAACATC-3'. The length of the PCR products was 228 bp. Primer Premier 5.0 software showed that this PCR product of the β-globin gene created two Hin1 DNA restriction sites in Hb Ube-1, and only one restriction site in the normal control. Hb Ube-1 could be cut by Hin1 into 59-, 105- and 164-bp bands, while the normal control could only be cut into 164-bp bands.

**Table I. Primers and length of globin amplification.**

<table>
<thead>
<tr>
<th>Primers</th>
<th>5'-3' sequences</th>
<th>Length of PCR products (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-B</td>
<td>CCATGCCTGGCACGCTTTGCTGAG</td>
<td>880</td>
</tr>
<tr>
<td>α1-L</td>
<td>TCCCCACAGACTCAGAGAGAACC</td>
<td>880</td>
</tr>
<tr>
<td>α2-D</td>
<td>AACACCTCCATTGTTTGGGCACATTCC</td>
<td></td>
</tr>
<tr>
<td>α2-L</td>
<td>TCCCCACAGACTCAGAGAGAACC</td>
<td>442</td>
</tr>
<tr>
<td>β-3F</td>
<td>GTGTACACATATTGACACAAA</td>
<td></td>
</tr>
<tr>
<td>β-4R</td>
<td>AGCACACAGACCAGCACGTT</td>
<td></td>
</tr>
</tbody>
</table>

**Table II. Summary of the hematological findings of the three Hb Koln carriers.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proband</th>
<th>Mother</th>
<th>Father</th>
<th>Brother</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender-age (years)</td>
<td>M-21</td>
<td>F-43</td>
<td>M-45</td>
<td>M-1.5</td>
</tr>
<tr>
<td>RBC (10^12/l)</td>
<td>4.61</td>
<td>3.32</td>
<td>4.84</td>
<td>4.84</td>
</tr>
<tr>
<td>Hb (g/l)</td>
<td>132.0</td>
<td>96.0</td>
<td>143.0</td>
<td>90.0</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>95.0</td>
<td>97.4</td>
<td>85.2</td>
<td>76.2</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28.6</td>
<td>28.9</td>
<td>29.5</td>
<td>22.9</td>
</tr>
<tr>
<td>RDW-CV (%)</td>
<td>15.8</td>
<td>17.2</td>
<td>12.4</td>
<td>20.4</td>
</tr>
<tr>
<td>Ret (%)</td>
<td>4.9</td>
<td>4.1</td>
<td>1.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Hb A (%)</td>
<td>89.9</td>
<td>89.8</td>
<td>96.6</td>
<td>87.7</td>
</tr>
<tr>
<td>Hb F (%)</td>
<td>1.0</td>
<td>0.9</td>
<td>0.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Hb A2 (%)</td>
<td>2.7</td>
<td>2.8</td>
<td>2.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Hb Koln (%)</td>
<td>6.4</td>
<td>6.5</td>
<td>-</td>
<td>6.1</td>
</tr>
<tr>
<td>Basophilic stippling of erythrocytes</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

RBC, red blood cell count; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; RDW, red blood cell distribution width; Ret, reticulocytes.
Exon 3 of the α1-globin was amplified with the sense primer 5'-CGGCCCCACTGACCCTCT-3' and antisense primer 5'-GTACGGGTGCAGGAAGGG-3'. The length of the PCR product was 232 bp. The PCR product was digested by Bsh1236Ⅰ. Hb Ube-4 could be cut into 72- and 160-bp bands, while the normal control could not be digested, and maintained the 232-bp band.

The digestive products were separated with DNA markers on 2% agar gels containing ethidium bromide. The bands were visualized under UV light.

**Results**

**Hb Ube-1 [β98 (FG5) Val → Met].** The proband was a 21-year-old Chinese male from Hunan, China. He was a medical outpatient seen for an abnormal hemoglobin on electrophoresis at Chaozhou Central Hospital, Guangdong, China. Physical examination revealed that the sclera was mildly jaundiced, the spleen was touchable below the left rib edge, and the liver was not touchable. Liver function tests revealed total bilirubin levels of 39.3 µmol/l (2-20 µmol/l) and indirect bilirubin of 35.6 µmol/l (2-14 µmol/l). The hematological data of the family is summarized in Table II. This variant migrated as a multiple Hb component between Hb A2 and Hb A on cellulose acetate electrophoresis at pH 8.6 (Fig. 1A). Its percentage was 6-7% of the total Hb, as measured by HPLC. Thermic instability and isopropanol precipitation tests were positive, revealing that this abnormal hemoglobin was unstable. Heinz bodies were observed in the peripheral blood (data not shown).

Sequencing of the three exons of the β-globin gene indicated a transition from G → A in the first position of codon 98 (Fig. 1B). The results of the PCR-RFLP are shown in Fig. 2A. These results revealed that the proband, his mother and his brother were Hb Ube-1 carriers.

**Hb Ube-2 [α68 (E17) Asn → Asp].** This abnormal hemoglobin was found in a 31-year-old female who was a medical outpatient for premarital thalassemia screening in Chaozhou Central Hospital. A complete blood count at the time of Hb analysis revealed the following: RBC 3.16x10¹²/l, Hb 113 g/l, MCV 99 fl, RDW 12.9%; WBCs and platelets were normal.
The Hb variant patterns were revealed by agar gel electrophoresis at pH 8.6 (Fig. 1C). This variant moved towards the node faster than Hb A, but slower than Hb H. The Hb variant, Hb A and Hb A2 were measured at 26.16, 71.3 and 2.54%, respectively. The Hb stability tests were normal.

This abnormal hemoglobin was dissociated by PCMB, and it was found that the hemoglobin variant was due to an α-globin mutation. The PCR products of the α-globin gene were sequenced and an AAC→GAC mutation at codon 68 of α1-globin was observed (Fig. 1D). PCR-RFLP identified Hb Ube-2 (Fig. 2B). These results were all suggestive of Hb Ube-2.

**Hb Ube-4 [α116 (GH4) Glu→Ala (α11)]**. The proband was a 30-year-old asymptomatic Chinese male medical outpatient from the Chaozhou area who was admitted for premarital thalassemia screening. The abnormal hemoglobin was found by Hb electrophoresis and the blood sample was sent to our laboratory for further identification. A complete blood count at the time of Hb analysis showed RBC 5.16x10^12/L, MCV 88 fl, MCH 30.2 pg, RDW 12.9%; WBCs and platelets were normal, as was his red blood cell morphology. The Hb variant patterns were revealed by agar gel electrophoresis at pH 8.6 (Fig. 1E). This variant moved slightly faster than Hb S at an alkaline pH. The sample was analyzed by automated HPLC, and the percentages of the Hb variant, Hb A2 and Hb A were 20.61, 1.66 and 77.73%, respectively.

The abnormal hemoglobin was dissociated by PCMB and found to be an α-globin mutation. The whole α1-globin gene was amplified by PCR, and sequence analysis showed a GAG→GCG substitution at codon 116, which resulted in a transition from glutamine (Glu) to alanine (Ala) (Fig. 1F). The results of PCR-PFLP are shown in Fig. 2C.

**Discussion**

The majority of hemoglobin variants do not have any hematological or clinical effects; they are often found incidentally during procedures such as thalassemia screening. To our knowledge, Hb Koln is the most common unstable hemoglobin variant, and causes hemolytic anemia. Mild anemia, jaundice, persistent excretion of dark urine and splenomegaly characterize the clinical manifestations of Hb Ube-1. The significant reduction of certain globin chains produces the effects of thalassemia, such as hemolytic anemia and microcytes (4). However, in our study, the proband had no anemia or microcytes, since Hb Koln increased the oxygen affinity during procedures such as thalassemia screening. To our knowledge, Hb Koln was 6-7%, less than that of Hb variants which resulted from previous reports, the content of Hb Koln in these three Hb Koln carriers was normal.


**References**


