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Low affinity and unstable hemoglobin variant caused by AAC→ATC (Asn→Ile) mutation at codon 108 of the β-globin gene

We describe the clinical presentation and DNA analysis of a patient who harbors the AAC→ATC (Asn→Ile) mutation at codon 108 (G10) of the β-globin gene. Our case represents the second report of this hemoglobin (Hb) variant that shows characteristics of both low oxygen affinity and unstable Hb.

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β108 (G10) Asn is located at the $\alpha_1\beta_1$ sub-unit interface at the central cavity of the Hb molecule. At this site, the asparagine residue is uncharged but through its amide group forms hydrogen bonds with other residues of the α- and β-globin chains. Four Hb variants have been described at this site: Hb Yoshizuka[1] Asn→Asp (negative charge), Hb Presbyterian[2-4] Asn→Lys (positive charge), Hb Shizuoka[5] Asn→His (positive charge), and Hb Schlierbach[6] Asn→Ile (hydrophobic). Both Hb Yoshizuka and Hb Presbyterian exhibit low oxygen affinity and high co-operativity, suggesting that any charge, positive or negative, at position β108 disrupts $\alpha_1\beta_1$ contact and alters the electrostatic properties of the central cavity. It results in destabilization of the Hb molecule, favoring the deoxy (T) over the oxy (R) conformation.[7] Other aspects of the pathophysiology of these Hb variants are different. Hb Presbyterian shows an increased Bohr effect while Hb Yoshizuka shows a decreased Bohr effect.[8] Moreover, the oxygen affinity of Hb Yoshizuka is insensitive to changes in chloride concentration while Hb Presbyterian shows a pronounced chloride effect, exhibiting a P^{50} almost identical to HbA at low chloride concentrations.[9] We describe the clinical presentation and DNA analysis of Hb Schlierbach (β108(G10) Asn→Ile; AAC→ATC) in a Chinese female.

A 53-year old housewife with long-standing anemia underwent a cholecystectomy for gallstones at the age of 40. Her blood counts showed: Hb 10.5 g/dL, mean corpuscular volume 101 fL, reticulocytes 3.3%, white blood cells 9.1×10^9/L, and platelets 295×10^9/L. Although not obviously cyanotic, pulse oximetry showed a low oxygen saturation (SaO_2) of 83% that was increased to 95% with oxygen given at a flow rate of 2 L/min. The cardiac and respiratory systems were normal. Arterial blood gas analysis performed in room air showed a normal pH and partial pressures of oxygen and carbon dioxide. Arterial blood gas co-oximetry revealed: oxyHb 83.1% (normal range: 94-97%), carboxyHb 0.2%, MetHb 0.2% and deoxyHb 16.5% (normal range: 0-5%). The low SaO_2 coupled with low oxyHb and increased deoxyHb suggested the presence of a low oxygen affinity Hb variant. Hb analysis by high performance liquid chromatography (Variant Hb Testing System, Bio-Rad, Hercules, CA, USA) showed a Hb variant (29.7%) that was eluted at the HbA2 window. HbA and HbF levels were 68.7% and 1.5%, respectively. The variant was not separated from HbA on electrophoresis at alkaline and acidic pH. Red cell inclusion bodies were demonstrated on two-hour incubation with supravital dye. Tests for unstable Hb using heat and isopropanol precipitation both showed positive results. Other investigations including vitamin B_{12} and folate, total bilirubin, lactate dehydrogenase and haptoglobin were within normal limits. Her ferritin level was increased slightly at 336 pmol/L (normal range: 10–291 pmol/L). The patient had three children and two were found to carry the same Hb variant. Owing to the proportion of Hb variant among total Hb, a β-chain variant was anticipated. Direct sequencing of the β-globin gene based on a protocol previously described[10] showed that the patient was heterozygous for AAC→ATC (Asn→Ile) mutation at codon 108 (Figure 1). While the α-globin genes were not directly sequenced, they showed normal configuration on Southern blot analysis with ζ- and α-globin gene probes.

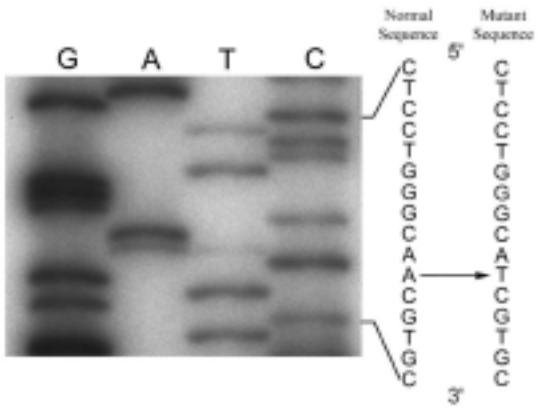

Figure 1. Direct nucleotide sequencing of the β-globin gene, showing AAC → ATC mutation at codon 108.

Ours is the second report of AAC→ATC (Asn→Ile) mutation at codon 108 (G10) of the β-globin gene, the first case being described in a Swiss family[6] and termed Hb Schlierbach. It is interesting that the same Hb variant originates from two separate geographic areas, especially that the nucleotide substitution involves replacement of asparagine by a hydrophobic amino acid isoleucine that is not used at all in the production of normal α- and β-globin chains. The $\alpha_1\beta_1$ contact is expected to be perturbed by mutations at β108 and may contribute to instability of the Hb molecule. This is evident by positive Hb instability tests reported in Hb Presbyterian[7,11] and Hb Schlierbach.[6] In our case, the presence of gallstones, slight reticulocytosis and positive Hb instability tests are in accordance with the unstable nature of AAC→ATC (Asn→Ile) mutation at codon 108 (G10) of the β-globin gene, although the hemolysis may be episodic in nature. The arterial blood gas results in the present case are similar to those previously reported for Hb Schlierbach,[6] and the proportion of Hb variant is also consistent in the two cases (29.7% in our case versus 31% in the previous case). While oxygen dissociation studies in the previous report of Hb Schlierbach clearly demonstrated reduced oxygen affinity, this has not been repeated in our case.

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Two more inv(16) acute myeloid leukemia cases with infrequent CBFβ-MYH11 fusion transcript: clinical and molecular findings

Ten different CBFβ-MYH11 fusion transcripts are reported. Two female patients with inv(16) acute myeloblastic leukemia were positive for type D and E CBFβ-MYH11 transcripts. We investigated the relationship of these rare transcripts with the clinical presentation and therapeutic outcome.

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The pericentric inversion of chromosome 16, inv(16), and the related translocation t(16;16), associated with acute myeloid leukemia (AML)-M4 with abnormal eosinophils (M4Eo), fuse the CBFβ (core binding factor β subunit) (16q22) to the MYH11 gene (16p13). Ten different CBFβ-MYH11 fusion transcripts have been reported. More than 85% of the positive patients have type A, and transcripts D and E account for many of the rest.[1]

Two female patients with inv(16) AML were positive for type D and E transcripts; at diagnosis, both our patients showed typical AML-M4 with eosinophilic abnormalities, and no atypical clinical or laboratory features were recognizable. We investigated the relationship of these type D and E transcripts with clinical presentation and therapeutic outcome (Table 1). CBFβ and MHY11 primers, specific amplifications and specific enzyme restriction digestions were used, as we have previously reported.[2,3]

In patient #1, after amplification, a 1157 base pairs (bp) product was obtained. Following digestion with two restriction enzymes, *Pst*I and *Acc*I, two fragments of 630 bp and 527 bp (PstI) and of 769 bp and 388 bp (AccI) were obtained, respectively. Both these analyses were compatible with a type D CBFβ (exon 5)-MYH11 (exon 8).[4] Similarly, in patient #2, a 1364 bp product was found, and after restriction digestions with PstI and AccI two bands of 769 bp and 595 bp and four bands of 630, 386, 285 and 63 bp, respectively were obtained: in this case a type E CBFβ (exon5)-MYH11 (exon7) was identified. Sequence analysis confirmed the breakpoints (GenBank accession numbers *AF249898, AF249897*).

The breakpoints of CBFβ gene in intron 5 (nucleotide 495) occur in nearly 99% of cases, comprising our two variant ones; in rare cases the breakpoints are located in intron 4 (nucleotide 399). Within the MYH11 gene, the breakpoint occurs in at least eight different points; seven different exons (from exons 7 to 13) are variably included in CBFβ-MYH11 fusion transcripts. Fusion breakpoints mostly occur at exon boundaries but rare case of intra-exonic breaks have also been recently reported.[5] Inv(16) positive AML is associated with a good prognosis, particularly after induction and consolidation chemotherapy including intermediate/high dosage aracytin.[3,6,7,9] In both our patients the response to chemotherapy was excellent with complete clinical and cytogenetic remission (overall survival: 29 and 40 months).

A firm conclusion can be drawn concerning the clinical value of molecular remission of type A CBFβ-MYH11 since patients with long-lasting clinical remission usually display negative qualitative[8] and quantitative polymerase chain reaction (PCR) results in their molecular follow up.[9,10] In contrast, given the limited number of patients with rare CBFβ-MYH11 fusion transcripts analyzed, to our knowledge, no information about the value of detection of such transcripts during follow-up can be obtained from literature. In this context, the present study is the first report of PCR negativity at remission in inv(16) AML patients without type A transcript. This finding, coupled with the typical clinical and morphologic features found at presentation, suggest that the clinical outcome of patients with type