A NEW SILENT β CHAIN VARIANT: Hb HOUNSLOW [β 80(EF4)Asn $\rightarrow$ Tyr]

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ABSTRACT

We report a new neutral hemoglobin (Hb) variant, found, during neonatal screening, in a child originating from Afghanistan. This variant was revealed by cation exchange and reversed phase high performance liquid chromatography (HPLC), but was silent in electrophoretic methods except for globin chain electrophoresis in the presence of urea and Triton X-100. The structural modification was determined by protein structure studies and the substitution established by tandem mass spectrometry (MS/MS). The mutation, located near to the 2,3-diphosphoglycerate (2,3-DPG) binding site, was without any hematological consequences. The pitfalls presented by the presence of neutral Hb variants as modulator factors of the main hemoglobinopathies is discussed.

Key words: Hemoglobin (Hb) variant; Hemoglobinopathies; Modulator factors

INTRODUCTION

Rare, or new, hemoglobin (Hb) variants, are regularly found during systematic programs of neonatal screening for the main hemoglobinopathies. Since such variants may cause hematological disorders or act as genetic modifiers of thalassemias or of sickle cell

anemias, their structural characterization is important. We report here on a new variant found in a newborn baby originating from Afghanistan, a country where several thalassemia mutations are present [1-3].

MATERIALS AND METHODS

In this program the samples were collected from a heelprick onto filter paper when the child was 7-10 days old. A dried blood spot of 0.3 cm diameter was obtained from which Hb was eluted to be analyzed by isoelectric focusing (IEF) and by cation exchange high performance liquid chromatography (HPLC) (VARIANT I; Bio-Rad Laboratories, Hercules, CA, USA). When an abnormal Hb is observed in a baby, venous blood from his parents is collected into EDTA vacutainers for further analysis.

Hematological parameters were obtained by routine methods (Coulter STKS; Beckman Coulter, Miami, FL, USA). Electrophoretic and chromatographic studies were done as previously described [4]. Procedures for separation of globin chains, aminoethylation, and digestion with trypsin are detailed elsewhere [4]. Electrospray mass spectrometry (ES-MS) studies on the globin and by tandem mass spectrometry (MS/MS) on the peptides were done as previously described [4].

RESULTS

An abnormal Hb was found during a neonatal screening in a child, and later in his 33-year-old father, both from Afghanistan. The father's red

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blood cell parameters were: RBC $5.41 \times 10^{12}/L$, Hb 14.9 g/dL, MCV 82.2 fL, MCH 27.5 pg. The variant, amounting to 37.5% of the total Hb, was observed eluting between Hb A and Hb A₂ at 2.96 min. by cation exchange HPLC, using the β -Thalassemia Short Program (Bio-Rad Laboratories) (Fig. 1). It was not separated from Hb A by IEF or by any other electrophoretic methods except for globin chain electrophoresis in the presence of urea and Triton X-100 where it was slightly more hydrophobic than normal (21.5 in an arbitrary scale where normal α -globin migrates at 10.0 and β at 20.0). This increased hydrophobicity of the β chain was confirmed by analytical reversed phase HPLC [5]: its retention time was 12.0 in a scale where normal β elutes at 10.0 and normal α at 20.0 (Fig. 2). Electrospray mass spectrometric analysis of the

Figure 1. Elution pattern of the hemolysate by cation exchange HPLC.

Figure 2. Reversed phase HPLC analysis of the globin chains.

globin showed an abnormal β chain with a mass increased by 49 ± 1 Da as compared to normal, which could correspond to Val $\rightarrow$ Phe, Asp $\rightarrow$ Tyr or Asn $\rightarrow$ Tyr.

Globin was prepared from the total hemolysate and the chains were separated by reversed phase HPLC. Since the two β chains could not be separated at the preparative level, a mixture containing the normal and abnormal β chains was aminoethylated, digested with trypsin and analyzed by reversed phase HPLC. The elution pattern of the tryptic peptides showed that there was an abnormal β T-9 peptide that eluted after the normal one (Fig. 3). Tandem

Figure 3. Reversed phase HPLC elution pattern of the peptides resulting from digestion with trypsin of a mixture of normal and abnormal β chains.

mass spectrometry analysis of this peptide and of the uncleaved β T-8,9, both of which displayed a 49 Da mass increase compared to the normal, showed that the mass difference started at ion Y3 (...Asn-Leu-Lys), for which the value was 423 Da instead of 374 Da. The structural change was therefore located at position 80 where the usual asparagine was replaced by a tyrosine (Fig. 4). This variant has been named Hb Hounslow for the place where the proband lives. According to the HUGO nomenclature, this amino acid exchange should correspond, at the DNA level, to the HBB: c.241A $\rightarrow$ T mutation.

DISCUSSION

Hb Hounslow is an example of a variant which has no charge difference from Hb A and is silent in

Figure 4. Y series of ions observed by MS/MS.

IEF. However, two other variants that carry the same amino acid exchange (Asn→Tyr), namely Hb Saint Mandé [β 102 (G4)] and Hb Aurora [β 139(H17)] are both well separated from Hb A by this technique (6). Hb Hounslow was detected because of its behavior on cation exchange HPLC, in which the retention time depends not only upon the difference in charge but also on other interactions with the stationary phase that result from local modifications induced by the mutation. Several variants, which behave like Hb Hounslow, include Hb Alzette [β 104(G6) Arg→Lys], Hb Puttelange [β 140(H18)Ala→Val] and Hb Rainier [β 145(HC2)Tyr→Cys] [6]. Many of the neutral variants described are clinically silent or have minimal hematological consequences in the heterozygous state but some interact with a thalassemia trait, resulting in a thalassemia intermedia phenotype or with a sickle cell trait and favor sickling. When screening for a specific abnormality, such as Hb S or a thalassemic phenotype, one method may be sufficient to reveal the defect, but it is important to be aware that another silent abnormality may coexist and act as a gene modifier. Thus, phenotype screening should include at least two methods that explore different properties in order not to miss a neutral variant. Additional techniques such as reversed phase HPLC and ES-MS may show the presence of a neutral variant when a combination of IEF and cation exchange HPLC fail to reveal it, while hematological arguments would suggest its presence. For example, these two last techniques were required for detecting Hb Zoetermeer [α 21(B2)Ala→Ser] [7].

Residue EF4, which is modified in Hb Hounslow, is located externally near to the 2,3-DPG binding pocket. A comparison of more than 1,100 sequences of molecules from the globin family (Hbs, myoglobins, cytoglobins and neuroglobins from various species) showed that this position is mostly occupied by an aspartic acid (35%) or an asparagine (30%) and means that conservation of the size of this residue and a possibility for hydrophilic interaction may be important in evolution [8]. Hb G-Szuhu (Asn→Lys), also clinically silent, is the other β chain variant that involves position EF4 [9].

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REFERENCES

1. Kornblit B, Taaning P, Birgens H. β -Thalassemia due to a novel nonsense mutation at codon 37 (TGG→TAG) found in an Afghanistani family. *Hemoglobin* 2005; 29(3): 209-213.
2. Krugluger W, Hopmeier P. Identification of a compound β -thalassemia homozygosity [codon 10 (GCC→GCA) and codon 16 (–C)] in an Afghan family. *Hemoglobin* 2002; 26(3): 317-320.

3. Khan SN, Riazuddin S. Molecular characterization of β -thalassemia in Pakistan. *Hemoglobin* 1998; 22(4): 333-345.
4. Wajcman H, Préhu C, Bardakdjian-Michau J, Promé D, Riou J, Godart C, Mathis M, Hurtrel D, Galactéros F. Abnormal hemoglobins: laboratory methods. *Hemoglobin* 2001, 25(6): 169-181.
5. Wajcman H, Riou J, Yapo AP. Globin chain analysis by reversed phase high performance liquid chromatography: recent developments. *Hemoglobin* 2002; 26(3): 271-284.
6. Hardison RC, Chui DHK, Giardine B, Riemer C, Patrinos GP, Anagnou N, Miller W, Wajcman H. HbVar: a relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. *Hum Mutat* 2002; 19(3):225-233 (<http://globin.bx.psu.edu/hbvar/menu.html>).
7. Harteveld CC, van Helden WCH, Boxma GL, van Delft P, Bakker-Verweij M, Wajcman H, Zanella-Cleon I, Becchi M, Giordano PC. Hb Zoetermeer. A new mutation on the α 2 gene inducing an Ala $\rightarrow$ Ser substitution at codon 21 is possibly associated with a mild thalassemic phenotype. *Hemoglobin* 2007; 31(3): (in press).
8. Marden MC, Dewilde S, Kiger L, Hamdane D, Uzan J, Burmester T, Hankeln T, Moens L, Baudin-Creuza V, Celier C, Wajcman H. Exploiting a list of protein sequences. *Gene* 2007 (in press).
9. Blackwell RQ, Yang HJ, Wang CC. Hemoglobin G Szuhu: β 80 Asn $\rightarrow$ Lys. *Biochim Biophys Acta* 1969; 188(1): 59-64.