Disorders of Erythropoiesis

Hb Bronovo, a new globin gene mutation at α2 103 (His→Leu) associated with an α thalassemia phenotype

Mild α-thalassemia, a common condition in many ethnic groups, presents with hematologic abnormalities almost identical to those found in iron deficiency. We report a new α globin chain variant associated with an α-thalassemia phenotype in two members of a Turkish family.

haematologica 2006; 91:570-571
(*http://www.haematologica.org/journal/2006/04/570.html*)

Most α-thalassemia conditions are mild and can only be ascertained by molecular analysis; indeed, one may argue whether such an analysis is needed. However, most of these patients remain undiagnosed or wrongly treated unless molecular analysis is done and combinations of α^0- and/or α^+- thalassemia defects may induce lethal HbBart's hydrops fetalis and intermediate to severe HbH disease, respectively. For these reasons a correct diagnosis of non-iron-deficient microcytic anemia is warranted.

The propositus was a 21-year old woman of northern Turkish origin (Black Sea coast) living in the Dutch city of The Hague. The propositus and her family were referred because of the woman's mild microcytic anemia in the absence of iron deficiency (Figure 1). Hematologic and biochemical analyses were done using standard procedures including alkaline gel electrophoresis, high performance liquid chromatography (Variant-II, Bio-Rad Laboratories, Hercules, CA, USA) and capillary electrophoresis (Capillarys 2, Sebia, Lisses, France). Genomic DNA was automatically purified (PUREGENE™, Gentra systems Inc., Minneapolis, USA).[1] Modified Gap-polymerase chain reaction (PCR) was used to screen for the seven common α-thalassemia deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{MED-I}$, $-\alpha^{20.5}$, $-^{-SEA}$, $--^{FIL}$, $--^{Thai}$).[2,3]

α globin genes were analyzed using our validated method which is firstly described in this paper. PCR were done on a GeneAmp9700 (Applied Biosystems, Foster City, CA, USA) using the QIAGEN® Multiplex PCR kit (cat.no.206143).

The primers used for sequence analysis were:

S13F (5'-tgtaaaacgacggccagtcgccagccaatgagcgcc-3') (α_2- and α_1-specific)

S6R (5'-caggaaacagctatgacctccattgttggcacattccg-3') (α_2-specific)

S13F and S8R (5'- caggaaacagctatgacctgtccacgcccatgctggcac-3') (α_1- specific).

The first amplification was followed by a second using the following primers:

Universal Forward (F: 5'-tgtaaaacgacggccagt-3')

S18R (5'-caggaaacagctatgacccgttgggcatgtcgtccac-3') (α_2 specific fragment A).

Universal Reverse (R: 5'-caggaaacagctatgacc-3')

S3F (5'- tgtaaaacgacggccagtcacggcaagaaggtggccgac-3') (α_2 specific fragment B).

Similarly, the first α_1 amplification was followed by a booster PCR (α_1 fragment A and B; respectively F and S18R, S3F and R). Amplification was performed in an end volume of 25 μL using 200 ng DNA template, 12.5 μL Mastermix MM, 2.5 μL Q solution (QIAGEN® Multiplex PCR kit cat.no.206143), 1 unit AmpliTaq Polymerase (Promega) and 25 pmol of primers. The following conditions were used: one cycle of 10 min at 97°C, 30 cycles of 30 sec at 97°C, 1 min at 65°C, 2 min at 72°C, followed by five cycles of 30 sec at 97°C, 1 min at 65°C, 3 min at 72°C ending with one cycle of 10 min at 72°C. Booster PCR was performed on 1 μL of the first PCR product in an end volume of 25 μL, using 2.5 μL of a 10×PCR buffer (Promega) and 25 pmol of both primers at the following conditions: 30 cycles of 30 sec at 97°C, 1 min at 55°C, 2 min at 72°C. Sequence analysis of the α_1- and α_2-genes was performed using universal F and R primers and the ABI PRISM® Big Dye Terminators v2.0 Cycle Sequencing Kit on an ABI PRISM™ 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

The propositus (II-3) presented with Hb 11.4 g/dL, MCV 76 fL and a serum ferritin of 80 μg/L. and no signs of hemolysis. Comparable microcytic hypochromic parameters were found in her brother (II-5) while these abnormalities were absent in her father and three sisters (Figure 1).

All separation patterns (alkaline electrophoresis, high performance liquid chromatography and capillary elec-

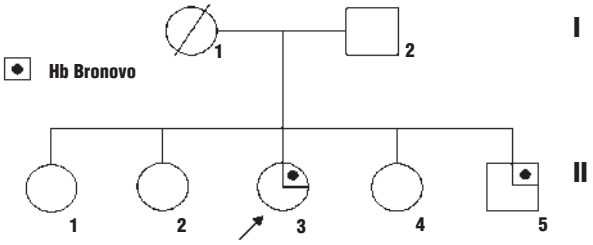

Figure 1. Pedigree of the family with Hb Bronovo.

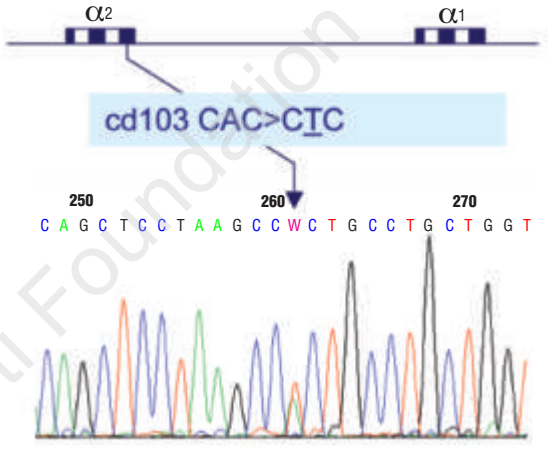

Figure 2. Sequence of the α2 globin gene showing a CAC→CTC transversion at codon 103.

Table 1. Hematologic, biochemical and molecular data.

Parameters	*I-2*	*II-1*	*II-2**	*II-3*	*II-4*	*II-5*
Age/gender	47/M	25/F	24/F	21/F	20/F	13/M
Hb (g/dL)	15.6	13.4	11.4	11.4	13.4	12.1
PCV (l/L)	0.47	0.41	0.35	0.36	0.41	0.37
RBC (10^{12}/L)	4.83	4.49	3.32	4.8	4.33	4.7
MCV (fL)	98	92	104	76	95	80
MCH (pg)	32.4	29.6	34.5	24.0	30.9	25.4
HPLC/capill. electr.	Nor.	Nor	Nor.	Nor.	Nor.	Nor.
Hb A_2 (%)	2.8	2.8	2.9	2.6	2.8	2.8
Hb F (%)	0.6	0.8	1.3	0.2	0.6	1.4
Haptoglobin (mg/dL)	32.3	88	41	83	122	32
ZPP(μmol/mol heme)	34	40	49	32	44	30
Hb-Bronovo	–	–	–	+	–	+

**: 27 weeks pregnant.*

trophoresis) were normal. None of the seven common α-thalassemia deletion defects were observed (*data not shown*). Direct sequencing of the α globin genes revealed a new CAC→CTC transversion at codon 103 of the α_2-globin gene in the propositus (II-3) and her brother (II-5) (Figure 2). We assume that the mutation must have come from the mother who died of a non-hematologic disease. The new Hb variant was named Hb Bronovo. Data are summarized in Table 1.

Both carriers of Hb-Bronovo presented with a typical α-thalassemia phenotype without iron deficiency. One of the sisters (II-2), also anemic at 27 weeks of gestation, did not carry the mutation. Hb Bronovo results in a single His→Leu amino acid substitution at position 103 of the α chain. A similar defect (Hb Contaldo, α 103 His→Arg) was described in association with moderate hemolysis in a quantity between 15 and 20% and was considered unstable.[4] Hb Bronovo was not detectable at any level in the absence of hemolysis and therefore we assume that this mutated chain could be unable to form stable dimers and tetramers generating a post-translational α^+-thalassemia phenotype. Similar phenotypes were observed in Hb Sallanches (α104 Cys→Tyr)[5] and Hb Oegstgeest (α104 Cys→Ser).[6] In both cases no abnormal Hb was observed in the carriers, who presented with a mild α-thalassemia phenotype. In conclusion, although Hb Bronovo seems to induce only mild clinical symptoms in the carrier, in association with α^0-thalassemia defects it might induce more severe clinical symptoms.[7]

Cornelis L. Harteveld, Gerard Steen,° L. Thomas Vlasveld,# Peter van Delft,* Piero C. Giordano**

**The Hemoglobinopathies Laboratory, Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; °Departments of Clinical Chemistry and #Internal Medicine, Bronovo Hospital, The Hague, The Netherlands*

Key words: abnormal hemoglobin, α thalassemia.

Correspondence: Piero C. Giordano, PhD, Clinical biochemical molecular geneticist, Hemoglobinopathies Laboratory, Human and Clinical Genetics, Leiden University Medical Center, Einthovenweg 20, PO BOX 9600, 2300 RC, Leiden, The Netherlands.
Phone: international + 31.71.5276064. Fax: international +31.71. 5276075. E-mail: p.c.giordano@lumc.nl

References

1. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
2. Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of α-thalassaemia deletions and α-globin gene triplication by multiplex polymerase chain reactions. Br J Haematol 2000;108:295-9.
3. Chong SS, Boehm CD, Higgs DR, Cutting GR. Single-tube multiplex-PCR screen for common deletional determinants of α-thalassemia. Blood 2000;95:360-2.
4. Sciarratta GV, Ivaldi G, Molaro GL, Sansone G, Salkie ML, Wilson JB, et al. The characterization of hemoglobin Manitoba or α (2)102(G9)Ser→Arg β2 and hemoglobin Contaldo or α (2)103(G10) Hisr→Arg β2 by high performance liquid chromatography. Hemoglobin 1984;8:169-81.
5. Morle F, Francina A, Ducrocq R, Wajcman H, Gonnet C, Philippe N, et al. A new α chain variant Hb Sallanches [α2 104(G11) Cys→Tyr] associated with HbH disease in one homozygous patient. Br J Haematol 1995;91:608-11.
6. Harteveld CL, Rozendaal L, Blom NA, Lo-A-Njoe S, Akkerman N, Arkesteijn S, et al. Hb Oegstgeest [α104 (G11)Cy→Ser (α1)]. A new hemoglobin variant associated with a mild α-thalassemia phenotype. Hemoglobin 2005; 29:165-9.
7. Kanavakis E, Papassotiriou I, Karagiorga M, Vrettou C, Metaxotou-Mavrommati A, Stamoulakatou A, et al. Phenotypic and molecular diversity of haemoglobin H disease: a Greek experience. Br J Haematol 2000;111:915-23.