Hb Foggia or α117(GH5)Phe→Ser: a new α2 globin allele affecting the αHb-AHSP interaction

We report a novel α2-globin gene allele with the mutation cod 117 TTC>TCC or α117(GH5)Phe>Ser detected in three carriers with α-thalassemia phenotype. The mutated mRNA was present in the reticulocytes in the same amount as the normal one, but no chain or hemoglobin variant were detected. Most likely the amino acid substitution impairs the interaction of the α-chain variant with the AHSP and prevents its stabilizing effect, thus leading to the α-chain pool reduction.

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The free α-globin chains are unstable and the α-hemoglobin stabilizing protein (AHSP), acting as chaperone, binds the α-chains and inhibits their precipitation.[1,2] The determination of the crystal structure of AHSP bound to Fe(II)-α-Hb has revealed that AHSP specifically recognizes the G and H helices of the α-globin chain through a hydrophobic interface that largely repeats the α1-β1 interface of hemoglobin. The interaction between α-globin chain and AHSP has been studied by creating 30 point mutants in α-globin chain. Only three substitutions of amino acids with alanine, Lys99, His103 and Phe117, hindered the interaction with AHSP.[1] It has been demonstrated that in the case of the Hb Groene Hart or α119(H2)Pro>Ser and of the elongated alpha chains (Hb Constant Spring and Hb Pakse) the α-thalassemia defect is due to the alteration of the interaction of the variant chain with the AHSP.[3,4] We report here the first mutant at the cod 117 (GH5) of the α-globin genes associated with α-thalassemia hematologic phenotype.

The novel α2 globin allele was detected in a family originating from Foggia, Southern Italy. The three carriers, the father and two sons, showed microcythemia with normal iron metabolism, Hb A2 in the normal range and no hemoglobin (Hb) variant (Table1). Hematologic parameters were obtained with standard methods. Hb analysis was performed with cation-exchange HPLC and with cellulose acetate electrophoresis Tris-EDTA-borate pH 8.4 buffer. No erythrocyte inclusion bodies were detected with standard methods and no unstable Hbs with isopropanol and heat stability tests. DNA extraction was performed with the salting-out method. The presence of the most common α-thalassemia deletions (-α3.7, -α4.2, --MED, -(α)20.5) and point mutations were excluded with gap-PCR[5] and multiplex ARMS[6] respectively. DGGE analysis identified an anomalous pattern in the amplicon containing the third exon of the α2 globin gene.[7] The sequencing of this amplicon revealed the presence of the mutation α2 cod 117 TTC>TCC in the father and in the sons (Figure 1A). No other mutation was detected in the sequencing of both α1 and α2 globin genes.[7] No globin chain variant was detected in the fresh hemolysate with Liquid Chromatography/Mass Spectrometry.[8] mRNA was purified from reticulocytes of carriers. The α2 globin gene cDNA was amplified with RT-PCR[9] and the sequencing revealed the presence of mutated cDNA (Figure 1B).The semi quantitative analysis of normal/mutated cDNA was carried out with the restriction analysis with the enzyme Ple I, for which the mutation creates a new site 5'-GAGTC$(N)_4$^-3'. The DNA and cDNA PCR amplification was carried out at 20 and 24 cycles on two normal and two heterozygous subjects with the primers (TGACCCTCTTCTCTGCACAGCTC)-forward (Gene Bank sequence NG_000006 position 34.315/37) and (GTCTGAGACAGGTAAACACCTCCAT)-reverse (34.618/42) for genomic DNA (328 bp); (GGCAAGAAGGTGGCCGACGC)-forward (34.070/89) and (GGGAGGCCCATCGGGCAGGAGGAAC)-reverse (34.482/506) for cDNA (295 bp). The PCR product was digested with Ple I restriction enzyme and separated with the electrophoresis on Nu Sieve gel (Figure 1C). In normal subjects only an undigested band was detected. In the carriers, two additional digested bands were detected. The ratio of undigested/digested bands (that is normal/mutated mRNA) was obtained with the KODAK Gel Logic 200 Imaging System and relative software. The average ratio for genomic DNA at 20 and 24 PCR cycles was 54:46 and 55:45 respectively and for cDNA 49:51 and 53:47. This result demonstrated that the mutation does not cause alterations of the mRNA processing and that processes taking place after the chain synthesis account for the absence of the chain/hemoglobin variant in the erythrocytes. The residue 117 (GH5) of the α-globin is in a random coil region and no variations of the secondary structure were predicted using SOPMA software for the Phe>Ser substitution (*http://www.expasy.org*). It has been reported that the same residue is involved in the formation of the binary complex 1:1 between AHSP and α-Hb.[1-2] In this chain variant the replacement of the non-polar bulky side chain of Phe with a much smaller and hydrophilic side chain of Ser (OHCH2–) is expected to alter the αHb-AHSP pocket. Because of this alteration, the α117 Phe>Ser replacement probably impairs the interaction of the new chain variant with AHSP, prevents the formation of the binary complex 1:1 between AHSP and αHb and accounts for the globin instability.[2] This suggests that the variant

Table 1. Hematologic, biochemical data and α-genotype of the carriers.

Parameters	*I-1*	*I-2*	*II-1*	*II-2*
Age(yrs)/sex	64/M	55/F	16/M	14/M
RBC (10^{12}/L)	6.9	4.88	6.15	5.75
Hb (g/dL)	16.4	13.1	14.6	13.8
Ht (L/L)	50	41.3	46.2	42.4
MCV (fL)	72.5	84.6	75.1	73.7
MCH (pg)	23.7	26.8	23.8	24.1
MCHC (%)	32.7	31.6	31.7	32.6
Hb A2 (%)	2.2	2.6	2.0	2.0
Hb F (%)	0.4	0.2	0.3	0.3
Ferritin (ng/mL)	nt	6	49	60
Bilir tot (mg/dL)	nt	0.68	0.75	0.55
Bilir dir (mg/dL)	nt	0.14	0.15	0.11
Bilir ind (mg/dL)	nt	0.54	0.60	0.44
LDH (U/L)	nt	305	259	496
RET (%)	nt	1.0	0.9	0.9
Haptoglobin (mg/dL)	nt	92	73	31
Heinz bodies	nt	absent	absent	absent
Stability tests	nt	normal	normal	normal
LC/MS	nt	normal	normal	nt
Genotype:				
$\alpha2\alpha1/\alpha2^{Hb\ Foggia}\alpha1$	yes	normal	yes	yes

nt: not tested; LC/MS: liquid chromatography/mass spectrometry.

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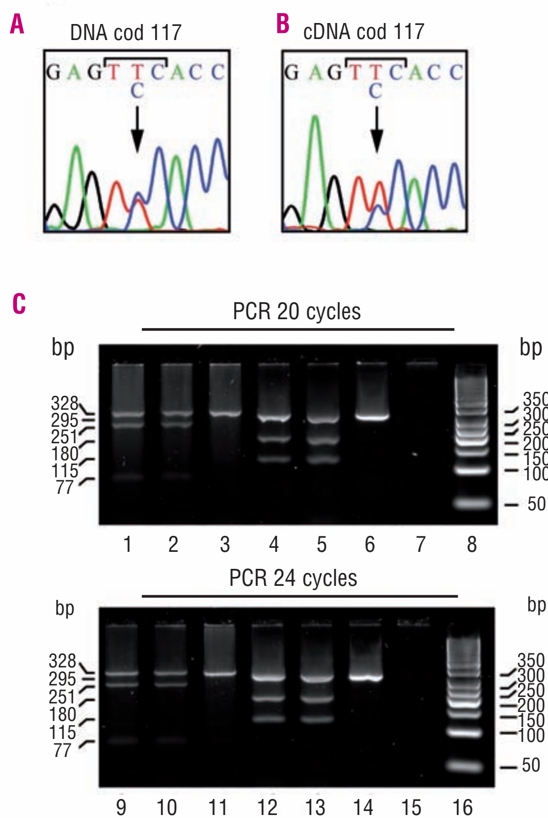

Figure 1. (A-B) DNA and cDNA sequence of the II-2 carrier of the Hb Foggia. (C) Electrophoresis on Nu Sieve 3:1 agarose 4% gel of DNA and cDNA amplicons digested with the restriction enzyme Ple I. Lanes 1-2, 9-10: DNA of the carriers I.1 and II.2; lanes 3, 11: DNA of the control subject 1; lanes 4-5, 12-13: cDNA of the two carriers; lanes 6, 14: cDNA of the normal control subject 2; lane 7, 15: PCR RT- on the carriers I.1 and II.2; lanes 8, 16: DNA 50 bp ladder. On the left and on the right, the fragment's length.

chain was synthesized but very rapidly degraded as free unprotected chain and this led to the reduction of the α-chain pool and to the α-thalassemia phenotype. Consideration must be given to the fact that the Hb Bushey or β122 Phe>Leu has been described in the corresponding position of the β-globin gene.[10] The corresponding globin variant was approximately 50% in peripheral erythrocytes and carriers showed normal red cell parameters. This supports the view that the ASHP plays a specific role in the stabilization of the α-chains.[1,2]

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