Hb Évora [α2-35 (B16), Ser→Pro], a novel hemoglobin variant associated with an α-thalassemia phenotype

We report a novel mutation in the α2-globin gene, codon 35 (T→C), detected in two unrelated Portuguese families. This mutation gives rise to a previously undescribed hemoglobin (Hb) variant, which we named Hb Évora. This variant seems to be responsible for the α-thalassemia phenotype present in its carriers. It cannot be detected by conventional laboratory techniques, probably because of its highly unstable nature.

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A range of conventional laboratory techniques are used to detect hemoglobin (Hb) variants. These techniques are based on the detection of alterations in physical or chemical properties of globin chains or Hb molecules. However, highly unstable Hb variants, commonly associated with hematologic abnormalities in carriers, cannot be detected using current procedures since, although the levels of mRNA are normal, newly synthesized mutant globin chains are rapidly destroyed by proteolysis.[1] In these cases, molecular studies are essential to identify the implicated missense mutation which then allows prediction of the variant's characteristics.

We detected a novel Hb variant in a Portuguese Caucasian family of five members (case A), and in a Portuguese Caucasian individual from another family (case B). The proband in case A, a female child, showed hematologic alterations consistent with an α-thalassemia like phenotype (moderate erythrocytosis, microcytosis, hypochromia and normal levels of HbA_2). The individual studied in case B, a male child, presented with a similar phenotype. Iron deficiency anemia was excluded in both cases.

Red blood cell indices were measured by a Coulter MAXM automated cell counter (Maxm Beckman Coulter, Miami, FL, USA). Hb biochemical studies included isoelectric focusing (IEF) and cation-exchange chromatography (HbGold; Drew Scientific Ltd., Barrow-in-Furness, Cumbria, England). Globin chains were separated and quantified by reversed phase high performance liquid chromatography (RP-HPLC) in a Gold Beckman liquid chromatograph (Beckman Instruments Inc., Fullerton, CA, USA). Inclusion bodies formation and isopropanol Hb stability tests were performed by standard methods. Molecular studies were conducted on genomic DNA isolated from peripheral blood cells by a salting-out procedure. Deletional α-thalassemia was studied by a Gap-polymerase chain reaction (PCR) methodology as previously described,[2] with negative results. The α2- and α1-globin genes were selectively amplified by PCR, and coding regions as well as exon/intron junctions were submitted to nested-PCR, as described elsewhere.[3] These amplified DNA fragments were sequenced using the ABI PrismR BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, according to the manufacturer's instructions. The case A proband was found to be heterozygous for a new mutation in the α2-globin gene, codon 35 T→C (Figure 1). Subsequently, her parents and siblings were studied. Her father and sister also presented an α-thalassemia-like phenotype, and were found to be heterozygous for the above mentioned mutation. Similar results were obtained for the case B proband. Table 1 summarizes these data. The T→C mutation at codon 35 of the α2-globin gene leads to a B16 serine to proline substitution, giving rise to a new Hb variant, which we named Hb Évora based on the geographic origin of family A. This alteration at the end of the B helix of the α2-globin chain is likely to introduce a severe conformational change in its structure, since proline is incapable of participating in α-helix formation, thus compromising α1:β1 contact.[4,5]

Another missense mutation was described at the same position of the α-globin chain and gives rise to an unstable Hb variant, Hb Shinagawa [α1-35 (B16) Ser→Tyr].[6] Additionally, other reported variants associated with mutations located near this position also present some degree of instability, such as Hb Boumerdès [α2-37 (C2) Pro→Arg],[7] Hb Heraklion [α1-37 (C2) Pro→0][8], Hb

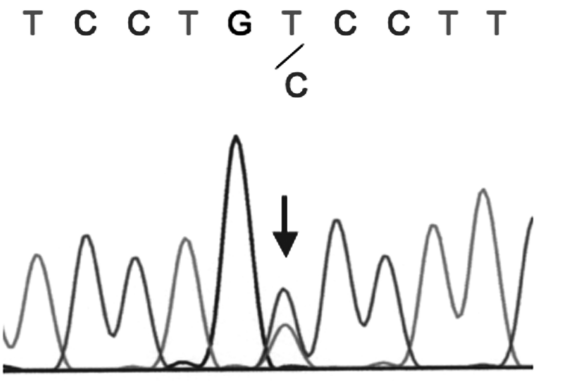

Figure 1. Sequencing of the DNA from the case A proband showing a new mutation in the α2-globin gene, codon 35 T→C, present in heterozygosity. This mutation gives rise to a novel Hb variant – Hb Évora.

Table 1. Hematologic, biochemical and molecular data of the individuals studied.

Parameters	*Proband*	*Father*	*Case A Mother*	*Brother*	*Sister*	*Case B Proband*
Sex-Age	F-Child	M-30	F-34	M-11	F-8	M-4
RBC (10^{12}/L)	5.69	6.03	4.74	4.80	5.19	5.88
Hemoglobin (g/dL)	12.7	15.1	15.6	14.4	12.5	12.8
MCV (fL)	69.7	76.8	98.4	90.1	73.4	66.9
MCH (pg)	22.3	25.0	32.8	30.1	24.1	21.7
MCHC (%)	32.0	32.6	33.4	33.4	32.8	32.4
Hematocrit (L/L)	0.396	0.463	0.467	0.433	0.381	0.394
RDW (%)	12.9	12.3	13.0	12.2	12.6	15.1
HbA2 (%)	2.2	2.2	2.3	2.5	2.4	2.5
Inclusion bodies formation assay	Neg	Neg	Neg	Neg	Neg	Neg
Isopropanol Hb stability	Norm	Norm	Norm	Norm	Norm	Norm
IEF	Norm	Norm	Norm	Norm	Norm	Norm
HPLC	Norm	Norm	Norm	Norm	Norm	Norm
RP-HPLC	Norm	Norm	Norm	Norm	Norm	Norm
α-genotype	$\alpha^1\alpha/\alpha\alpha$	$\alpha^1\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$	$\alpha^1\alpha/\alpha\alpha$	$\alpha^1\alpha/\alpha\alpha$

α^1=α^2-globin gene carrying the codon 35 T→C mutation associated with the α-thalassemia phenotype. RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width; Neg: negative; Norm: normal; IEF: isoelectric focusing; HPLC: high performance liquid chromatography; RP; reversed phase.

Manawatu [α2-37(C2) Pro→Leu],[6] and Hb Queens [α2 or α1-34 (B15) Leu→Arg]6. Some variants, such as Hb Quong Sze [α2-125 (H8) Leu→Pro] and Hb Suan Dok [α2→109 (G16) Leu→Arg] or Hb Plasencia [α2-125 (H8) Leu→Arg][6] were described as highly unstable α-globin variants affecting α1:β1 contact, just as the new Hb Évora.

It is important to note that the co-inheritance of Hb Évora with an α^0-thalassemia deletional determinant might generate HbH disease, as a consequence of the additional proteolytic stress on the abnormal α-globin chain.

As neither abnormal globin chains nor Hb fractions were detected in Hb Évora carriers, supplementary studies including globin biosynthesis assays could be performed to evaluate the deficiency of α-globin chains. However, it is known that biosynthesis studies of hyperunstable α-chain variants are difficult to interpret, producing variable results which probably reflect a complex underlying *in vivo* and *in vitro* physicochemistry; therefore, these studies are not useful for evaluating the status of globin chain imbalance.[9]

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