An identical mutation carried by different α genes: Hb Frankfurt [α50(CE8) His Gln]

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The development of molecular biology methods for characterization of α-chain hemoglobin (Hb) variants leads frequently to the finding of known mutation but affecting a different α-gene.[1] For example Hb G-Philadelphia [α68Asn->Lys] is known since long to be frequently carried by an $\alpha^{-3.7}$ gene in African peoples, where it is expressed at a level of 30-35% while, in other populations, it is an α2-mutant expressed at around 25%. Such a situation has been described for several other α-chain variants. Others were found, according to the case, to be α1- or α2-mutants (1-2). Identical variants may also result from different nucleotide changes encoding for the same amino-acid.[3] We report here three unrelated cases of a new variant, Hb Frankfurt [α50(CE8)His->Gln], found under different α gene situations. When this variant was posted on the HbVar database, it had only been characterized by protein chemistry methods in a single individual from Germany, and the α-gene carrying the mutation has not been determined. In the following years, two additional unrelated cases of this variant were identified in our laboratory. DNA studies revealed that in each case a different α-gene was involved. The first observation of Hb Frankfurt was made during a glycated Hb measurement done in a diabetic German woman aged 85 who had no hematological features.(RBC $4.6x10^{12}$/L, Hb 14.3 g/dL, MCV 85 fL, MCH 31 pg). Table 1 shows the electrophoretic mobility of this Hb under the various experimental conditions used in our laboratory for presumptive diagnosis of Hb mutants.[4] Using the Hb Variant system with the β Thal short program (Biorad Laboratories, Hercules, CA, USA), the variant Hb eluted in the P2 window at 1.5 min and amounted to 24% of the total Hb. RP-HPLC of the globin showed an -chain slightly more hydrophobic than the normal.[5] For structure determination, this abnormal Hb was isolated by flatbed preparative IEF and the globin chains separated by RP-HPLC.[4] The tryptic digest of the aminoethylated -chain showed an αT-6 peptide more hydrophobic than normal, eluting after αT-13. Tandem mass spectrometry of this peptide, which mass was decreased by 9 Da (1824 vs 1833), showed that His at position α50 was replaced by a Gln. Residue α50(CE8) is external, without special function. When, later on, the DNA of this patient was sequenced it was found to be heterozygous at CD50 in the α1-gene (CAG/C).

Table 1. Electrophoretic parameters of Hb A and Hb Frankfurrt

Hb			Citrate agar	Urea		
		pH 6.2		pH 9.0	pH 6.2	Tris pH 10.2
A	0.0	0.0	0.0	0.0	0.0	0.0
Frankfurt	0.23	0.0	0.0	0.0	0.0	0.0

The second case found under similar conditions concerned a 85-year-old woman from Corsica. In this case the abnormal Hb fraction amounted to 27% and the abnormality carried by the α2-gene. In the third patient, from Portuguese origin, an abnormal Hb with the same electrophoretic and chromatographic behavior was observed but differed in its level of expression reaching 36%. His hematological data showed a mild microcytosis (RBC $5.67x10^{12}$/L, Hb 15.2 g/dL, MCV 83 fL, MCH 26.8 pg) suggesting that an α+ thalassemia was associated. DNA analysis revealed, indeed, that the patient carried an $\alpha^{-3.7}$ deletion. His three -genes were sequenced : α1 and α2 were normal but, in the recombinant $\alpha^{-3.7}$ gene, CD50 was found to be CAG (instead of CAC), which encodes for Gln. From a formal genetic point of view, despite that these mutations lead to the same altered protein, they must be considered as different events since the nucleotide change occurs in another gene, even if it is homologous. An open question is to know if they arise from independent mutation in a hot spot or from a gene conversion mechanism. These situations are cause of difficulties when these variants are reported in genetically oriented databases such as HbVar.[6] It is not yet clearly determined if an α1 variant should be distinguished from the corresponding α2 variant by a specific name (or an appropriate suffix such as I for α1, II for α2) and, furthermore, of what should be done when the mutation is carried by an recombinant gene (III). From a phenotypic point of view these situations may also differ because of the level of expression, which varies with a ratio near to 1.2 from one type of α gene to another.[1] Mutants carried by a recombinant -thalassemic gene display levels of expression similar to those of b-chain variants and, in homozygous cases, could mimic homozygosity for β chain variants. In Hb J-Sardegna CD50 encodes for an Asn, which is spontaneously deamidated into Asp,[7] while in Hb Frankfurt as

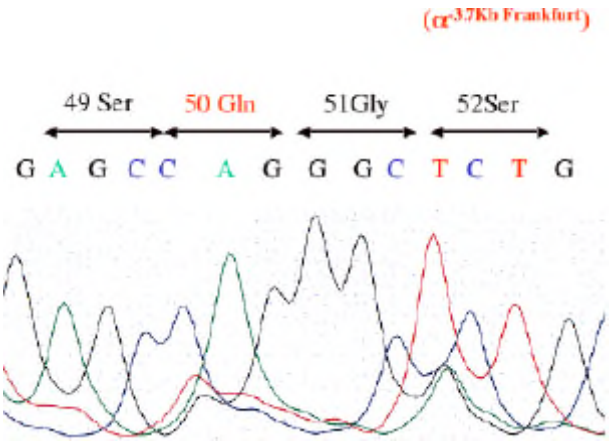

Figure 1: DNA sequence of the region near to CD50 in the -3.7Kb gene. The mutation appears as hemizygot and leads to CAG, which encodes for Gln.

well by DNA sequencing as by protein study a Gln is found, demonstrating that Gln is less prone to post-translational modifications.

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