Further characterisation of Hb Okazaki [β93 (F8) Cys-Arg], a rare hemoglobin variant found in a family from Naples, Italy

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Hb Okazaki [β93(F8)Cys-Arg], was first described in 1984 as unstable hemoglobin variant having high oxygen affinity but normal phenotype, and appearing by isoelectric focusing of the hemolysate, under the Author's analytical conditions, as a sharp band migrating near to the position of HbA.[1] We have encountered this variant in the course of an hemoglobinopathy survey, in three members of an italian family (from Naples).

In the last years the possibility of detecting abnormal hemoglobins was improved by automated High-Performance Liquid Chromatography (HPLC) systems used for the quantification of HbA_2 and HbF, and the detection, provisional identification and quantification of variant hemoglobins. The automated cationic-exchange HPLC Diamat™ (BioRad, Richmond, Ca, USA) used in our laboratory for variant hemoglobin screening, revealed in this case a peak eluted in the so-called *HbS window*; this is a finding common to several Hb variants but the proportion of about 41% of the observed peak and the comparison with a HbS peak (Figure 1) obtained from a sample analysed under the same analytical condition (e.g. amount of sample applied on the column) can suggest the presence of a Hb variant different from HbS. In our case, a simple method, as Hb electrophoretic analysis performed on cellulose acetate with Tris-EDTA-Boric acid buffer at pH 8.5,[2] showed the occurrence of a band migrating between HbF and HbS, demonstrating that the hemoglobin variant was not HbS. On the contrary, on agar gel with sodium maleate buffer at pH 6.1 (Beckman Paragon acid Hb gel kit from Beckman Instruments INC., Fullerton, CA, USA) a band migrating as HbS was observed. In the Hb Okazaki the amino acid Arg take the place of Cys in the β chain of normal HbA; the Arg is a positively charged amino acid and in our electrophoretic condition, at pH 8.5 the protein migrate faster than HbS toward the anode; in fact because of the loss of extra charged groups when Valine is substituted for Glutamic acid, the HbS has a slower electrophoretic mobility in an alcaline media.

The difference between the two variants is resulted also evident using reverse-phase HPLC (RP-HPLC) analysis[3] performed on a Vydac C4 Large-Pore column (Vydac, Esperia, CA. USA); globin separation was achieved in 50 min. by a linear gradient; solvent B (H_2O-Acetonitrile-Trifluoroacetic acid 40:60:0.1) increased from 44 to 70 % in solvent A (H_2O-Acetonitrile-Trifluoroacetic acid 80:20:0.1) in 60 min. at 38°C and pH<3. In this case we have observed, a β chain variant with a decreased retention time due to less hydrophobic character of the Arg than Cys; in fact the elution profile shows the appearence of the most hydrophilic heme group at about 14 min., followed in 39 min. by the β-variant (β^v) peak, in 41 min. by the normal β-chain (β^A), in 43 min. by the β-chain and in 48.5 min. by the β-chain. On the contrary, in the case of HbS, the substitution of Glu by Val in the β chain (β^S) being this latter aminoacid more hydrophobic, bring about an increased retention time of the variant, eluting just behind the β^A (Figure 2).

The absorbance ratio at 220 nm between the abnormal β chain (β^v), the normal β chain (β^A) and the α chain were similar in all carriers: α/β^A was 2.02 and α/β^v 2.24; these ratios indicated that the variant chain was a β globin variant because α / (β^A + β^v) reached a value of about 1, as expected for the α/ non-α globin ratio.

Finally the structural characterisation of the variant was achieved by fast atom bombardment mass spectrometry (FAB/MS).

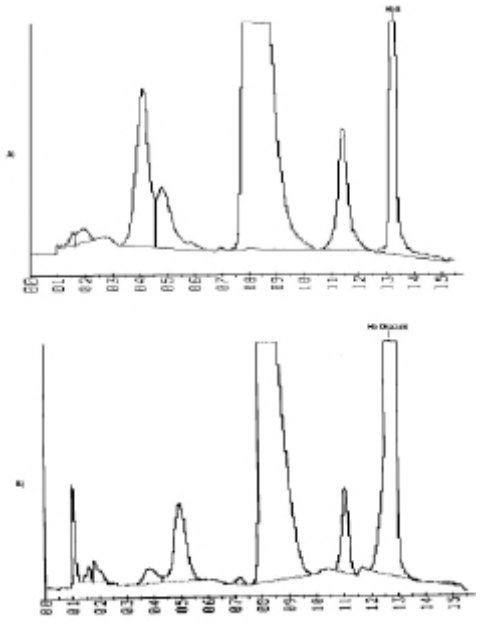

Figure 1. BioRad Diamat™ cationic-exchange HPLC elution profiles. Panel A: HbS (total area 6512; HbS proportion: 35%). Panel B: Hb Okazaki (total area 5723; Hb Okazaki proportion: 41%).

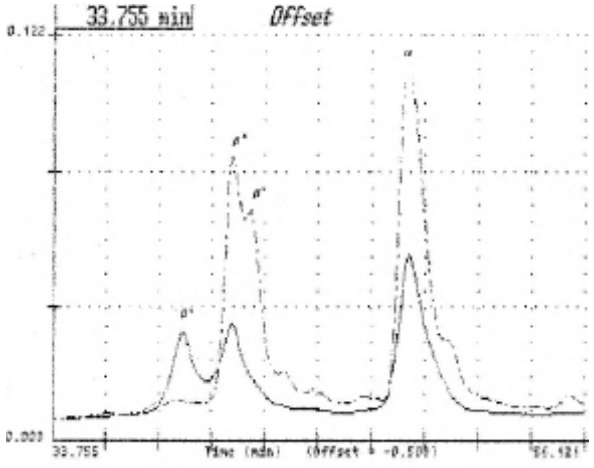

Figure 2. Detail of reverse-phase HPLC elution profile of globin chains separation. The profiles from HbS (dotted line) and from Hb Okazaki (continuous line) were superimposed.

Table 1. Laboratory data of the family members.

	RBC ($x10^{12}$/l)	HGB (g/dl)	MCV (fl)	HCT (%)	MCH (pg)	MCHC (g/dl)	HbA2 (%)	HbF (%)	HbX (%)	Iron (μg/dl)	Ferritin (ng/ml)
Father	4.72	14.6	88.1	44	31	33.2	2.5	0.3	0.0	150	200
Mother	4.50	13.5	87.0	41	30	32.9	2.4	0.2	41.2	100	85
Son 1	4.00	11.7	88.0	35	29	33.4	2.6	0.3	41.4	86	97
Son 2	4.10	12.0	90.0	36	30	33.3	2.8	0.2	40.9	98	100

HbX = Hb Okazaki

The family studies suggested that the Hb Okazaki trait in the two children affected (5 and 7 years old), was inherited from the mother (30 years old), being the father unaffected. Since the mother is of italian origin, probably the abnormal gene may have originated in Italy. The three family members heterozygous for the variant were not anemic, showed normal HbA_2 and other hematologic values (sex and age related) (Table 1) and did not exhibit any severe clinical disadvantages, thus indicating that the variant is phenotypically silent.

The case described here represent to our knowledge, the first report of Hb Okazaki in a non-Japanese family and the second description with further characterisation of these variant even if, probably, as it occurs for hemoglobins with mild hematological consequences, new cases are not always reported.

The presence in areas with high HbS incidence, of Hb Okazaki and other most commons variants having in many screening methods an analytical behaviour like to HbS, should suggest always further investigations: by no way a single electrophoretic or chromatographic test should allow one to make diagnosis of HbS. However we think it right to specify that the pattern characteristic described in this study may not be applicable as a reference for future uncharacterised variants which may present in HbS window; in fact different laboratory setting will hamper identification of any abnormal globin variant based on hemoglobin pattern recognition.

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