HAEMOGLOBIN COVENTRY (β 141 DELETED) IN IRAN

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1. Introduction

Haemoglobin Coventry is a mildly unstable variant with a deletion of leucine at position 141 in what is chemically a β-chain, but must be considered an anti-Lepore β/δ fusion chain for genetical reasons [1]. It occurs at well below 10% in the haemolysate, and in haemolysates of straight forward carriers does not precipitate on routine lability tests for unstable haemoglobins. In the first case studied it was present together with the unstable Hb Sydney and co-precipitated in lability tests yielding an enriched sediment, and this led to its discovery. We now report a finding of this variant in two Iranian sisters where it was associated with α-thalassaemia.

2. Methods

Haematological investigations and determination of oxygen dissociation followed routine practice [2]. Preparation of haemolysate, electrophoresis on cellulose acetate at pH 8.9, agar gel (pH 6.0), and starch gel (pH 8.5), quantitation of Hb F and Hb A_2, heat and isopropanol lability tests, preparation of globin, preparation of two-dimensional diagnostic and preparative tryptic peptide chromatograms, specific and ninhydrin staining of peptides, their elution and amino acid analysis have been summarised in [3]. Separation of globin chains was carried out in the presence of 8 M urea on CM 23 cellulose columns [4]. The methods used for determining the α/non-α globin synthesis ratio in reticulocytes and bone marrow cells have been fully described [1,5].

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3. Results

The propositus was a 36 years old Iranian woman from Kermanshah who came to attention because of a haemolytic anaemia with jaundice and hepato-splenomegaly which had become aggravated after a delivery. She was treated with iron unsuccessfully, but responded to folic acid treatment, and improved steadily. She still had a hypochromic anaemia with reticulocytosis associated with a normal serum iron level and a low percentage of Hb A_2. In addition her blood picture was that of thalassaemia with anisocytosis, poikilocytosis and basophilic stippling. On the basis of these findings an α-thalassaemia was diagnosed. No Hb H inclusion bodies were found however and no Hb H was seen on electrophoresis. The α-thalassaemia was classified as α-thalassaemia type 1 (2 α-chain genes not expressed). For details of the last haematological findings see table 1. The α/non-α globin synthesis ratio in reticulocytes and bone marrow cells labelled with [^{3}H]leucine was 0.77 as compared with a ratio of 0.97–1.1 in 4 normal controls. This is a value expected for α-thalassaemia type 1. The bone marrow showed erythroid hyperactivity with normoblasts and ample iron storage; megakaryocytes were numerous, and the *m/e* ratio was within normal limits. These findings again are compatible with α-thalassaemia type 1. The PO_{50} of the peripheral blood was 19 mm Hg compared with 23 of a normal control, i.e. it was normal.

Table 1
Haematological findings

	Hb (g/dl)	RBC (× 10^{12}/l)	Reticuloc. (× 10^{9}/l)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Serum Fe (μmol/l)	Hb F (%)	Hb A_2 (%)
Propositus	10.0	5.03	500	0.32	63.6	19.9	31.2	25.1	0.8	1.7
Sister	9.2	4.73	–	0.31	65.5	19.5	29.7	–	–	–

3.1. *Hb Coventry*

Some years ago when the anaemia of the patient was severe after pregnancy and delivery, an unstable haemoglobin disease was suspected and a 10 min isopropanol precipitate was sent to Cambridge. On examination of the 'fingerprint' of the isolated β-chain an additional peptide was discovered moving on the fingerprint with the same electrophoretic mobility as β^{A}TpXIV (β 133–144) but lying below as if it was more hydrophilic. Both peptides showed a positive reaction for histidine (fig.1). On amino acid analysis the companion peptide was found to have the same amino acid composition as β^{A}TpXIV, except that the residue derived from β141 Leu was absent. The fingerprint and the amino acid analysis were identical with those found for Hb Coventry [1]. In the case of the original propositus Hbs Coventry and Sydney had co-precipitated. It was assumed that in this case Hb Coventry had co-precipitated with the haemoglobin derivatives arising from the α-globin chain imbalance of α-thalassaemia, which, though not giving rise to Hb H inclusion bodies, were the cause of stippling seen in the thalassaemic cells. When fresh blood was obtained in Kermanshah and sent to Cambridge no precipitate was obtained from the haemolysate, but on freezing and thawing it was noted that a faint precipitate developed which on cellulose acetate electrophoresis remained at the origin. Fingerprints of the β-chain derived from globin of whole blood red cell lysate showed the abnormal peptide only very faintly, and it was just recognisable on staining for histidine. A considerably more deeply stained abnormal peptide was obtained when the globin was prepared from the lysate of reticulocyte enriched suspensions.

In view of the distance of Kermanshah from Tehran it was not possible to conduct an adequate family study. One anaemic sister of the propositus however showed almost identical haematological features (see table 1) with an α/non-α globin synthesis of 0.8. She also possessed a βTpXIV companion peptide typical for Hb Coventry and there was again a difference in the quantity of the abnormal peptide

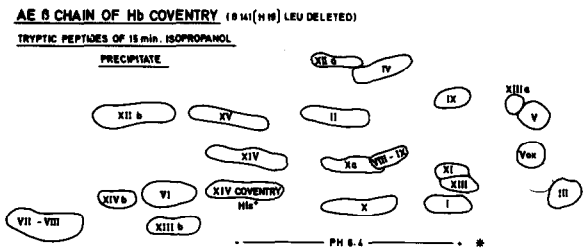

Fig.1. Peptide map of 'β'-chain tryptic peptides of the peptides of the propositus' haemoglobin. Electrophoresis at pH 6.4. Below βTpXIV another less hydrophobic peptide is seen. This peptide is additional to the peptides expected to be found in the β-chain fingerprint. AE: aminoethylated.

Table 2
Amino acid composition of the less hydrophobic companion peptide of β^{A}Tp XIV (see fig.1)

Amino acid	Abnormal peptide nmol	Abnormal peptide molar ratio	Expected for β^{A}Tp XIV
Asp[a]	1.46	1.04	1
Gly	1.76	1.25	1
Ala	5.18	3.67	4
Val	3.87	2.74	3
His	1.46	1.04	1
Lys	1.76	1.25	1
Leu	0	0	1

[a] From the electrophoretic properties of the peptide it can be concluded that this Asp is derived from Asn which has been deamidated during the hydrolysis of the peptide which precedes amino acid analysis

The peptide has the amino acid composition of β^{A}Tp XIV, except that one residue of leucine is missing

when fingerprints were compared from whole blood globin and from globin derived from reticulocytes and young erythrocytes.

4. Discussion

The straightforward heterozygote for the Hb Coventry showed no haematological symptoms and the variant was discovered fortuitously in the propositus because of its co-precipitation with the unstable Hb Sydney which was also present [1]. It seems that in the present case the α-thalassaemia associated with an imbalance of polypeptide chains assisted in the preferential precipitation of Hb Coventry and permitted its subsequent identification. As stated on the first occasion [1], one wonders whether this and similar haemoglobin variants might not be more frequent than is at present realised.

References

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