Hb F Kingston ($^{G}\gamma$55 [D6] Met →Arg)

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A fetal haemoglobin variant was noted in a healthy Jamaican infant of mixed African/Chinese extraction. A two-dimensional chromatogram of the soluble tryptic peptides (Tp) showed 2 'new' ones. One was composed of the last 4 residues of the usually insoluble Tpγ41–59. To permit a tryptic split this required a change of residue γ55 Met to Lys or Arg. The other new Tp contained arginine and was in the position expected for a Tpγ41–55 (55 Arg). As the material was limited it could not be analysed. When after more than 6 years no example of Hb F Kingston had become available it was decided to describe the variant on the basis of the present evidence.

Hb F Kingston *Cord blood screening* *Fetal haemoglobin* *Chain $^{G}\gamma$* *Position γ55 (D6)* *Tryptic peptide mapping*

1. INTRODUCTION

A cord blood screening programme for the detection of sickle-cell disease has been in progress at the main Government Maternity Hospital, the Victoria Jubilee Hospital, in Kingston, Jamaica. Since mid-1973, 95% of cord blood samples from non-surgical deliveries have been examined by haemoglobin electrophoresis on cellulose acetate (CAM) at pH 8.9 and electrophoretically abnormal samples re-examined on agar gel at pH 6.0 [1,2]. A sample manifesting, on alkaline electrophoresis, a band in addition to the normally present Hbs A and F was found which on further investigation was seen to be a new fetal haemoglobin variant which has been designated Hb F Kingston.

2. METHODS

Red cell indices were measured in an electronic cell counter (Coulter 2B 16, Coulter Electronics) and the packed cell volume estimated as the haematocrit. Haematological investigations followed established procedures [2]. Analytical procedures of haemoglobin have been summarised in [3], notably they are those used for the preparation of haemolysates, separation of haemoglobin by paper and cellulose acetate electrophoresis at pH 8.9, and agar gel electrophoresis at pH 6.0, lability tests, preparation of globin, its tryptic digestion and the two-dimensional separation of the tryptic peptides by high-voltage electrophoresis and paper chromatography, the specific staining of the separated tryptic peptides for arginine, methionine, tyrosine, histidine as well as for tryptophan and cysteine, and finally the analysis of their amino acid content. Haemoglobin and globin chain separation by chromatography followed established techniques [4,5].

3. RESULTS

The infant was a healthy female weighing 2.6 kg at birth. Her haematological and clinical development has been entirely uneventful. Haematological observations are summarised in table 1.

On electrophoresis of the cord blood haemolysate an additional haemoglobin band was noted at pH 8.9, but was not seen on electrophoresis on agar gel at pH 6.0. In the cord blood this band con-

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Table 1

Haematological values, serum iron and haemoglobins found in the Casus propositus and her parents

Age	HB F (%)	Hb A_2 (%)	Hb (g/dl)	PCV (%)	MCHC (%)	RBC ($\times 10^{12}$/l)	MCV (fl)	MCH (pg)	Retics (%)	Serum Fe (μg/dl)	% Sat.	Electrophoresis CAM	Agar
Propositus													
10 d	–	–	14.4	49.5	29	4.91	99	29	5.0	–	–	AF+X	AF
1 m	–	–	10.7	32.5	33	4.72	86	23	3.0	–	–	–	–
2 m	–	–	9.5	30.0	32	3.80	77	25	2.0	–	–	–	–
5.25 y	0.3	3.0	11.3	33.0	34	4.56	76	25	1.2	81	18	AA_2	A
Mother													
30 y	0.2	3.0	12.3	37.0	33	4.16	88	30	0.4	88	14	AA_2	A
Father													
28 y	0.3	2.3	14.3	45.0	32	4.66	92	31	0.6	152	30	AA_2	A

tributed ~⅓rd of the haemolysate but at the age of 3 months it was <10%. This suggested that the electrophoretically abnormal haemoglobin was a fetal haemoglobin variant. On electrophoresis at alkaline pH the variant moved more slowly toward the anode than Hb F, at a distance which suggested that it differed from Hb F by one additional positive charge or by one less negative charge per γ-chain.

The variant was separated from the other haemoglobins by paper electrophoresis and eluted. After vacuum concentration, the globin was prepared and submitted to tryptic digestion. The two-dimensional chromatogram (fingerprint) of the tryptic peptide had almost all the features of a fingerprint of the soluble tryptic peptides of Hb F (fig. 1). There were however two differences:

(A) A 'new' peptide not usually seen in the fingerprint of the soluble tryptic peptides of Hb F was seen with the electrophoretic mobility of small peptides with one positive charge and with a very low chromatographic mobility suggesting that it was strongly hydrophilic. It stained with ninhydrin only and not with any specific amino acid stain (Arg, Met, Tyr, His, Trp, Cys);

(B) An additional arginine positive peptide was noted in the neutral region, less hydrophobic than the tryptic peptides γTpIX-a (γ67–76) and γTpIX-b (γ77–82) but more hydrophobic than γTPXIII (γ121–132).

Analysis of the new peptide A showed it to be composed of one residue each of glycine, aspartic acid, proline and lysine (table 2). Amino acid

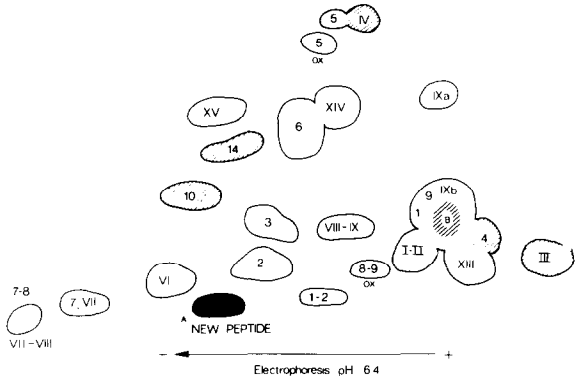

Fig. 1. Fingerprint of the soluble tryptic peptides of Hb F Kingston. Arabic numbers denote α-chain peptides and Roman numbers denote γ-chain peptides. Peptides staining positive for arginine are hatched. The fingerprint differs from that of normal Hb F by two additional peptides A and B. For further details see text.

Table 2

Amino acid analysis of a new tetrapeptide found in Hb F Kingston

Residue	Found (nmol)	Molar ratio	Expected for γ56–59
Asp	6.77	1.3	1
Pro	4.19	0.8	1
Gly	5.29	1.0	1
Lys	6.84	1.3	1

Amounts of <0.3 of a residue were considered contaminants

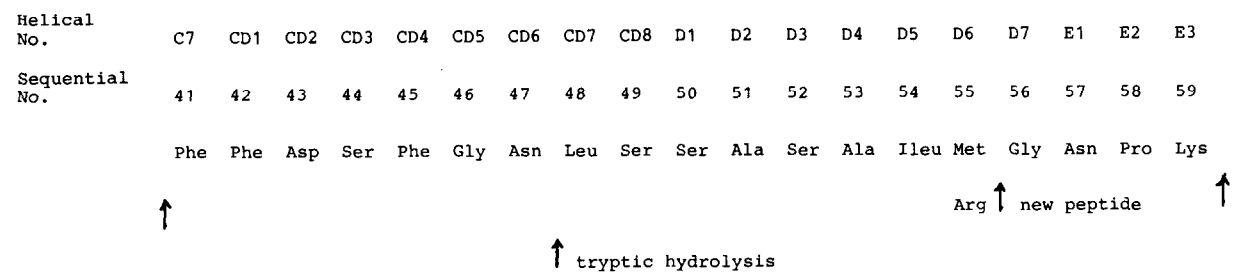

Fig. 2. Diagram of the amino acid sequence of the 5th tryptic peptide of the γ-chain of human haemoglobin. This large peptide is usually insoluble and remains in the tryptic 'core'. In the case of Hb F Kingston a new tetrapeptide corresponding to residues 56–59 appears in the two-dimensional chromatogram of the soluble tryptic peptide as well as a new arginine-containing peptide in the position where a neutral peptide with the electrophoretic and chromatographic properties of γ41–55 (55 Arg) would be expected.

analysis of a peptide involves acid hydrolysis which converts asparagine into aspartic acid. From the electrophoretic mobility of peptide A and the fact that the haemoglobin carried one additional positive charge per γ-chain, it could be deduced that the aspartic acid residue was derived from asparagine. The composition of this peptide (Gly, Asn, Pro, Lys) corresponded to that of residues 56–59 of the γ-chain, the four terminal residues of the tryptic peptide γTpV (residues 41–59). This large peptide does not usually appear in the fingerprint of the soluble tryptic peptides of fetal haemoglobin and remains in the 'insoluble core'.

The appearance of a tryptic peptide with the composition of residues γ56–59 indicates that the residue at position 55 had been replaced by one which made the peptide γ41–59 subject to tryptic digestion at position 55–56 and thus allowed the products of such a digestion to become soluble (fig. 2). Residue 55 is one of methionine; a mutation permitting a tryptic break would either be Met→Lys or Met→Arg. The second abnormality in the fingerprint was an additional neutral peptide (B) which stained positive for arginine. It was closely allied to the other neutral peptides and we did not succeed in getting a satisfactory amino acid analysis of this peptide. Its electrophoretic and chromatographic position and its staining properties would be those expected from a peptide γ41–55 in which γ55 Met was replaced by γ55 Arg. The only methionine positive spot in the fingerprint was seen in the position of γTPXIV (γ133–144) whose N-terminal residue is one of methionine. We conclude that the mutation had been γ55 Met→Arg and name the variant Hb F Kingston.

Man possesses two γ-chains and position 136 of the tryptic peptide γ133–144 (γTpXIV) already referred to above may either be occupied by glycine or by alanine [6]. Therefore, it had to be determined whether residue γ136 was one of alanine or one of glycine in Hb F Kingston. In the tryptic peptide XIV of the $^{G}\gamma$-chain there are two alanines and one glycine (136 Gly, 130 and 140 Ala) and the glycine/alanine ratio is 0.5. In the $^{A}\gamma$-chain peptide there are 3 alanine residues and the Gly/Ala ratio is 0. The tryptic peptide γTpXIV of Hb F Kingston was isolated and found to contain 5.4 ng of Gly and 11.8 of Ala; i.e., the Gly/Ala ratio was that expected for $^{G}\gamma$ (table 2).

The $^{A}\gamma$-chain variant Hb Sardinia (γ75) possesses at position γ75 a threonine instead of an isoleucine. This mutation is very frequent but is associated with the $^{A}\gamma$-chain. Nevertheless, we analysed the tryptic peptide γTpIX-a (γ67–76) of Hb F Kingston and confirmed that it contains the expected residue of isoleucine.

4. DISCUSSION

The mutation Met→Arg is placed at γ55, which is the 6th residue in the D helix. The proline at position H2 of the α-chain has numerous intradimeric ($\alpha 1\beta 1$) contacts with the non-α chain of the haemoglobin molecule, and one of these contracts is with D6 methionine [7]. A mutation of an $\alpha 1\beta 1$ (in this case $\gamma 1$) contact might be expected to destabilise the dimer and cause an unstable

haemoglobin. The simple isopropanol (Carrell) test for unstable haemoglobins was slightly positive, but no more so than it is with any haemolysate containing a large proportion of fetal haemoglobin. Unstable fetal haemoglobins are compatible with the life of a new born but the only one known to us, Hb F Poole ($^{G}\gamma$ 130 [H8] Trp→Gly), caused considerable anaemia, not seen in the case of Hb F Kingston [8]. On examining the model of haemoglobin it can be seen that on replacing the methionine side chain with one of arginine, the substitute can still make one atomic contact with H_2 of $\alpha 1$, and the guanidinium group of the arginine can swing out to the surface of the molecule without interfering with the basic structure of the dimer or of the tetramer.

5. THE FAMILY

The mother of the propositus was predominantly Chinese, 3 out of 4 grandparents being Chinese and the fourth of African–Caucasian origin. The father has one Chinese grandparent, the other three being of predominantly African origin. Their haematological indices were normal (table 1).

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