Haemoglobin K Woolwich: a study of the family of a homozygote

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SUMMARY A family is described in which the proband is homozygous and several relatives are heterozygous for Hb K Woolwich ($\beta 130$ [H10] Lys $\rightarrow$ Gln). These people are clinically and haematologically normal. The relationship between the presence of Hb K Woolwich and β^+ -thalassaemia is discussed. The distribution of Hb K Woolwich in West Africa is discussed and it is seen to be closely associated with the Akan group.

Haemoglobin K Woolwich (Hb KW) was first described in a West Indian family¹ and was then described from Nigeria² and Ghana,³ mainly in the heterozygous form but also in double heterozygous combination with Hb S or Hb C.

In the Ivory Coast it has been seen from 1969 and it is in this part of Africa that most of the subjects with Hb KW are observed.⁴ We have now studied a family of which six members are heterozygotes for Hb KW and one is a Hb KW homozygote.

Methods

Blood samples were collected in EDTA or in ACD and transported on ice to Abidjan. Routine haematological studies were done using standard methods. Reticulocytes were counted after incubating blood with brilliant cresyl blue. Serum iron was measured by the Mecktest method and glucose-6-phosphate dehydrogenase activity by the technique of Motulsky

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and Campbell-Kraut.⁵ Blood grouping was done by standard methods, using test sera from the Centre National de Transfusion de Paris. All the methods were those recommended by the International Committees for Standardization in Haematology (1975).

Initial identification of haemoglobin variants was made by cellulose acetate (cellogel) electrophoresis in tris-EDTA-borate buffer, pH 8.9 to 9.6 and by agar gel electrophoresis in citrate buffer, pH 6.1.⁷

Separation of globin chains by cellulose acetate electrophoresis was carried out in urea-mercapto-ethanol⁸ or PCMB.⁹

Hb A₂ was measured by elution after electrophoresis on cellogel, and by microcolumn chromatography on DEAE-cellulose.¹⁰ Alkali resistant Hb was evaluated by the method of Singer *et al.*¹¹

The haemoglobin solubility was tested by the method of Itano¹² and the heat stability by the isopropanol method.¹³

Globin was prepared from the total haemolysate obtained from III.3 (fig 1) by acid acetone

FIG 1 Pedigree of proband (III.3).
OAB⁺/— = ABO and Rh blood groups.
G⁺/— = glucose-6-phosphate dehydrogenase normal/deficient.
□, ○, AA; ⊗, ⊗, dead; ■, ●, AC;
■, ●, AK; ■, ●, KK; □, ○, not examined.

precipitation. A small portion of the globin was digested with trypsin and the remainder was separated into α -, β -, and δ -chains according to Clegg *et al.*¹⁴ The isolated chains were dialysed against 0.5% (v/v) aqueous formic acid at 4°C and recovered by freeze drying. Fingerprints of the soluble tryptic peptides were prepared from both the unmodified β -chain and the total globin and were stained with ninhydrin and reagents for specific amino-acid residues.¹⁵ Relevant peptides were eluted from lightly stained preparative fingerprints and hydrolysed in sealed tubes at 108°C for 24 or 48 hours with constant boiling HCl containing 1% (w/v) phenol. Where necessary, peptides were purified by paper electrophoresis at pH 3.5 (53V/cm, 2 h) before elution and hydrolysis. After removal of the HCl in vacuo, the hydrolysates were analysed using a Locarte amino-acid analyser.

Results

The fingerprints of the soluble tryptic peptides of the total globin from III.3 showed that peptides β^A TpXIII and β^A TpXIV, which normally give staining reactions for tyrosine and histidine, respectively, were both absent, indicating the absence of normal Hb A from the total haemolysate of III.3. This was confirmed by CM-cellulose chromato-

graphy of the total globin from III.3, which showed that all the β -chains present in the sample eluted earlier than normal β^A -chains.

The fingerprint of the soluble tryptic peptides from these β -chains also showed the absence of β^A TpXIII and β^A TpXIV and the presence of a new peptide containing histidine and tyrosine (peptide 1 in fig 2). This suggested that the haemoglobin was Hb K Woolwich (β 132 [H10] Lys $\rightarrow$ Gln), in which there can be no tryptic hydrolysis at position β 132 and in which β^A TpXIII and β^A TpXIV are replaced by β^K TpXIII-XIV (see fig 3).

Amino-acid analysis of the peptide 1 (table 1) confirmed that the composition was that of residues β 121 to 144 but with only one lysine and with an additional glutamic acid. The electrophoretic mobility of the haemoglobin (and of the abnormal peptide) indicated that lysine must have been substituted by glutamine (which is converted to glutamic acid during acid hydrolysis).

The following additional features of the fingerprint are worthy of mention.

- (1) The minor dipeptide β TpXIIIb (β 131 to 132, Gln—Lys) which results from hydrolysis on

TABLE 1 Amino-acid analyses of peptides 1, 2, and 3 from Hb K Woolwich

Amino-acid	1	Expected for β^A TpXIII + β^A TpXIV	2	3
Asp	1.1 (1)	1	1.1 (1)	1.2 (1)
Thr	1.0 (1)	1		0.9 (1)
Glu	3.8 (4)	3	2.1 (2)	3.8 (4)
Pro	2.0 (2)	2		1.9 (2)
Gly	1.2 (1)	1	1.3 (1)	1.3 (1)
Ala	6.3 (6)	6	3.7 (4)	4.1 (4)
Val	3.9 (4)†	4	2.5 (3)*	4.2 (4)†
Leu	1.0 (1)	1	1.2 (1)	
Tyr	0.9 (1)	1		0.8 (1)
Phe	1.0 (1)	1		1.1 (1)
His	1.0 (1)	1	1.3 (1)	
Lys	1.2 (1)	2	1.1 (1)	

*Value from a 24 h hydrolysate.

†Value from a 72 h hydrolysate.

FIG 2 Fingerprint of the soluble tryptic peptides from the β -chain of Hb K Woolwich. † indicates point of application.

FIG 3 Amino-acid sequence of the residues β 121 to 144 from haemoglobins A and K Woolwich. (↓) = major points of tryptic hydrolysis, (↑) = minor points of tryptic hydrolysis.

TABLE 2 Haematological findings of the proband and his family

Total Hb (%)												
Age	Hb type	KW	A	A ₂	F	RBC ($\times 10^{-12}/l$)	Hb (g/dl)	MCHC (g/dl)	MCV (fl)	Reticulocytes (%)	Serum iron ($\mu g/dl$)	
II.1	72	AKW	36.0	62.0	2.0	—	4.0	13.0	32.5	100	1.0	58
II.2	57	AKW	34.0	65.0	1.0	<1	3.9	12.0	30.7	100	1.6	90
II.3	62	AKW	37.0	61.5	2.5	—	4.4	14.0	35.9	90	1.0	76
III.3 (Proband)	37	KWKW	95.6	—	4.4	—	5.2	16.0	30.7	92	1.5	82
III.4	34	AKW	37.0	62.0	1.0	—	4.9	16.2	33.1	85	1.8	54
III.8	44	AKW	38.0	61.5	0.5	—	5.3	15.7	29.6	81	0.9	103
IV.2	16	AKW	31.0	68.0	1.0	—	4.3	14.2	31.2	79	1.4	64

the carboxyl side of tyrosine $\beta 130^2$ is absent while β TpXIIIa ($\beta 121$ to 130) is present.

- (2) The minor peptide β TpXIVb ($\beta 140$ to 144) which results from a non-specific split on the carboxyl side of asparagine $\beta 139^{16}$ is present.
- (3) Two new peptides are present; peptide 2 is positively charged at pH 6.4, and gives a staining reaction for histidine, while peptide 3 is negatively charged at pH 6.4 and gives a staining reaction for tyrosine. Amino-acid analysis (table 1) shows that peptides 2 and 3 represent the sequences β^K 131 to 144 and β^K 121 to 139, respectively (see fig 3). The recovery of these minor peptides was about 10% of that of peptide 1 (β^K 121 to 144).

Fig 3 shows that the presence of β^A TpXIIIa and β^A TpXIVb, together with the absence of β^A TpXIIIb, are consistent with the substitution $\beta 132$ Lys $\rightarrow$ Gln. The amino-acid analyses of peptides 2 and 3 provide useful confirmatory evidence that the variant is Hb K Woolwich.

The family belongs to the Attié (or Akié) tribe, which is part of the Akan people. The pedigree is shown in fig 1. The proband (III.3) is a 37-year-old male who is married and has eight children. He is in good health, except for spasmodic malarial crises. His mother (II.2), his father (II.3), the sister of his father (II.1), his brother (III.4), one of his cousins (III.8), and this cousin's daughter (IV.2) were also examined. They too are all in good health. Table 2 shows the results of the haematological examinations and the haemoglobin patterns of these seven people. (The presence of Hb KW was detected by cellulose acetate electrophoresis; Hb KW moves slightly faster than Hb A and is easily distinguishable from it.) It is noteworthy that all of the Hb KW heterozygotes have low levels (31 to 38%) of Hb KW, as has previously been noted by Lang *et al.*¹⁷ The solubility test for Hb KW in both the oxygenated and deoxygenated form gave normal results, as did the isopropanol stability test. Functional examination of the haemoglobin from III.3 showed no abnormality (J Rosa, personal communication).

Discussion

Double heterozygotes for Hb KW and Hb S or Hb C have been described before; this, however, is the first description of a Hb KW homozygote. The family, which was discovered during a survey, lives in a traditional environment in the middle of a forest. Although Hb KW coexists with Hb C in a branch of the family, the combination Hb KW/Hb C was not observed.

Lang *et al.*¹⁷ consider the β^{KW} gene to be a β^+ -thalassaemia gene on the basis of results from a family in which α -thalassaemia and Hb KW interacted, and in which defective synthesis of β^{KW} -chains was shown. One might then expect that a Hb KW homozygote would show signs of β -thalassaemia. The proband (III.3) does show an increased level of Hb A₂, but other haematological parameters are normal. All of the Hb KW heterozygotes show a low proportion of Hb KW (31 to 38%) with normal or decreased levels of Hb A₂ and normal haematology. Clearly, without studies of globin chain synthesis, no conclusions can be drawn from this family concerning the relationship of Hb K Woolwich to β -thalassaemia. We hope to carry out a study in the future.

Most of the Hb KW carriers have originated from the Akan, an ethnic group of Ghana and the Ivory Coast. In Ghana, Ringelmann *et al.*³ examined 24 cases of various genotypes (AKW, SKW, CKW), all of which belong to tribes of the Akan group (Kwahu-Busanga). In the Ivory Coast, of 42 subjects having Hb KW, 41 belong also to the Akan group (Baule, Appolo, Attié, Agni) but one subject is of Voltaic origin (Lobi); it is known that the Akan remained for a long time in the Volta Plateau during their migration. Thus it seems that Hb K Woolwich is closely associated with the Akan group.

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