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Haemoglobin D Ouled Rabah (β19[B1] Asn→Lys) in a Tuareg tribe of the Southern Sahara

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SUMMARY Haemoglobin Ouled Rabah found only once before in an Algerian has been discovered at high frequency in the Kel Kummer Tuaregs in Mali. The tribe represents a 'genetic isolate'.

In November to December 1971, an anthropological and genetic study was carried out of the Kel Kummer Tuaregs. These Tuaregs, numbering 367 individuals, belong to a local leader clan nomadising around Menaka (Mali). The clan is highly endogamous, 85% of the genes in the community being derived from 25 'founders' who can be traced to the beginning of the genealogies spanning 15 generations. Thus, the Kel Kummer Tuaregs constitute a 'genetic isolate' built up some 300 years ago (Chaventre, 1971, 1972; Degos, 1973).

This report concerns the finding of an unusual variant of human haemoglobin (Hb) at a high frequency in this community. This variant, Hb Ouled Rabah (β19[B1] Asn→Lys) has been reported once before in an Algerian from the Aures mountains (Elion *et al.*, 1973) and may prove to be a useful anthropological marker.

Methods

(a) THE SURVEY

The sample studied represented about 76% of the Tuareg population.

Genealogical tables showing the family relations between carriers of Hb Ouled Rabah were drawn by A. Chaventre (Fig. 1).

The following methods were used routinely: (i) Electrophoresis of haemolysate using paper, cellulose acetate, starch gel (pH 9·2), and agar gel (pH 6·2) (Lehmann and Huntsman, 1974); (ii) the Itano solubility test (Itano, 1953); (iii) Hb F estimation by alkali denaturation (Singer *et al.*, 1951); and (iv) Hb A_2 estimation (Marengo-Rowe, 1965).

(b) HAEMOGLOBIN STRUCTURAL STUDIES

Studies were carried out on haemolysate prepared from an individual who had been shown, from the survey, to be heterozygous for the haemoglobin variant.

DEAE-Sephadex chromatography of the haemolysate was carried out according to Huisman and Dozy (1965); the variant haemoglobin was further purified, after DEAE-Sephadex chromatography, by paper electrophoresis.

The purified Hb D was converted to globin (Winterhalter and Huehns, 1964) and the latter was separated into α- and β-chains by CM-cellulose chromatography in the presence of 8M urea (Clegg *et al.*, 1966). The α- and β- chains were aminoethylated (Raftery and Cole, 1966), freed of urea and salts by dialysis against 0·5% (v/v) formic acid in water at 4°C, and recovered by freeze-drying. The isolated aminoethylated chains were digested with trypsin (1/50 (w/w), 37°, 2h, in 0·08 NH_4HCO_3); insoluble material was removed by centrifugation and the supernatant was freeze-dried and 'fingerprinted' in the usual manner (Beale, 1967).

Appropriate peptides (see results) were eluted from a preparative-scale 'fingerprint' which had been lightly stained with 0·02% (w/v) ninhydrin in acetone (containing 1% (v/v pyridine)) with constant-boiling HCl and hydrolysed at 108°C in a sealed tube for 16 hours. After removal of excess HCl *in vacuo* over NaOH pellets, the amino acid composition of the dried hydrolysate was determined (Spackman *et al.*, 1958) using a Locarte amino acid analyser.

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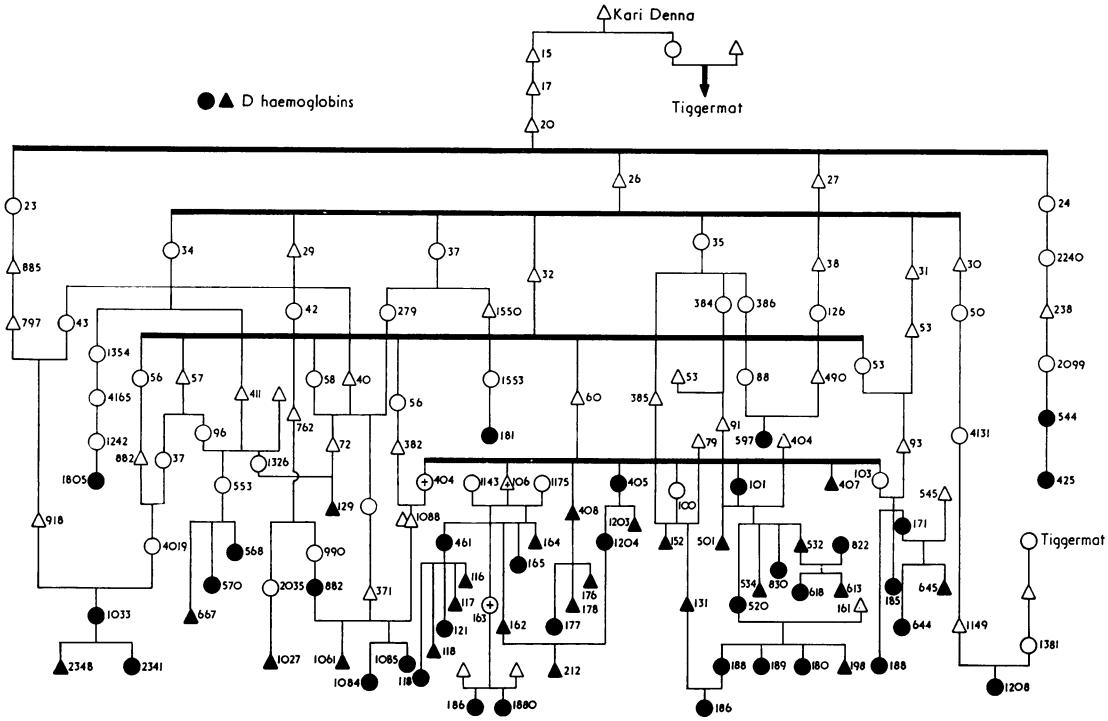

Fig. 1 *Genealogical tree of the Kel Kummer tribe studied.*

Results

(a) THE SURVEY

In 285 samples studied the abnormal haemoglobin was found together with Hb A in 57; a single Hb D band was found in 3 cases, presumably representing the homozygous state. None of the specimens showed a raised Hb A_2, but 3 (1%) showed a raised level of Hb F.

(b) THE HAEMOGLOBIN VARIANT

Examination of the haemolysate by paper electrophoresis showed the presence of Hb A, a single Hb A_2, and a slow-moving (D) haemoglobin. The relative proportions of the three fractions from DEAE-Sephadex were Hb A_2, 2·5%, Hb A, 50·5%, and Hb D, 47·0%. The presence of only one Hb A_2 suggested that the amino acid substitution was in the β-, rather than the α-, chain.

CM-cellulose chromatography in 8M urea of the globin from the purified Hb D showed the α-chains to elute in the normal position; the β- chains, however, eluted later than normal, suggesting a loss of one negative charge, or a gain of one positive charge, per β^D-chain.

The 'fingerprint' of the aminoethylated (AE) α-chains was normal. Comparison of that of the AEβ^D-chains with the pattern obtained from AEβ^A-chains, however, showed the presence of a new, negatively-charged peptide which gave a positive staining reaction for arginine (β^D18-30 in Fig. 2). The amino acid composition of this peptide resembled that of β^A Tp III, except that one of the two residues of aspartic acid normally found on analysis of β^A Tp III had been replaced by a residue of lysine (Table 1). The electrophoretic mobilities of the variant peptide and the whole haemoglobin made it clear that there had been an increase of one positive charge per

Table 1 *Amino acid composition of peptide βTPIII from Hb A and peptides β18–30 and β20–30 from Hb Ouled Rabah*

Amino acid	*β^D18-30*	*β^ATpIII*	*β^D20-30*
Asp	1·0	2	1·0
Glu	2·1	2	2·0
Gly	2·7	3	2·8
Ala	1·0	1	1·0
Val	2·9	3	2·0
Leu	1·0	1	1·0
Lys	1·2	—	—
Arg	1·0	1	1·0

One amino acid residue is approximately 48 nmol for β^DTp 18-30 and 16 nmol for β^D20-30.

Table 2 *Amino acid sequence of the tryptic peptide β18–30 from Hb D Ouled Rabah*

Residue No.	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*
Helical No.	*A14*	*B1*	*B2*	*B3*	*B4*	*B5*	*B6*	*B7*	*B8*	*B9*	*B10*	*B11*	*B12*
Residue:													
Hb^A	↓Val	Asn	Val	Asp	Glu	Val	Gly	Gly	Glu	Ala	Leu	Gly	Arg↓
Hb^D Ouled Rabah	↓Val	Lys↓	Val	Asp	Glu	Val	Gly	Gly	Glu	Ala	Leu	Gly	Arg↓

↓ Indicates the positions of hydrolysis by trypsin.

β-chain, indicating that asparagine β19, rather than aspartic acid β21 (see Table 2) had been substituted (asparagine is converted to aspartic acid during the acid hydrolysis which precedes amino acid analysis).

There was apparently no peptide absent from the 'fingerprint' of the AEβ^D chain; it is, however, to be expected that some tryptic hydrolysis would normally take place between lysine β^D19 and valine β20, and the products, β^D18-19 (Val-Lys) and β20-30 (see Table 2), would be expected to appear on the 'fingerprint' at positions overlapping β^A Tp VI (β60-61, Val-Lys) and β^A Tp III (β18-30), respectively. The spot which apparently corresponded to β^A Tp III was, therefore, eluted, hydrolysed, and analysed; its amino acid composition (Table 1) was that expected for β20-30, showing that limited hydrolysis had occurred between lysine β19 and valine β20 (Table 2). From the relative yields of the two peptides β^D18-30 and β^D20-30, it could be calculated that the extent of hydrolysis at lysine β19 was about 25% of that at arginine β30. This slower rate of hydrolysis probably results from the proximity of aspartic acid β21 and glutamic acid β22 (Table 2), as pointed out by Elion *et al.* (1973).

These results show that this variant is Hb Ouled Rabah (β19(B1) Asn→Lys) (Elion *et al.*, 1973).

Discussion

In the Tuareg of Arabo-Berber tribes so far investigated, the only unusual haemoglobins to have been identified (electrophoretically) have been Hb S, Hb C, and Hb K. The frequency of these variants (2 to 3%) is low compared with that observed in the sedentary people of the oases (10 to 20%) (Cabannes, 1964; Cabannes and Ruffie, 1961; Cabannes *et al.*,

Table 3 *Abnormal haemoglobins in the Tuareg tribe*

Total	*AD*	*DD*	*%*	$q^2\beta^D$	*Thalassaemia*				
					A_2↑	F↑	(A_2+F)↑	*Total*	*%*
285	57	3	21	0·1105	—	3	—	3	1

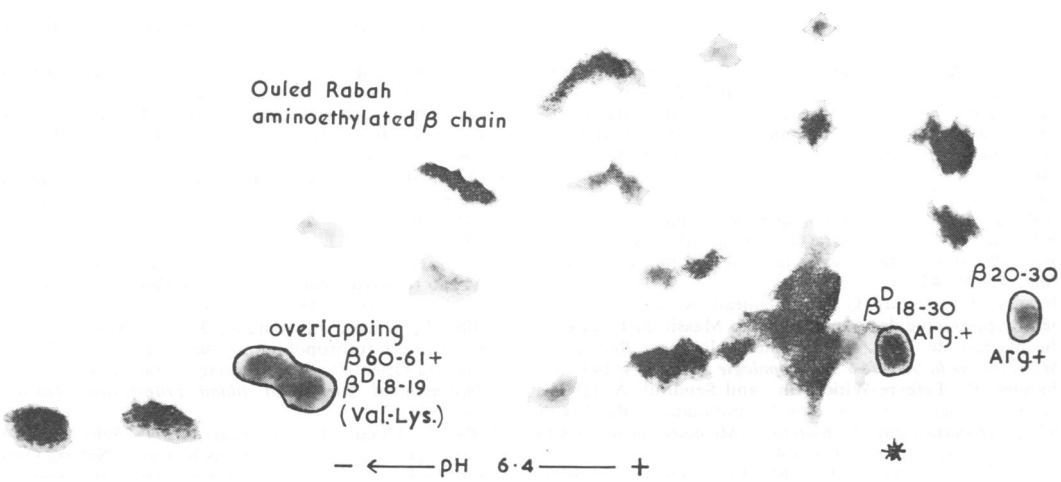

Fig. 2 *'Fingerprint' of the tryptic peptides of the AEβ chain from Hb D Ouled Rabah. Electrophoresis at pH 6·4, 53 V/cm for 1 hour. Ascending chromatography in pyridine–isoamyl alcohol–water (6:6:7, by vol.) for 20 hours. Peptides located by staining with 0·2% ninhydrin in acetone containing 1% v/v pyridine.*

*=origin.

1967, 1969a, b; Ruffie *et al.*, 1959a, b, 1962, 1963; Treilhou, 1969). Table 3 shows the haemoglobin distribution observed in the present survey (see also Mauran-Sendrail and Lefevre-Witier, 1973), from which it can be seen that the frequency of Hb D in the Tuareg tribe studied is very high. Though the Hb D was identified as Hb Ouled Rabah in only a limited number of cases, it is likely that the variant affects the bulk of the tribe, which is a genetic isolate with extremely intricate family ties, as shown in Fig. 2 and previously discussed elsewhere (Chaventre 1971, 1972; Landre and Valat, 1972; Langaney *et al.*, 1973). While it is possible that the variant might have a certain selective value for example against *Plasmodium falciparum* infection (from which the Kel Kummer Tuaregs suffer every year), thus leading to its observed high frequency, the fact that the population is highly inbred could be the sole reason for the high incidence of Hb Ouled Rabah.

The study of different blood markers has revealed a clearly Mediterranean genetic structure in the Kel Kummer Tuaregs, close to that of Moroccan Chleuh or Mauritanian Reguibat (Degos, 1973; Constans and Lefevre-Witier, 1973). The finding of Hb D Ouled Rabah would fall within that genetic pattern; but while it is noteworthy that the male founder of the tribe came from Northern Sahara, the probability of transmission of Hb D Ouled Rabah by that founder (5·7%) should be compared with the probable contribution of the Tuareg group already settled in that area (24·2%) (Jacquard, 1972).

In conclusion, it is felt that Hb Ouled Rabah may prove to be a useful anthropological marker in the study of the tribes of the Sahara.

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