Haemoglobin J in the French Canadian

SALLY KELLY, LUCILLE DESJARDINS, and DONALD JUCKETT

From New York State Department of Health, Birth Defects Institute, Albany, New York, U.S.A.

Haemoglobin J occurs in several ethnic groups, including the French Canadian. At least two such families are known (McCabe, Lange, and Crosby, 1957; Chernoff and Perillie, 1964), one carrying a variant with the structure of haemoglobin J β Baltimore, $\alpha_2\beta_2^{A\beta_2^{16} Asp}$ (Chernoff and Perillie, 1964; Gilcher *et al.*, 1968), the other with the variant not identified further. We describe a third French Canadian family with Hb J and present evidence that the variants in the three families are similar.

Materials and Methods

The variant, herein referred to as Hb Lewiston, was discovered when we noted a fast-moving anodal band in a control sample from a French Canadian chemist in a haemoglobin electrophoresis on cellulose acetate in barbitone buffer of pH 8.6 (Beckman, 1967) (Fig. 1). It was distinguished from other haemoglobins by its differential electrophoretic mobilities in buffers of pH 8.6 and 6.5 (Thorup *et al.*, 1956), its reactions to heat (Dacie *et al.*, 1964), sickling test (Wintrobe, 1967), alkali (Wintrobe, 1967), and its solubility (Itano, 1953).

Structural studies were carried out on toluene-extracted haemolysates (Huisman, 1969) of Hb Lewiston, separated from other haemoglobins by electrophoresis through and elution from polyacrylamide gels (Technical Bulletin No. 130; Heideman, 1964).

The mutation site was localized to the β -chain by hybridization of lyophilized eluate with canine haemoglobin and electrophoresis of the recombinants on cellulose acetate in Tris ethylenediamine tetra-acetic-borate (TEB) buffer of pH 9.0 (Gammack, Huehns, and Shooter, 1960; Huehns and Shooter, 1962).

The altered peptides of Hb Lewiston were identified by combined electrophoresis and paper chromatography (Ingram, 1958; Baglioni, 1961; Lehmann and Huntsman, 1966) of trypsin-digested globin fractions (Anson and Mirsky, 1930; Ingram, 1958) from eluates concentrated by vacuum dialysis (Everall and Wright, 1958). Peptide composition was also revealed by chromatography of trypsin-digested β -chain fractions of globin, isolated on 10 cm. carboxymethyl cellulose columns (Clegg, Naughton, and Weatherall, 1966) on a 20 cm. column of Beckman PA-35 resin in a Beckman 120 C analyser (Crestfield, Stein, and Moore, 1963).

Haematology of the proband was by standard methods (Wintrobe, 1967). The following phenotypic traits were examined: blood group, haptoglobin pattern (Davis, 1964), immunoglobulin concentrations (Stiehm and Fudenberg, 1966), leucocyte alkaline phosphatase (Kaplow, 1955), and creatine kinase activities (Tanzer and Gilvarg, 1959), glucose-6-phosphate dehydrogenase activity (Hsia and Inouye, 1966), and isozymes (Sparkes, Baluda, and Townsend, 1969), galactose-1-phosphate uridyl transferase activity (Beutler and Baluda, 1966) and isozymes (Ng *et al.*, 1969), and serum lactic dehydrogenase isozymes (Fisher and Nixon, 1967). Leucocytes were cultured for cytogenetic analysis (Arakaki and Sparkes, 1963) and palm prints made.

Samples of blood were obtained from survivors in the first reported French Canadian family with Hb J (McCabe *et al.*, 1957). The variant was further characterized by 'peptide mapping', as described above.

The frequency of Hb J in the French Canadian population was estimated by electrophoretic screening of haemolysates from 239 individuals with French Canadian antecedents. 15 were laboratory personnel of the Albany area, 200 were patients at a veterans' hospital near the family homes of the proband and of McCabe's patient, and 24 were members of 10 families with the proband's surname who lived in St. Pascal, the Canadian origin of the paternal line.

Results

Structural studies. Hb Lewiston resembled Hb AJ in its anodal mobility in alkaline buffer and immobility at pH 6.4, heat stability, lack of sickling, alkali denaturation, and ferrohaemoglobin solubility.

The electrophoretic pattern of the variant-canine haemoglobin hybrid was consistent with a mutation in the β chain (Fig. 2), as bands with mobilities of hybrids with β_2^A chains were absent. The proportion of variant, 50% of the total haemoglobin, was also characteristic of β -chain mutants of Hb J.

The peptide map (Fig. 3) was similar to that of Hb J β Baltimore (Baglioni and Weatherall, 1963; Lehmann and Huntsman, 1966). β^A Tp II was missing and β^A Tp III was diminished. There were two peptides with mobilities and staining properties of the new peptides of Hb J β Baltimore: a

FIG. 1. Haemoglobin pattern of proband (top and bottom) showing A and J components after electrophoresis on cellulose acetate in barbitone buffer of pH 8.6.

FIG. 2. Electrophoretic patterns of Hb J Lewiston hybrids. From the top: unhybridized Hb A-canine; Hb A-canine hybrid; Hb J-canine hybrid, in which band of $\alpha_2^{can}\beta_2^A$ is absent; unhybridized Hb J-canine.

FIG. 3. Diagram of peptide map of tryptic digest of Hb J Lewiston globin after electrophoresis in pyridine-acetic acid buffer at 2500 mV. for 60 minutes and ascending paper chromatography with 1:1 pyridine: isoamyl alcohol solvent system for 18 hours.

neutral one, containing tryptophane and only faintly visible with ninhydrin, resembling the mutant peptide $\beta^J\text{Tp II}$, and the other, migrating anodally and containing both tryptophane and arginine, resembling the combination peptide, $\beta^J\text{Tp II-III}$. Column chromatography of the tryptic-digested β -chain polypeptides also revealed two new peptides, which were confirmed by Dr. Samuel H. Boyer as those of Hb J β Baltimore.

The peptide map of the Hb J from McCabe's family was similar to that of Hb Lewiston.

Pedigree. The proband, a healthy woman in her early twenties, several members of her immediate family, and relatives in the paternal line carried Hb Lewiston in heterozygous form (Fig. 4). The mutation was traceable through five generations through the distant cousin, V.17a. Most of the family lives in New England and emigrated from the St. Lawrence River county of Kamarouska, Quebec, in the eighteenth century.

Haematology. Laboratory data of the proband's blood were as follows: white cell count of 6700 cells per cu. mm.; a differential count of 70% neutrophils, 25% lymphocytes, 3% monocytes, and 2% eosinophils, with normal numbers of platelets and normal red cell morphology. There were fewer than 1% reticulocytes and no inclusion bodies. Haemoglobin was 14.7 g.; haematocrit, 43%; red cell count, 4,500,000/cu. mm. Indices were mean corpuscular volume of $96 \mu^3$, mean corpuscular haemoglobin of $32.7 \mu\mu\text{g.}$, and mean corpuscular

haemoglobin concentration, 35%. There was no fetal haemoglobin. Osmotic fragility tests were normal.

Phenotype. The blood type and haptoglobin pattern of the proband were consistent with the pedigree (Table). Concentrations of immunoglobulins, Ig G, 1390 mg./100 ml.; Ig M, 150 mg./100 ml.; and Ig A, 260 mg./100 ml. were within normal ranges for the age. Leucocyte alkaline phosphatase, creatine kinase, galactose-1-phosphate uridyl transferase, and glucose-6-phosphate dehydrogenase (G6PD) activities were normal, as were the isozyme patterns of galactose-1-phosphate uridyl transferase, G6PD, and serum lactic dehydrogenase.

The chromosome complement was normal, 46,XX. Dermatoglyphs included fingertip patterns of ulnar loops and whorls and palm prints with axial triradii in the τ position, ad angles of 38° and 40° , loop patterns in the thenar and hypothenar areas, and normal flexion creases, bilaterally. There were two digital triradii in the left palm, with two adjacent interdigital triradii, cd , and a loop in interdigital area III.

Frequency. The families of the proband and of McCabe's patient lived in adjacent towns in Maine; no relationship was known, and paternal lines carrying the variant emigrated from different counties in Quebec. Hb J was not found in other families in the area, when 200 patients with French Canadian surnames at a nearby Veterans' Administration Hospital were screened, nor was it found in 24

FIG. 4. Pedigree of the proband with Hb J Lewiston.

TABLE
BLOOD GROUPS AND HAPTOGLOBIN PATTERNS
IN PROBAND AND FAMILY

Subject	Hb	Blood Group System					Haptoglobins
		ABO	Rhesus				
			C	D	E	hr	
Proband	AJ	A	+	+	-	+	2-1
Father	AJ	O	+	+	-	+	2-1
Mother	A	AB	+	+	-	+	2-1
Sister	AJ	B	-	-	-	+	1-1

French Canadians of St. Pascal with the proband's surname. Screening of these samples and of 15 controls gave a frequency of Hb J in the heterozygous state of less than 1 in 240 in individuals with French Canadian surnames.

Discussion

The identification of Hb J β Baltimore as the variant in the proband's family and in that of McCabe's patient brings to three the number of French Canadian families in whom Hb J has been described and identified. The three families carry the same molecular aberration. Ten other families or individuals with Hb J β Baltimore have been recorded, to our knowledge: three in negroes (Thorup *et al.*, 1956; Itano and Robinson, 1959; Baglioni and Weatherall, 1963; Weatherall, 1964; Gammack *et al.*, 1961), five in Caucasians of British stock (Gammack *et al.*, 1961; Holman *et al.*, 1964; Lie-Injo *et al.*, 1968; Wilkinson *et al.*, 1967), one in a Dane (Sick *et al.*, 1967) and one in a part negro who is believed to have received his Hb J β Baltimore from a North American Caucasian father (Went and MacIver, 1959).

The coincidence of the same variant in all three of the French Canadian families so far recognized as carriers of Hb J suggests relatedness. None, however, is known. It is also easy to postulate a common origin of Hb J β Baltimore in the French Canadian families and in the six other Caucasian families reported, five of British stock, perhaps in some old Norman who came across with William. Nor is it difficult to reconcile its appearance in these European families with that in the African, if geographic and historic ties are recalled.

The variant haemoglobin may also have arisen independently in these groups by sporadic mutations. A wider distribution in Caucasians and other races, however, would lend credence to this view. A predilection for mutation at the specific gene site might well be a corollary.

The casual manner in which Hb Lewiston was discovered, in one of us, and the lack of association of the variant in previously described families with related disease are evidence of the physiologically adequate role of Hb J β Baltimore in its carriers.

Summary

Haemoglobin J was found in the paternal line of a third French Canadian family. Its structure was similar to Hb J β Baltimore and to the Hb J variants in the French Canadian families previously reported. No cluster of Hb J carriers was evident in a screening of French Canadians in the area where two of the families lived nor in the paternal ancestors' Canadian birthplace. The frequency of the variant in heterozygous form was estimated as less than 1 in 240 French Canadians. Hb J in the proband was associated with normal haematology and biochemical phenotypes.

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