

Haemoglobin Lepore Boston and elliptocytosis in a family of Indonesian-German ancestry

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Summary. A family is presented in which Hb Lepore Boston was found in six individuals over three generations. The gene must have had its origin either in Java (Indonesia) or in what is now the Federal Republic of Germany. The haemoglobin was characterized by amino-acid analysis of the six tryptic peptides that have a different composition in the β - and the δ -chain. The ratio of glycine to alanine in position 136 of the fetal haemoglobin, which was somewhat raised in the Hb Lepore carriers, averaged 31:69.

In addition an elliptocytosis gene was found, which was inherited independently from Hb Lepore; the simultaneous presence of elliptocytosis in three family members did not seem to aggravate the mild anaemia caused by Hb Lepore.

The Lepore haemoglobins are interesting from a molecular-, clinical-, and population-genetical point of view. Structurally they are characterized by the presence of globin chains which are fusion products of δ - and β -chains. These $\delta\beta$ -chains seem to be the result of unequal crossing-over between the closely linked δ - and β -genes (Baglioni, 1962). Three different types of Hb Lepore are known, which differ in the position of the crossing-over. The regions of $\delta\beta$ fusion in Hb Lepore Hollandia, Hb Lepore Baltimore, and Hb Lepore Boston are located between residues 22 and 50, 50 and 86, and 87 and 116, respectively. Hb Lepore Hollandia is rather common among the Papuans of New Guinea (Barnabas and Muller, 1962). Hb Lepore Baltimore has been characterized once in a North American negro (Ostertag and Smith, 1969). Hb Lepore Boston is rather wide spread in middle- and eastern-Mediterranean populations (Fessas, Stamatoyannopoulos, and Karaklis, 1962; Quattrin, 1967; Duma *et al*, 1968) and is also present in Jamaican negroes (Ahern *et al*, 1972). Definite biochemical characterization of the $\delta\beta$ -chain of Hb Lepore Boston has only been performed in some individuals of Italian ancestry (Baglioni, 1965; Labie,

Schroeder, and Huisman, 1966; Baglioni and Ventruto, 1968), in three Jamaican negro families (de Jong, 1969; Ahern *et al*, 1972), in two Macedonians (L. F. Bernini, personal communication), and in a case from Greece (Baglioni and Ventruto, 1968). We now report the finding and characterization of Hb Lepore Boston in a Dutch family of Indonesian and German ancestry. This family possessed in addition a gene for hereditary elliptocytosis, which provided the opportunity to study the interaction between Hb Lepore and elliptocytosis.

Methods

Haematological data were obtained using standard methods. Starch gel electrophoresis of haemolysates was carried out in Tris-EDTA-borate buffer, pH 8.9 (Smithies, 1965). Fetal haemoglobin was estimated using the alkali-denaturation procedure of Singer, Chernoff, and Singer (1951). Quantitation of Hb Lepore and Hb A₂ was done on DEAE-cellulose columns according to the method of Bernini (1969). The methods for the structural characterization of Hb Lepore were the same as described previously (de Jong, Bernini, and Khan, 1971) and comprised the isolation of pure Hb Lepore, conversion to globin, separation of α - and $\delta\beta$ -chains, aminoethylation and tryptic digestion, peptide mapping, and amino-acid analyses of tryptic peptides.

The proportions of the two types of γ -chains, $\Lambda\gamma$ and $\text{C}\gamma$ were determined in the fetal haemoglobin present in

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the carriers of Hb Lepore by amino-acid analysis of the C-terminal cyanogen bromide fragment of the γ -chains (de Jong, 1971).

Results

The index case (III.7, Fig. 1) was first seen at the age of 7 because of a fever of unknown but possibly viral origin. On physical and laboratory examination the only abnormality was a mild anaemia. After recovery from the fever the mild anaemia persisted (iron was administered without success) and was analysed in greater detail. This analysis showed: Hb 10.5 g/100 ml., haematocrit 36%, red cells $4.4 \times 10^6/\mu\text{l.}$, white cells $2300/\mu\text{l.}$ with normal differential count, platelets $150\text{--}000/\mu\text{l.}$, sedimentation rate 2 mm/hour, total bilirubin 0.6 mg/100 ml. (conjugated 0.1 mg/100 ml.), free serum iron 178 $\mu\text{g}/100$ ml., latent iron binding capacity 158 $\mu\text{g}/100$ ml. (saturation 53%). The morphology of the red cells was reported abnormal with anisocytosis and some basophil stippling. The direct Coombs test was negative. The osmotic resistance of the red cells was slightly increased. The bone marrow was somewhat hypoplastic with a mild iron deficiency. The examination of the urine gave normal results.

Following the finding of an abnormal haemoglobin, migrating in the position of HbS, more detailed studies were performed on the blood of the

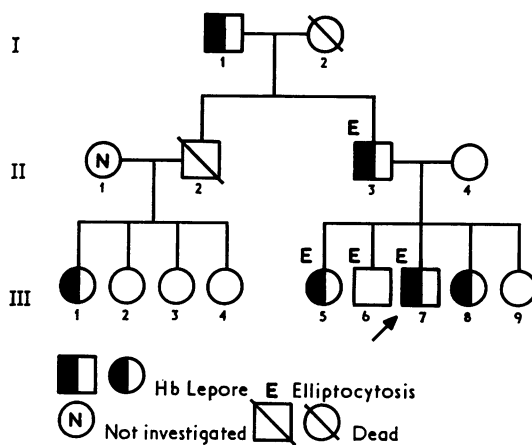


FIG. 1. Family pedigree.

index case and of his living family members. The same abnormal haemoglobin was found in a further five family members in an amount of around 9–10% of all haemoglobin. The family history revealed that III.5 had as a young girl been chronically anaemic, while the father (II.3) when younger had frequently had iron supplementation. The deceased mother (I.2) of the father was Indonesian. The mother of the grandfather, I.1, was likewise

TABLE I
FINDINGS OF THE HAEMATOLOGICAL STUDIES, THE HAEMOGLOBIN

Pedigree Number	Sex	Year of Birth	Hb (g/100 ml.)	Ht (%)	Red Cells ($\times 10^6/\mu\text{l.}$)	MCV (μ^3)	MCHC	Reticulo-cytes (%)	Hb F (%)	G γ :A γ
I.1	M	1884	15.4 <i>14.9</i>	46.8 <i>47.5</i>	6.13	77	33.5 <i>31.5</i>	1.7 <i>1.7</i>	3.5 <i>2.6</i>	39:61
II.3	M	1923	13.7	40.8	6.32	65	34.0	0.6	1.5	28:72
II.4	F	1930	13.2	39.0	3.98	98	33.0	1.0	0.7	
III.1	F	1950	12.9	38.7	5.53	70	34.0	2.9	2.8	23:77
III.2	F	1951	12.7	38.7	4.37	89	33.5	nd	0.8	
III.3	F	1954	14.1	42.7	4.60	93	33.5	nd	0.6	
III.4	F	1958	13.5	40.6	4.66	87	34.0	nd	0.5	
III.5	F	1954	12.5 <i>11.0</i>	38.5 <i>34.0</i>	5.30 <i>4.84</i>	72 <i>70</i>	33.0 <i>32.5</i>	1.8 <i>1.8</i>	3.2 <i>3.2</i>	nd
III.6	M	1957	13.0	37.0	4.30	88	34.5	1.2	1.1	
III.7	M	1960	12.4	39.0	5.55	70	32.0	2.0 <i>0.6</i>	3.5 <i>4.0</i>	35:65
III.8	F	1964	11.6	35.5	5.10	69	33.0	1.3 <i>0.9</i>	4.0 <i>3.2</i>	nd
III.9	F	1967	13.4	40.0	nd	na	33.5	0.8	0.8	

The haematological results date from 1971, with the exception of the italicized data which were obtained in 1974. na=no abnormalities; nd=not done; sp=sporadically present.

Indonesian, from the middle of Java. The father of I.1 had been born like his ancestors in Arnberg (near Düsseldorf, Federal Republic of Germany). The results of a haematological and blood group analysis of the family members are presented in Table I. From this analysis and the morphological appearance of the red cells it will be apparent that apart from the abnormal haemoglobin fraction, hereditary elliptocytosis seems also to occur in the family. No aggravation of the anaemia appears to have resulted from the simultaneous presence in II.3, II.5, and II.7 of the abnormal haemoglobin and the elliptocytosis as compared to the abnormal haemoglobin carriers only (I.1, III.1, and III.8). If the conclusion about the presence of an elliptocytosis gene is correct, this gene must have been inherited from the deceased grandmother I.2, who was Indonesian.

Characterization of the abnormal haemoglobin. The abnormal haemoglobin was isolated from the blood of the propositus. The peptide map of the aminoethylated non- α chain showed the presence of the typical δ -chain peptides T2, T3, T5, and T10 and the typical β -chain peptides T12b and T13; the other peptides are common to both the β - and δ -chains. Amino-acid analyses of these peptides showed also the characteristic δ -like composi-

tion up to T10, and a β -like composition from T12b onward (Table II). This proves that the non- α chain is a $\delta\beta$ fusion product in which the crossing-over is located between residues 87 in T10 and 116 in T12b, characteristic of Hb Lepore Boston.

Since the discovery by Schroeder *et al* (1968) of the multiplicity of the human γ -chain genes, considerable attention has been given to the proportion of the two types of γ -chain, $^A\gamma$ and $^G\gamma$, in different haematological conditions (Huisman *et al*, 1974). We therefore determined the $^A\gamma$: $^G\gamma$ ratio in the fetal haemoglobin which is present in low amounts (3–4%) in the carriers of Hb Lepore. This ratio is estimated from the amino-acid analysis of peptide γ CB3, which is the C-terminal fragment of the γ -chain obtained after treatment with cyanogen bromide. The only difference between $^A\gamma$ and $^G\gamma$ is the presence of alanine in position 136 of the $^A\gamma$ -chain, where $^G\gamma$ has glycine. Careful analysis of the amounts of glycine and alanine in peptide γ CB3 therefore enables the ratio of $^A\gamma$ and $^G\gamma$ in the total γ -chain pool to be determined. The results are given in Table I.

Discussion

The haematological findings of heterozygotes for Hb Lepore Boston and β -thalassaemia are closely comparable (Weatherall and Clegg, 1972), the only

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ANALYSIS AND BLOOD GROUPS OF THE 12 FAMILY MEMBERS STUDIED

Hb A ₂ (%)	Starch Gel Electrophoresis	Hb Lepore (%)	Elliptocytosis	Red Cell Morphology			Blood Groups	
				Poikilocytosis	Anisocytosis	Basophil Stippling	ABO	Rhesus
2.5	A + Lepore	7.8	na	sp	+	+	A	R ₁ R ₂
1.9	A + Lepore	9.8	++	sp	++	na	A	R ₁ r
2.8	A		na	na	na	na	A	R ₁ r
2.2	A + Lepore	9.0	na	sp	na	++	O	R ₁ R ₁
2.6	A		na	na	na	na	O	R ₁ R ₁
2.5	A		na	na	na	na	O	R ₁ R ₁
2.6	A		na	na	na	na	O	R ₁ R ₁
2.7	A + Lepore	10.5	++	+	++	na	A	R ₁ r
2.3	A		+	na	na	na	A	R ₁ r
2.8	A + Lepore	9.0	++	+	+	+	A	R ₁ r
2.9	A + Lepore	9.5	na	sp	+	±	A	R ₁ R ₁
2.5	A		na	na	+	na	A	R ₁ r

TABLE II
AMINO-ACID ANALYSES OF THOSE TRYPTIC PEPTIDES FROM THE AMINOETHYLATED $\delta\beta$ -CHAIN OF Hb LEPORE WHICH HAVE A DIFFERENT COMPOSITION IN THE β - AND THE δ -CHAIN

Amino Acid	$\delta\beta T_2$	Expected		$\delta\beta T_3$	Expected		$\delta\beta T_5$	Expected		$\delta\beta T_{10}$	Expected		$\delta\beta T_{12b}$	Expected		$\delta\beta T_{13}$	Expected	
		δ	β		δ	β		δ	β		δ	β		δ	β		δ	β
Lys	0.8	1	1				0.9	1	1	1.0	1	1	0.9	1	1	0.9	1	1
His										0.9	1	1	2.1		2			
Arg				1.0	1	1								1				
Asp	1.0	1		2.0	2	2	3.3	3	3	1.0	1	1		1				
Thr	0.7	1	1					1		1.2	1	2				1.0	1	1
Ser			1				3.2	3	2	2.0	2	1						
Glu				1.1	1	2	1.1	1	1	2.3	2	1				2.9	4	3
Pro							1.8	2	2							1.6	1	2
Gly	1.1	1	1	3.1	3	3	2.0	2	2	0.8	1	1	1.1	1	1			
Ala	2.1	2	2	2.0	2	1	0.9	1	1		1	1	1.0	1	1	2.2	2	2
Cys										+	1	1						
Val	1.0	1	1	2.8	3	3	0.8	1	1				0.9	1	1	1.1		1
Met							0.7	1	1									1
Leu	1.0	1	1	1.0	1	1	0.9	1	1	1.9	2	2	0.9	1	1			
Tyr																0.7	1	1
Phe							3.1	3	3	1.0	1	1	1.0	1	1	1.1	1	1
Trp	+	1	1															

differences being the level of HbA₂—normal in Hb Lepore and increased in β -thalassaemia—and the presence or absence of the abnormal haemoglobin. The findings in the six carriers of Hb Lepore in our family are as expected: a mild, more or less well-compensated microcytic normochromic anaemia; some increase in fetal haemoglobin and in reticulocytes; mild morphological abnormalities of the red cells; mild to marked increase of basophil stippling (in four of our six patients). The amount of the haemoglobin Lepore in the six carriers of our family ranges between 7.8 and 10.5%. Our finding that the $\alpha\gamma$ chain of the HbF in our Hb Lepore carriers makes up between 23 and 39% of all γ chains is in good agreement with the findings of Huisman *et al* (1972). They found between 27 and 51% $\alpha\gamma$ chains in the HbF of three heterozygotes for Hb Lepore. Our own findings in two unrelated negroes from Jamaica with Hb Lepore Boston, who had $\alpha\gamma$ to $\alpha\gamma$ ratios of 36:64 and 28:72, respectively, also fall within the same range.

In our six patients there is in addition marked heterogeneity in the morphological appearance of the red cells. Three of the individuals, I.1, III.1, and III.8, are characterized by an increased coarse basophil stippling and some anisocytosis or poikilo-

cytosis. The other three have in addition a pronounced ellipto-ovalocytosis, which is much more marked than can be found in thalassaemia. The hypothesis that the difference is caused by the simultaneous presence of a gene for hereditary elliptocytosis in II.3 and his two children, III.5 and III.7, is supported by the finding of ellipto-ovalocytosis only in a further child (III.6), who does not carry the Hb Lepore. Under this hypothesis the elliptocytosis must have been inherited from I.2 who was Indonesian, born in Java.

Linkage with a Rhesus gene complex might present additional evidence in this respect. Although II.3 must have inherited the Rhesus r from his mother together with the elliptocytosis, II.2 most likely inherited a Rhesus R₁ (all his four daughters are R₁R₁). The children of II.3 are not very informative because he and his wife both have the genotype R₁r. Only III.8 who has the genotype R₁R₁ is informative as a possible non-recombinant, having inherited the R₁ gene from her father without the elliptocytosis. More definite conclusions about the existence of this elliptocytosis gene cannot therefore be made.

If the presence of an elliptocytosis gene is accepted it is clear from the haematological data that the

simultaneous presence of Hb Lepore and elliptocytosis genes does not lead to an aggravation of the anaemia. This combination has not previously been described; combination of elliptocytosis with Hb S or Hb C (Wolman and Özge, 1957) and Hb E (Lie-Injo Luan Eng, Bolton, and Gilman, 1972) does not produce any haematological symptoms. Association with β -thalassaemia does seem variably to enhance or not to enhance the thalassaemic symptoms (Aksoy and Erdem, 1968).

It should be emphasized that the different types of Lepore haemoglobins can only be distinguished with certainty by amino-acid analysis of the tryptic peptides $\delta\beta T5$ and $\delta\beta T10$. The latter has to be isolated following aminoethylation of the $\delta\beta$ -chain. A crossing-over between residues 87 and 116, characteristic of Hb Lepore Boston, has definitely been shown by Baglioni (1965) in two unrelated individuals of Italian extraction, by Labie *et al* (1966) in another person of Italian ancestry, and by Baglioni and Ventruto (1968) in a southern Italian. The occurrence of Hb Lepore Boston in Jamaican negroes has been shown unambiguously by de Jong (1969) and by Ahern *et al* (1972). Cases of Hb Lepore Boston from Macedonia and Greece have been characterized by L. F. Bernini (1974, personal communication) and Baglioni and Ventruto (1968).

The geographic origin of the cases of Hb Lepore from Cyprus (Beaven *et al*, 1964), Rumania (Rowley, Barnes, and Williams, 1969), and Iran (Rahbar, Golban-Moghadam, and Saoodi, 1974) make it likely that these also belong to the Boston type, although peptide $\delta\beta T10$ has not been analysed in these instances. The Lepore haemoglobin described by Ranney and Jacobs (1964) in a North-American negro might just as well be a case of Hb Lepore Baltimore.

Thus there seem to be two distinct sources of Hb Lepore Boston: the eastern Mediterranean area extending north into Macedonia and Rumania and east into Iran, and negroes in Jamaica. The finding in our family of Hb Lepore which must have originated either in Java or in the Federal Republic of Germany might possibly present another source.

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