Haemoglobin Lepore Boston-Washington in Sicily: clinical, haematological, and biosynthetic studies

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SUMMARY In the south-east of Sicily 23 children from 14 unrelated families have been diagnosed as suffering from haemoglobin Lepore. Such a high incidence shows that Sicily is an important focus of haemoglobin Lepore. The results of haematological and biosynthetic studies in 18 carriers of Hb Lepore and in five double heterozygotes for Hb Lepore and β -thalassaemia are presented.

In the carriers the haematological and biosynthetic data are compared with carriers of β -thalassaemia, while the five double heterozygotes are compared with β^- - and β^+ -thalassaemia major subjects. In the carriers of Hb Lepore no synthesis of $\delta\beta$ -chains was observed in peripheral blood cells; in fact we found a peak in the bone marrow. Double heterozygotes with circulating nucleated red cells showed $\delta\beta$ -chain synthesis in peripheral blood.

Haemoglobin Lepore is a group of abnormal haemoglobins composed of normal α -chains and fused $\delta\beta$ -chains. The Lepore chains result from an unequal crossing over between linked δ - and β -genes during meiosis.¹

Three different types of Hb Lepore result from three different crossing over points, Hb Lepore Hollandia (δ^{22} to β^{50}), Hb Lepore Baltimore (δ^{50} to β^{86}), and Hb Lepore Boston-Washington (δ^{87} to β^{116}).

These Hb Lepore are found chiefly among Mediterranean populations, but have also been found among other ethnic groups.^{2,3} In Italy, Campania has the highest number of cases of Hb Lepore^{4,5} and in Sicily only one case has been found up to now.⁶

We report clinical, haematological, and biosynthetic studies in 14 unrelated Sicilian families with Hb Lepore, seen at the Department of Pediatrics of Catania from 1974 to 1979.

Materials and methods

In these families 18 members are carriers of Hb Lepore and five are double heterozygotes for Hb Lepore and β -thalassaemia. All the cases studied came from south-east Sicily. In all cases the disease was diagnosed by genetic studies and haematological data. In three randomly chosen families the

fused $\delta\beta$ -chains of one member were examined biochemically by Professor Tentori and his co-workers at the Istituto Superiore di Sanità, Centro Riferimento per le Talassemie ed Emoglobinopatie, Rome. Routine haematological studies were performed using standard methods. Estimation of Hb F was done by the method of Betke *et al.*⁷ Haemoglobins were analysed by electrophoresis on cellulose acetate in glycine buffer at pH 9. Quantitative analysis was carried out by elution of the various haemoglobins from cellulose acetate strips. Globin chain synthesis was performed by incubating washed red cells with ³H leucine. In one case with Hb Lepore trait the globin synthesis was done in the bone marrow. After 2 hours the incubation was stopped and the cells were washed and lysed with distilled water. Globin was prepared by the acid acetone precipitation method of Rossi Fanelli *et al.*⁸ and the globin chains were separated chromatographically on CM cellulose according to the method originally described by Clegg *et al.*⁹ Routine haematological tests and haemoglobin synthesis in the double heterozygotes were done at the time of diagnosis before transfusion; in only one of them haemoglobin synthesis was also done after a high transfusion regimen.

Results

The haematological data of the 18 carriers are shown in table 1. They are compared with carriers

TABLE 1 *Haematological findings in β -thalassaemia trait and Hb Lepore*

Genotype	No of cases	Hb (g/dl)	RBC ($\times 10^6/\text{mm}^3$)	MCHC (g/dl)	MCH (pg)	MCV (μ^3)	Haemoglobins (%)			α -non- α -ratio
							Hb A ₂	Hb F	Hb L	
Hb Lepore trait	18	11.63 ± 1.59	5.02 ± 0.70	31 ± 2.38	23.14 ± 1.79	75 ± 6.38	1.82 ± 0.47	2.93 ± 1.43	10.14 ± 1.87	2.05 ± 0.33
β -thalassaemia trait	15	11.49 ± 0.98	4.55 ± 0.69	30.08 ± 4.87	23.21 ± 4.63	75.83 ± 14.04	5.18 ± 1.15	1.60 ± 1.21		1.76 ± 0.35

of β -thalassaemia. In both groups the morphological red cell alterations, osmotic fragility, MCV, MCHC, and MCH were very similar. However, the values of Hb A₂ were constantly low in subjects with Lepore trait (mean value 1.81 ± 0.47), while

in β -thalassaemia trait the mean value was 5.18 ± 1.15 . This difference was statistically significant ($p < 0.01$).

The levels of fetal haemoglobin were slightly raised, with a mean value of 2.93 ± 1.46 . In δ -thalassaemia trait the mean value was 1.60 ± 1.21 . However, this difference was not statistically significant ($p < 0.10$). The mean value of Hb Lepore was 10.14 ± 1.87 . Haemoglobin synthesis was similar in both groups showing a mild imbalance of δ -chain synthesis. No radioactivity was observed corresponding with an optical $\delta\beta$ peak in any subject, but we found a $\delta\beta$ peak (fig 1) when haemoglobin synthesis was studied in the bone marrow. In the bone marrow, synthesis was more balanced (α -/ β -ratio 1.27) than in peripheral blood (α -/ β -ratio 1.90).

The haematological data of the five double heterozygotes are compared with β^0 - and β^+ -thalassaemia major subjects in table 2. In three classes of patients the parameters were similar and the differences were not statistically significant. In the double heterozygotes the levels of haemoglobin Lepore were lower than in those with Hb Lepore trait, with a mean value of 5.6 ± 1.06 . In the haemoglobin synthesis of four double heterozygotes we found a small radioactive peak corresponding with an optical peak (fig 2, table 3). One of them had measurable, although very low, levels of β -chain synthesis (β^+ -thalassaemia-Hb Lepore) while the others were characterised by the absence of β -chain synthesis (β^0 -thalassaemia-Hb Lepore). The $\delta\beta$ -synthesis was very low, approximately 2 to 4%

FIG 1 Carboxymethylcellulose chromatography of peripheral blood (top) and bone marrow in Hb Lepore trait.

TABLE 2 *Haematological findings in β^0 - and β^+ -thalassaemia major and double heterozygotes for β -thalassaemia and Hb Lepore*

Genotype	No of cases	Hb (g/dl)	RBC ($\times 10^6/\text{mm}^3$)	MCHC (g/dl)	MCH (pg)	MCV (μ^3)	Haemoglobins (%)			α -non- α -ratio
							Hb A ₂	Hb F	Hb L	
Hb-Lepore	5	6.7 ± 1.48	3.38 ± 0.46	27 ± 2	21 ± 3.8	71.4 ± 7.12	1.82 ± 0.49	78.07 ± 2.74	5.6 ± 1.06	306 ± 0.38
β^0 -thalassaemia major	16	5.5 ± 2.28	2.49 ± 1.80	26.01 ± 1	21.45 ± 1.30	71 ± 0.40	1.3 ± 0.5	80 ± 0.10		4.22 ± 1.88
β^+ -thalassaemia major	33	5.39 ± 2.46	2.60 ± 2	26.17 ± 0.5	20.5 ± 0.90	71 ± 1	1.4 ± 0.6	81 ± 0.56		3.46 ± 1.36

FIG 2 Carboxymethylcellulose chromatography of peripheral blood in double heterozygotes at diagnosis and after high transfusion regimen.

TABLE 3 Globin chain synthesis in peripheral blood cells in double heterozygotes for HB Lepore- β -thalassaemia

Patients	Nucleated red cells (RBC/mm ³)	α -/ β -ratio	α -/ γ -ratio	α -/ $\delta\beta$ -ratio	α -/ $\text{non-}\alpha$ -ratio
1	25 000		2.96	22.79	2.62
2	30 000		3.39	20.34	2.90
3	30 000		3.49	42.11	3.22
4	40 000	15.85	5.12	37.80	3.51

of α -chain synthesis. Also γ -chain synthesis was reduced with an α -/ γ -ratio of 2.96 to 5.12. This is similar to that found in homozygous β -thalassaemia. The four patients showed severe imbalance with a mean value of α -/ $\text{non-}\alpha$ -ratio of 3.06 ± 0.49 . These mean values were slightly lower than in those suffering from β^0 - and β^+ -thalassaemia major, but this difference was not statistically significant ($p < 0.10$).

Discussion

Because of the low numbers of subjects with Hb Lepore in the world, Lehmann and Huntsman¹⁰ stated that while this anomaly is very important

from the theoretical point of view it is less important from the practical point of view. However, this paper shows a high number of subjects with Hb Lepore in Sicily, where β -thalassaemia trait is present with a frequency of 7.7%, and other β -chain anomalies like Hb S are present.^{11 12} Moreover, the interaction between Hb Lepore and β -thalassaemia or Hb S presents a very severe and interesting clinical and haematological picture.² Thus, this anomaly is of practical significance in Sicily. We found five double heterozygotes for β -thalassaemia and Hb Lepore with a clinical, haematological, and biosynthetic picture similar to Cooley's disease.

In three subjects where the isolation, purification, and identification of Hb Lepore were made, the crossing over was found between residue 87 of the δ -chain and 116 of the β -chain. This is consistent with Hb Lepore Boston-Washington,¹³ which has been found almost exclusively in Mediterranean populations.

The clinical and haematological findings in carriers of Hb Lepore and β -thalassaemia are identical and these conditions can be distinguished only by haemoglobin electrophoresis.

Therefore, screening of haemoglobin without electrophoresis cannot detect subjects with Hb Lepore trait. Haemoglobin synthesis in peripheral blood was constantly imbalanced and no $\delta\beta$ -chains were found. In the bone marrow of one carrier the synthesis of haemoglobin was almost balanced and $\delta\beta$ -chains were synthesised. These results are in accordance with previously published findings.¹⁴⁻¹⁶ In four double heterozygotes we found an imbalance of haemoglobin synthesis similar to that seen in thalassaemia major, and they have severe transfusion dependent anaemia. These patients showed $\delta\beta$ -chain synthesis in the peripheral blood and they had circulating nucleated red cells (25 000 to 40 000³) which could cause the production of these fused chains. When the haemoglobin synthesis was repeated in one of these subjects after a high transfusional regimen without circulating nucleated red cells no $\delta\beta$ -chains were found (fig 2).

Our data confirm that $\delta\beta$ -chain synthesis takes place during the early stages of erythroid cells and ends in reticulocytes. This phenomenon could be the result of instability of $\delta\beta$ mRNA which then cannot be translated in the reticulocytes.^{17 18} In fact the fused $\delta\beta$ (Lepore) and $\beta\delta$ (anti-Lepore) chains are not synthesised in reticulocytes but only in erythroblasts.^{15 16 19-21} Other fused chains without the δ -chain sequence, like the $\gamma\beta$ -chains of Hb Kenya, are synthesised in reticulocytes also.^{22 23}

Recently Benz *et al*,²⁴ in four double heterozygotes for β^0 -thalassaemia-Hb Lepore, did not

find $\delta\beta$ -chain synthesis in peripheral blood cells. Although not stated, these subjects had probably been polytransfused and so had no nucleated red cells. In the same subjects variable amounts of β -like mRNA were detected, but it is conceivable that this mRNA could have been contributed by the β^0 -thalassaemia gene.

Forget *et al*¹⁹ and Ramirez *et al*,²⁵ in homozygous Hb Lepore with circulating nucleated red blood cells, found that the amount of β -like ($\delta\beta$)mRNA in peripheral blood cells is proportional to the chain synthesis in the same cells.

We are grateful to Drs R Curreri and R Testa for their technical assistance.

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