α -thalassaemia in Apulia: biosynthetic studies

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SUMMARY Analysis of haemoglobin chain synthesis was performed in 15 Apulian patients with Hb H disease and in their parents and offspring. The Apulian carriers of Hb H disease show a marked imbalance of α and β chain synthesis (0.39 ± 0.1) with variable clinical and haematological manifestations. However, we are dealing with an intermediate form similar to that described in Italians from other regions. A significant difference was found between the mean α/β ratio values (0.81 ± 0.13) of parents and offspring of Hb H patients and those of the normal controls (1.05 ± 0.09); however, extensive overlapping between these two groups exists.

These results have led us to the conclusion that the forms of α -thalassaemia found in Apulia are similar to the α defects observed in Sicily; in both cases, in fact, haemoglobin chain synthesis was an unreliable test for discriminating between α -thalassaemia-1 trait and α -thalassaemia-2 trait.

The α -thalassaemia syndromes are inherited anaemias caused by defective function of one or more α globin genes. In most human populations,^{1,2} α globin structural gene loci are duplicated so that each diploid cell contains four copies of α globin genes. Four main α -thalassaemia syndromes of increasing clinical severity are recognised: (1) the silent carrier state (α -thalassaemia-2) with no clinical manifestations; (2) α -thalassaemia trait (α -thalassaemia-1) characterised by microcytic red blood cells but little or no anaemia; (3) haemoglobin H disease, which manifests as haemolytic anaemia; and (4) homozygous α -thalassaemia in which the affected fetus dies at or around term from hydrops fetalis. These four syndromes are the consequence of dysfunctional expression of from one to all four α globin genes, respectively.

Patients with α -thalassaemia are usually of Asian, Mediterranean, or Negro descent. Gene counting experiments indicate that one or more α globin genes are deleted in blacks^{3,4} and in most⁴⁻⁶ but not all Asians^{4,7} with α -thalassaemia. By restriction endonuclease patterns the presence of deletions as well as more complex defects have been demonstrated⁸ in people of Mediterranean origin. These observations suggest that α -thalassaemia may be heterogeneous among people of different ethnic backgrounds. In this paper, in order to define the molecular characteristics of the dysfunctional expression of the α globin genes in the population of Apulia, as a first approach we investigated the

globin chain synthesis in families of patients with Hb H disease, observed by one of us⁹ during the last few years.

Materials and methods

The families described here were selected for globin chain biosynthetic studies whenever a patient with Hb H was found.

Routine haematological investigations were carried out by standard methods. Full blood counts were made with the Coulter Counter. Haemoglobin electrophoresis was performed on cellulose acetate strips at pH 8.6. The different fractions were measured after elution of the bands and read spectrophotometrically at 415 nm. Haemoglobin A₂ was quantified by DE 52 microchromatography and Hb F was determined by alkali denaturation according to the method of Betke *et al.*¹⁰ Preparations for detection of Hb H inclusion bodies were made after incubation of equal volumes of blood and 1% Brilliant Cresyl Blue at 37 °C for 1 to 2 hours. Red cell osmotic fragility was assessed according to Silvestroni and Bianco.¹¹ Serum iron and iron binding capacity was determined by the method of Lauber.¹²

Globin chain synthesis was performed by incubation of washed red cells (0.8 ml) with an equal volume of a leucine free amino-acid reagent mixture, identical to that of Lingrel and Borsook,¹³ except that human dialysed AB plasma was used in place of rabbit plasma. Addition of Krebs Ringer phosphate buffer (KRP) at pH 7.4 completed the

incubation mixture which was placed at 37°C in a water bath. To this mixture 100 μ l 3 H-leucine (1 mCi/ml, specific activity 130 Ci/mmol, the Radiochemical Centre, Amersham) were added and the cells incubated for 2 hours at 37°C. Whenever the reticulocyte counts were low (<1%) reticulocyte enrichment with Percol gradients was performed. After 2 hours of incubation the cells were washed three times at 4°C in KRP to remove free radioactivity and lysed with three volumes of distilled water. The lysate was thawed and added in drops to an acid-acetone mixture. Globin was fractionated on CM cellulose chromatography in 8 mol/l urea.¹⁴ Optical density was continuously monitored by an LKB Uvicord at 280 nm. The radioactivity of each fraction was measured with a Beckman scintillation counter. The total radioactivity incorporated into the α and β chains was determined.

Results

Clinical and haematological data are summarised in tables 1 to 4. The results of the globin chain biosynthetic studies are illustrated in the figure.

All 15 patients with Hb H disease had a decreased α chain synthesis with a mean α/β ratio of 0.39 ± 0.1 (table 1, figure c). The α/β ratio in 24 parents of the patients with Hb H disease was 0.79 ± 0.15 (table 2, figure b). The mean α/β ratio in the 36 control subjects was close to unity (figure a). Although there was no overlap in the mean α/β ratio between carriers of α -thalassaemia and controls, at least seven obligate heterozygotes had α/β ratios in the normal range.

Offspring of patients with Hb H disease, all obligate carriers of α -thalassaemia, excluding the cases with parent-child transmission of Hb H disease,

TABLE 1 Haematological values in 15 subjects with Hb H disease.

Case No	RBC ($\times 10^{12}/l$)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hb A ₂ (%)	Hb H (%)	Inclusion bodies	Erythrocyte morphology	α/β ratio	
										A P H		
1	3.70	6.2	28.7	76	16	21	1.5	14.2	+	+	+	0.40
2	4.91	9.9	34.8	71	20	28	1.9	5.0	+	+	—	0.46
3	5.80	8.5	35.0	60	14	24	1.8	5.0	+	+	—	0.60
4	3.75	10.0	37.0	98	36	27	1.4	22.0	+	+	+	0.33
5	3.20	7.0	22.0	68	21	31	1.4	38.0	+	+	—	0.26
6	6.10	11.4	36.0	59	18	31	2.6	10.0	+	+	+	0.35
7	5.00	8.0	25.0	50	16	32	3.4	7.0	+	+	+	0.62
8	5.20	9.2	37.0	86	18	29	1.2	5.0	+	+	—	0.37
9	4.80	7.1	29.0	72	19	25	2.2	6.1	+	+	—	0.41
10	3.95	7.3	32.0	86	24	26	1.9	11.1	+	+	+	0.40
11	4.58	8.1	33.0	89	22	25	2.7	7.5	+	+	—	0.38
12	3.54	7.6	31.2	78	23	28	2.5	9.5	+	+	—	0.39
13	4.00	8.1	27.0	68	23.5	26	2.0	6.3	+	+	—	0.36
14	4.95	7.9	30.0	85	25	27	2.1	8.0	+	+	—	0.26
15	3.39	7.3	31.5	81	17	25	2.6	9.9	+	+	+	0.30

TABLE 2 Haematological values in parents of Hb H disease patients.

Case No	RBC ($\times 10^{12}/l$)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hb A ₂ (%)	Inclusion bodies	Erythrocyte morphology A P H	α/β ratio		
1	5.73	13.3	39.9	69	23.0	33.0	1.6	± — —	+	+	+	0.58
2	4.30	10.6	36.5	82	23.0	29.0	2.3	± — —	+	—	—	0.67
3	5.51	13.2	43.7	79	23.0	30.0	1.8	— + +	—	—	—	1.20
4	4.65	13.1	38.4	82	28.0	34.0	2.3	± — —	+	—	—	0.67
5	6.10	15.3	50.0	81	25.0	30.0	2.3	+	—	—	—	0.70
6	6.60	17.1	50.0	87	25.0	34.0	2.7	± — —	+	—	—	0.72
7	4.10	12.5	35.0	85	30.0	35.0	2.5	+	—	—	—	0.65
8	4.50	15.2	40.0	88	33.0	38.0	2.6	— — —	—	—	—	1.00
9	4.50	13.0	43.0	95	28.0	30.0	2.7	— — —	+	+	—	0.69
10	3.50	11.5	37.0	105	32.0	31.0	2.5	— — —	+	—	—	0.81
11	5.31	12.1	40.2	91	22.1	32.1	2.6	± — —	+	+	±	0.66
12	4.97	11.9	37.8	88	23.8	30.8	2.4	— — —	+	±	±	0.71
13	5.45	13.2	39.7	88	32.0	33.0	2.7	— — —	—	—	—	0.94
14	4.99	10.8	38.2	89	30.0	34.0	2.7	— — —	+	—	—	0.73
15	5.02	13.0	37.0	87	29.1	32.4	2.5	— — —	+	—	—	0.90
16	4.82	12.2	35.0	95	27.4	30.1	2.6	— — —	+	±	—	0.83
17	5.32	12.4	39.2	78	26.6	30.0	2.3	— — —	+	+	±	0.72
18	5.27	11.9	38.0	99	28.0	35.0	2.7	— — —	+	—	—	0.80
19	5.53	11.3	39.0	91	24.0	30.0	2.2	— — —	—	—	—	0.99
20	5.00	11.6	40.0	89	27.0	32.0	2.4	— — —	—	—	—	0.90
21	5.60	11.8	38.0	85	26.0	29.9	2.6	± — —	+	+	—	0.70
22	5.43	10.3	37.0	89	26.0	31.0	2.3	— — —	+	+	+	0.81
23	5.15	12.9	40.0	88	27.0	33.0	2.7	— — —	—	—	—	1.00
24	5.06	13.2	40.0	87	29.0	28.0	2.2	+	—	—	±	0.66

TABLE 3 Haematological values in seven offspring of Hb H patients.

Case No	RBC ($\times 10^{12}/l$)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hb A ₂ (%)	Inclusion bodies	Erythrocyte morphology A P H	α/β ratio
1	4.60	13.5	39.0	84	29.0	34.0	2.4	— — —	— — —	0.83
2	5.50	16.0	47.0	85	29.0	34.0	2.8	— — —	— — —	0.85
3	5.00	14.7	43.0	68	29.0	34.0	2.9	— — —	— — —	0.80
4	6.24	11.6	40.4	69	28.6	28.7	2.2	— — —	— — —	0.87
5	5.30	13.6	41.8	79	25.7	32.5	2.5	— — —	± + —	0.90
6	6.13	11.7	39.8	65	19.1	29.4	1.8	— — —	+ + —	0.88
7	4.40	10.7	34.5	88	30.0	33.0	2.7	— — —	— — —	0.92

TABLE 4 Summary of haematological mean values.

	RBC ($\times 10^{12}/l$)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hb A ₂ (%)	α/β ratio
Normal controls (n=36)	4.46 ± 0.20	15.40 ± 1.00	44.40 ± 3.70	92.71 ± 2.50	32.22 ± 1.20	34.68 ± 2.60	2.69 ± 0.11	1.05 ± 0.10
α -thalassaemia trait (α -thal-1 and α -thal-2) (n=31)	5.15 ± 0.66	12.70 ± 1.60	39.93 ± 3.76	85.00 ± 8.81	27.04 ± 3.18	31.96 ± 2.28	2.43 ± 0.30	0.81 ± 0.13
Hb H disease	4.46 ± 0.89	8.24 ± 1.37	31.30 ± 4.43	75.13 ± 12.93	20.83 ± 5.53	27.00 ± 2.95	2.08 ± 0.60	0.39 ± 0.10

FIGURE Results of globin chain synthesis study in (a) normal controls, (b) parents of patients with Hb H disease, (c) patients with Hb H disease, (d) parents and offspring of Hb H patients.

showed a mean α/β chain synthesis ratio of 0.86 ± 0.04 (table 3). However, four out of seven did not show alterations of red cell morphology or of α/β ratio.

Discussion

Haemoglobin H disease in Apulia is characterised by a marked imbalance of α and β chain synthesis similar to that observed by Galanello *et al*¹⁵ in Sardinia, Musumeci *et al*¹⁶ in Sicily, and Kan *et al*¹⁷ in non-Negro patients.

It is well known that carriers of Hb H disease show variable clinical and haematological manifestations in different racial groups; the most severe form has been observed in the Far East¹⁸ and the mild form in American blacks.¹⁹ In our patients an intermediate form was present, very similar to that described by Galanello *et al*¹⁵ and Musumeci *et al*¹⁶ in Sardinian and Sicilian subjects respectively. None of the families studied showed abnormal haemoglobins like Hb Constant Spring or Hb Icaria observed in about 50% of patients with Hb H disease in the Far East,^{20,21} both determined by genes which act like an α -thalassaemia-2 gene.

Since Hb H disease is known to occur from double heterozygosity for α -thalassaemia-1 and α -thalassaemia-2, parents and offspring of patients with this disease must be heterozygous for either α -thalassaemia-1 or α -thalassaemia-2 traits. Usually one of the parents of a patient with Hb H disease has a thalassaemia-like disorder, while the other, the so called 'silent carrier', has either minimal red cell changes or is haematologically normal. Less frequently both parents are affected by the more severe form (α -thalassaemia-1).

According to Kan *et al*¹⁷ and Pootrakul *et al*²²

the 'silent carriers' can be detected through the analysis of haemoglobin chain synthesis. In fact, in both of these studies, presumed obligatory carriers of the milder form of α -thalassaemia were shown, as a group, to have a mean α/β ratio different from both the value of normal controls and the value of the group with the more severe type of disorder. In our case, too, if we pool (figure d) the results of the Hb chain synthesis observed in parents (12 couples) and offspring (seven cases) of patients with Hb H disease, the α/β ratios segregate, without overlapping, around two different means of 0.90 ± 0.09 and 0.68 ± 0.04 , which could represent the values of α -thalassaemia-2 and α -thalassaemia-1 traits, respectively. However, the normal α/β ratio we found in several silent carriers demonstrates that a carrier of the α -thalassaemia-2 gene will be overlooked by random screening of the population. Furthermore, if the parents of Hb H patients are considered one by one, as should be done in every case, it can be seen that in two instances both members of the couple show an α/β ratio corresponding to the α -thalassaemia-2 trait. Apart from these, we were not able to single out two different groups among the 12 couples of parents of patients with Hb H disease.

These data, in agreement with the observations of Musumeci *et al.*¹⁶ in the Sicilian population, differ from those reported by Pootrakul *et al.*²² and Kan *et al.*¹⁷

From the clinical point of view, failure to diagnose silent carriers is of no consequence, but different considerations apply to the detection of the silent carrier state for the purpose of genetic counselling. It is also necessary to have a method for the identification of such persons in any population survey to determine the frequency of the α -thalassaemia gene.

Further studies of cellular DNA fragments containing the defective α loci and mRNA analysis, according to the recent observations of Orkin and Goff,²³ could permit the characterisation of specific mutations responsible for abnormal globin gene expression in the different cases.

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