Sickle cell disease in Sicily

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SUMMARY The chemical and physical properties of haemoglobin S derived from homozygotes for this haemoglobin in Sicily were examined, as well as some erythrocytic characteristics. Sicilian Hb S was identical to that found in USA black patients in electrophoretic mobility on both starch and citrate agar media, solubility, mechanical precipitation rate of oxyhaemoglobins, and minimum gelling concentration, as well as by peptide mapping and amino-acid analysis of all β -chain peptides. Taken together with the presence in Sicily of African blood group markers and certain historical considerations, it seems clear that the source of Hb S in Sicily is Africa. While the clinical severity in nine Sicilian children did not seem remarkably different from the disease in the USA, the most severe and fatal complications were not seen. Mean Hb F was 10.5% and 2,3-diphosphoglycerate (2,3-DPG) values were higher in Sicilian homozygotes than in black USA counterparts (21.79 μ mol/g Hb vs 15.16). Red cell ATP values were also slightly higher in Sicilian patients. The presence of concomitant thalassaemia was excluded by both family studies and globin chain synthetic ratios. In conclusion, haemoglobin S in Sicilian homozygotes is identical to Hb S found in USA blacks. Although the severity of the disease seems quite similar in both groups of patients, other erythrocytic properties were found to be different. Whether these factors influence severity remains to be elucidated.

In the decades after the first description of sickle cell anaemia, it became apparent that some people of non-black ancestry also had this disease. By 1959, approximately 30 cases of white patients had been reported.¹ Since then, substantial numbers of Italians, Sicilians, Greeks, Arabs, Turks, and Indians have been found to have haemoglobin S (Hb S) as homozygotes, simple heterozygotes, or as double heterozygotes with haemoglobin C or β -thalassaemia trait.^{2,3} The finding of a putative African blood trait in non-African populations has been of great interest to both medical scientists and anthropologists. In addition, the finding that sickle cell disease may have a milder clinical course in non-black patients⁴ may be of direct concern to the physician as well. It is likely that other heritable traits which accompany the presence of Hb S are capable of modifying the expression of sickle cell disease. One such trait which has been identified is the synthesis of large amounts of fetal haemoglobin (Hb F) in adult life. It appears that concentrations of Hb F in the range of 20% or more in Saudi Arabs

and Persian Gulf populations may produce a mild clinical course in patients homozygous for Hb S, whereas among Galilee Arabs, who appear to have a comparable benign course, the level of Hb F is below 5%. In the latter case, other as yet unknown factors must be invoked to account for the clinical course.^{5,6}

By studying another non-black population of sickle cell patients, we hoped to contribute some understanding to the origin and spread of the Hb S gene. In addition, the clinical presentation of the disease, as well as the red cell biochemistry associated with it, was also of great interest.

Methods

Patients were seen in the Pediatric Clinic of the University of Catania, Catania, Sicily, and in the Istituto di Clinica Pediatrica, Palermo, Sicily. In addition to routine haematological studies, blood was taken in heparin for determination of 2,3-DPG content and ATP content. Blood samples were placed on ice and shipped to the United States for the following studies: Hb solubility in dithionite-

phosphate buffers, alkali resistant fraction (Hb F), starch gel electrophoresis pH 8.6, citrate agar electrophoresis pH 6.5, quantitative Hb A₂ determination, minimum gelling concentration (MGC), peptide mapping^{6a} and amino-acid analysis of all β -chain peptides, as well as mechanical stability of oxyhaemoglobin. The methods used have all been described previously.^{6,7} For comparison, clinical and biochemical studies were performed on patients seen in the paediatric haematology clinics of the Montefiore Hospital, Bronx, New York and the Jacobi Hospital, Bronx Municipal Hospital Center, New York. These patients were of black or Puerto Rican origin. In some cases, in order to exclude the presence of concomitant β -thalassaemia, family studies as well as globin chain synthetic ratios were performed as described previously.⁶

Results

The chemical and functional identity of Sicilian Hb S with that of North American Hb S was confirmed in the following studies: reduced solubility in dithionite-phosphate buffer, identical migration on both starch gel and citrate agar electrophoresis, mechanical precipitability of oxyhaemoglobin (fig 1), and peptide mapping and amino-acid analysis of all peptides from a tryptic digest of the β -chains. In addition, a gelation study of deoxy Hb S from one of the Sicilian patients yielded an MGC of 24.5 g/dl with a concomitant USA MGC of 23.0 g/dl, which within the error of the method indicates a similar polymerising tendency of the two haemoglobins. There can therefore be little doubt that Sicilians do indeed possess haemoglobin S with identical properties to that found in black patients in North America.

A study of certain erythrocytic properties of Sicilian Hb S homozygotes in comparison with those of North American patients (table 1) showed a significantly lower mean haemoglobin level and higher fetal haemoglobin, 2,3-DPG, and ATP. The difference in ATP was significant only at the 5% level, whereas the other differences noted were significant at the 0.1% level using the *t* test with Bessel's correction for small samples.¹⁰ The blood smears of all Sicilian patients showed polychromasia,

FIG 1 Mechanical precipitation curve of oxyhaemoglobins A and S: a comparison of Hb S from Sicily and the USA. The samples of haemoglobin were at an initial concentration of 200 mg/dl in 0.15 M KPO₄ buffer, pH 7.35 at 24°C.

many target cells, irreversible sickle forms, and some hypochromia. Thalassaemia syndromes were ruled out in five of the cases by chain synthesis studies which in every case failed to disclose any imbalance in synthetic ratios (table 2).

Physical examination was notable chiefly for the fair complexion and light brown to blond colour of the hair of several of the patients who were generally well developed (except for one boy of 17 with

TABLE 2 α /non- α globin chain synthetic ratios in 5 Sicilian patients with sickle cell anaemia

Patient	α /non- α ratio	
1	1.09	0.98
2	1.03	1.05
3	0.96	
4	1.08	
5	1.10	

TABLE 1 Some haematological values from American and Sicilian patients with sickle cell anaemia (mean $\pm$ SD)

	Hb (g/dl)	Hb F (%)	2,3-DPG (μ mol/g)	ATP (μ mol/g)	Age (range)
USA children (n = 9)	9.69 $\pm$ 1.1	2.8 $\pm$ 1.6	15.16 $\pm$ 1.16	3.51 $\pm$ 0.89	11.5 (7-16)
Sicily children (n = 9)	7.58* $\pm$ 0.67	10.5* $\pm$ 3.2	21.79* $\pm$ 2.55	4.27† $\pm$ 0.45	9.8 (3-17)
USA adults‡ (n = 14)	9.00 $\pm$ 1.8	5.3 $\pm$ 3.9	17.90 $\pm$ 3.2	4.06 $\pm$ 1.0	—
Normal adults	13-16	< 2.0	13.2 $\pm$ 2.6	3.65-4.45	—

* $p < 0.001$ (2 vs 1); † $p < 0.05$ (2 vs 1); ‡ from Steinberg *et al.*⁹

TABLE 3 *Clinical findings in 9 Sicilian homozygotes for Hb S*

Patient	Age	Splenomegaly	Crises	% Hb F	Transfusions	Pneumonia	Reticulocytes (%)	% ISCs	Howell-Jolly bodies
1 GDIG	6	0	Rare	10.5	Occasional	1	8.0	—	+
2 GP	17	0	1/24 months	6.9	Frequent	1	8.5	4	+
3 MS	3	1FB	Monthly	16.1	Frequent	0	16	—	—
4 GI	9	4FB	Rare	10.6	None	0	9.6	—	—
5 VR	8	0	2/36 months	11.5	Rare	0	7.4	5	+
6 AM	13	0	5-6/12 months	6.2	Frequent	0	10.0	10.6	+
7 AT	9	0	1/24 months	7.6	0	0	7.0	19	+
8 GR	3	3FB	3/36 months	13.9	0	0	8.0	4.6	+
9 PM	13	2FB	1/12 months	10.9	0	0	3.0	—	—

delayed onset of puberty). Four of the nine patients had readily palpable spleens. Pallor and jaundice were also apparent, but there was no evidence of active or healed leg ulceration or joint deformities. Table 3 summarises some clinical information on the nine patients seen in Catania and Palermo who ranged in age from 3 to 17 years. Only one of these patients experienced more than one crisis per year. Three others were receiving frequent transfusions, and two reported a single episode of pneumonia. The well known hand-foot syndrome was not reported in these patients. In the five cases where it was possible to inspect the blood smear, irreversibly sickled cells (ISCs) were always seen; they ranged from 4 to 19%. Howell-Jolly bodies were also seen frequently on these smears indicating hyposplenism. Patient 8 with a three finger-breadth spleen also had many Howell-Jolly bodies. Because of the small sample size, it was not possible to assay the clinical severity of the disease in Sicily in any quantitative

manner; however, a pattern of typical bouts of abdominal and joint pain were noted as well as frequent intercurrent infections. On the other hand, the severe and fatal complications were not seen by us. Tentatively, it can be stated that the clinical picture of sickle cell disease in Sicily is not more severe than in the United States.

Discussion

The history of Sicily is one of multiple invasions and occupations by peoples from parts of northern and southern Europe as well as North Africa.¹¹ Of particular concern to the discussion of the spread of the Hb S gene is the extensive occupation of Sicily by Carthaginians, descendants of the Phoenicians from North Africa. Although these people were eventually driven out during Roman times, there continued to be contact with Africa.

Fig 2 shows a mosaic representation from the Villa

FIG 2 Mosaic figure from the Villa del Casale, Piazza Armerina, Sicily (AD 360 to 400). The black man is seen in the Great Hunting mosaic in which he is leading a Zebu drawn cart with cages for the captured wild animals.

del Casale, Piazza Armerina, Sicily (AD 360 to 400) in which a black African man is depicted. The artisans themselves were imported from North Africa to make these mosaics. The figure indicates the antiquity of Sicilian contact with black Africa.

Continued contact with Africa occurred during the Arab conquest of Sicily in the 9th century AD owing to the mixture of Arab and Sudanese troops who participated in the invasion. During the subsequent Norman period (AD 1071 to 1200), at least one king, William II, had a personal corps of body guards consisting of black slaves. In fact, slavery and the importation of slaves from North Africa continued well into the 1600s.¹²

Biological evidence of admixture of black Africans into Sicilian populations has been obtained by Sandler *et al*⁸ who found erythrocyte blood group markers characteristic of African origin in 17% of Hb S-bearing Sicilians and their families. The present study extends the findings of Sandler by confirming the identity of Sicilian Hb S with that found in blacks from the USA. The claim of identity is based on numerous physical and chemical properties which have been described above.

While no clear distinction between Sicilian and American sickle cell patients can be made on clinical grounds, there were certain differences in red cell properties. The Sicilian children have approximately 2 g/dl less haemoglobin content, which may be explained by nutritional factors since thalassaemia was ruled out by family studies and chain synthesis ratio studies in five of the patients (table 2). Because the patients were not under the primary care of the referral centres in Palermo and Catania it was not possible to assess the number of infective episodes and minor crises with any degree of accuracy. It can be safely asserted, however, that deaths attributable to sickle cell anaemia were not seen. In comparison with North American children with this disease, some features appear quite similar. For example, in a study of 420 Brooklyn, New York homozygote children, Robinson¹⁴ found the incidence of splenomegaly to be 54.5%. In this much smaller sample, the incidence was 44% (table 3). However, the percentage of fetal haemoglobin was higher in the Sicilian patients although not high enough to confer protection against sickling as it appears to do in Saudi Arabs. The slightly higher values of red cell ATP in Sicilians may be attributed to the previously described racial differences in red cell ATP content.¹⁵ The largest difference is seen in the 2,3-DPG levels which are approximately 40% higher in Sicilians than in USA black patients. These results are reminiscent of our study of Galilee Arab Hb S patients in whom the 2,3-DPG level was 55% higher than the level of the American black patients.⁶

These studies illustrate that there is no correlation between severity and 2,3-DPG content despite the theoretical consideration that a substance which facilitates the T (deoxy) conformation of haemoglobin is likely to potentiate sickling and intensify symptoms. In this regard, it should be noted that the recent work of Bookchin *et al*¹⁶ has shown that 2,3-DPG per se does not enhance gelation (polymerisation) of Hb S when due regard for pH changes are taken into account. When the pH was maintained in the presence of 2,3-DPG, there was no effect on gelation. Nevertheless, 2,3-DPG may affect sickling in vivo by shifts in the Donnan equilibrium, which would tend to lower intracellular pH and favour sickling. The actual effects of this compound in vivo remain to be elucidated, but simple increments of 2,3-DPG may be well tolerated by certain patients with sickle cell anaemia.

In conclusion, a typical syndrome of sickle cell anaemia-related symptoms with moderate severity is seen in Sicilian children who are homozygous for haemoglobin S. Their levels of 2,3-DPG appear to be intermediate between Galilee Arabs and American blacks with this disease. No single ancillary factor can be found which significantly affects the course of the illness in Sicily. The chemical and physical identity of Hb S in both USA blacks and Sicilians, as well as the presence of African blood group markers, suggests that Hb S may have spread from Africa to Sicily.

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Note added in proof

Thirteen Sicilian carriers of haemoglobin S underwent globin gene mapping by means of restriction endonuclease techniques in the laboratory of Professor Y W Kan, San Francisco, USA. All 13 subjects were found to have the 13 kilobase β -globin gene fragment. This finding is consistent with an African origin of this gene.