α -thalassaemia in Cyprus¹

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SUMMARY The frequency of α -thalassaemia in Cyprus was determined with studies of haemoglobin Bart's in 1200 Greek Cypriot and 132 Turkish Cypriot newborn babies. Of the Greek newborns, 12.4%, and of the Turkish newborns, 6.8% had raised Hb Bart's (from 0.6% to 12.9% of the total haemoglobin) suggesting that they were carriers of either α -thalassaemia-1 or α -thalassaemia-2 genes. The findings suggest that the population of Cyprus has the highest frequencies of α -thalassaemia among Caucasian people.

Although haemoglobin H disease (the compound heterozygous state for α -thalassaemia-1 and α -thalassaemia-2 genes) is found in almost every population in the Mediterranean area, frequencies of α -thalassaemia genes among the Mediterranean people are relatively low (Weatherall and Clegg, 1972). An exception to this appears to be the population of Cyprus in which haemoglobin H disease has a frequency of one case per 200 to 500 people (unpublished observations). A previous small survey of newborns has provided evidence that the frequency of α -thalassaemia genes in the population of Cyprus is of the order of 10% (Ashiotis *et al.*, 1973). The purpose of the present study was to obtain more accurate data about the frequency and the types of α -thalassaemia genes in the population of Cyprus by measuring Hb Bart's in a large number of Greek and Turkish Cypriot newborns.

Methods and results

Umbilical cord samples were collected from infants born at the Nicosia General Hospital and the Turkish Hospital of Nicosia. Screening for haemoglobin Bart's (Hb γ_4) was done with cellulose acetate electrophoresis in two buffer systems (TEB pH 8.6 and phosphate pH 7.0). With this method, amounts of Hb Bart's over 0.3 to 0.5% can be seen as distinct bands by visual inspection of the electrophoretic strips. When an electrophoretic band migrating in the position of haemoglobin Bart's was seen in the cellulose acetate electrophoresis of a sample, the amount of this haemoglobin in the sample was

quantified by CM-Sephadex chromatography using a tris malleic acid (0.05M) buffer system. All quantifications were done in triplicate and the average of three measurements was used in the analyses shown below.

Of the 1200 Greek Cypriot newborns tested, 149 (12.4%) had raised Hb Bart's; the amounts of this haemoglobin ranged from 0.6% to 12.9% (Fig. 1). Of the 132 Turkish Cypriot newborns, 9 (6.8%) had raised Hb Bart's, ranging from 0.9 to 5.1% of the total haemoglobin.

Discussion

The phenotypic expression of α -thalassaemia genes has been well defined (reviewed by Weatherall and Clegg, 1972). The genes for α -thalassaemia-2 (α -thal-2) usually fail to be expressed haematologically in the heterozygous adult, or they produce only a

Fig. 1 Distribution of levels of Hb Bart's in 149 Greek Cypriot newborns who had increased amounts of this haemoglobin.

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minimal change in the haemoglobinisation of the red cells. In addition, in the heterozygous newborn the gene for α -thal-2 produces a rise of haemoglobin Bart's at the range of 1 to 2%. On the other hand, heterozygosity for α -thalassaemia-1 (α -thal-1) is haematologically detectable in the adult and, in the newborn period, manifests with a rise of Hb Bart's at the range of 5 to 10%. Studies in Thailand have shown that the values of Hb Bart's in newborns form a trimodal distribution corresponding to the three common genotypes, that is, heterozygous α -thal-2, heterozygous α -thal-1, and compound heterozygous Hb H disease (Na-Nakorn and Wasi, 1970). The observed frequency of Hb H disease in the Thai population fits the expected one on the basis of the frequency of α -thal-1 and α -thal-2 genes calculated from the distribution of Hb Bart's in the population of newborns (Na-Nakorn and Wasi, 1970).

The distribution of Hb Bart's in the Greek Cypriot newborns shows a tendency to trimodality (Fig. 1, 2). There is a large group of subjects (126 of the 149 newborns) with levels of Hb Bart's between 0.6 and 5.5%; a second group of 21 newborns with Hb Bart's from 5.6 to 9.5%; and two newborns with over 12% Hb Bart's. If each of the three groups correspond to heterozygosity for α -thal-2, α -thal-1, and α -thal-2/ α -thal-1, respectively, the frequencies of the three types of heterozygous Cypriots will be α -thal-2, 10.5%; α -thal-1, 1.75%; α -thal-1/ α -thal-2, 0.17%. These possible frequencies fit with other observations on α -thalassaemia on the island. Thus, the frequency of the α -thal-1/ α -thal-2 compound

heterozygotes (0.17%) is close to the frequency of Hb H disease among adult Cypriots (1:200 to 1:500 persons). Also, a low frequency of heterozygous α -thal-1 (1.7%) could explain the scarcity of Hb Bart's hydrops fetalis in Cyprus in spite of the fact that Hb H disease is so common. We have not seen a case of Hb Bart's hydrops fetalis during the five years of the operation of our Thalassemia Center, although the obstetric services of the island have been alerted about the syndrome. However, Hb Bart's hydrops fetalis has been observed or retrospectively diagnosed in the offspring of Greek Cypriot couples (Diamond *et al.*, 1965; Kattamis and Lehman, 1970). If the frequency of heterozygous α -thal-1 is 1.75%, the expected incidence of Hb Bart's hydrops fetalis is 7.6 per 100 000 births. Since, per year, there are about 10 000 births among Greek Cypriot couples, one case of Hb Bart's hydrops fetalis is born every 10 months and presumably these cases are still not brought to our attention.

There are, however, several reservations concerning the accuracy of distinction between heterozygosity for α -thal-1 and heterozygosity for α -thal-2 using the levels of Hb Bart's in the Cypriot newborns. Firstly, the levels of Hb Bart's in the group of newborns that we considered to be heterozygous for α -thal-2 are higher than those observed in the Thai newborns (Na-Nakorn and Wasi, 1970). Secondly, we have re-examined children who at birth had less than 5% Hb Bart's and found that they have developed the haematological stigmata of α -thal-1 (Table). Furthermore, studies in Chinese infants have also failed to produce sharp discrimination of α -thal-1 from α -thal-2 heterozygotes on the basis of the levels of Hb Bart's at birth (Todd and Chan, 1978). Thus, it is possible that there is a large overlap between the levels of Hb Bart's in the α -thal-1 or in the α -thal-2 heterozygous newborns, so that a value of Hb Bart's lower than 5% does not permit an accurate distinction between these two conditions in the newborn.

Genotyping the α -thalassaemias on the basis of the levels of Hb Bart's in newborns has been further

Fig. 2 Cumulative distribution of the values of Hb Bart's measured in 149 Greek Cypriot newborns.

Table Haematological findings at 3 years of age in 5 Cypriot children who had less than 5% Hb Bart's at birth.

	Case 1	Case 2	Case 3	Case 4	Case 5
Hb Bart's at birth (%)	0.85	3.5	3.7	4.1	4.2
PCV (%)	37.0	30.0	32.0	34.0	32.0
RBC ($\times 10^6$)	4.6	3.9	4.1	4.5	4.3
Hb (g/dl)	11.8	9.2	9.2	10.5	9.6
MCV (μ^3)	80.0	77.0	78.0	75.0	74.8
MCH (pg)	25.7	23.5	22.5	23.5	22.4
MCHC (g/dl)	32.0	30.5	28.8	31.0	30.0
Hb H inclusions	+	+	+	+	+

complicated by the recent findings of molecular heterogeneity of α -thalassaemia in Cyprus (Kan *et al.*, 1978). It has been established that the α -globin genes are duplicated in man and that α -thalassaemia in Orientals results from α -globin gene deletions (Ottolenghi *et al.*, 1974; Taylor *et al.*, 1974). The molecular hybridisation data are compatible with the interpretation that both α -globin loci are deleted in α -thal-1 but only one in α -thal-2, and that patients with Hb H disease have only one active α -globin gene, while Hb Bart's hydropic infants have no α -globin genes. A non-deletion form of α -thalassaemia-1 has been observed in Orientals (Kan *et al.*, 1977), but it is rare. A recent study of Cypriot families with Hb H disease disclosed that one third of the α -thal-1 genes in these families are of the non-deletion type (Kan *et al.*, 1978). The haematological and haemoglobin phenotype of newborns with non-deletion of α -thal-1 has not yet been worked out; thus, it is not known in what range of Hb Bart's distribution newborns heterozygous for this gene fall.

An interesting observation of this study is that α -thalassaemia is frequent in both Cypriot cultural communities, the Greek and the Turkish. Since only 13 generations have passed since the arrival of the original Turkish settlers, it is unlikely that the high frequencies of α -thalassaemia in the two communities were attained independently by natural selection during such a short period of time. It is more likely that the similarity in frequency of α -thalassaemia genes reflects the common origin of the cultural communities, at present segregated, from the people that inhabited the island 400 years ago.

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