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Prevention of Homozygous β-Thalassemia by Carrier Screening and Prenatal Diagnosis in Sardinia

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SUMMARY

We report here results of a 3-year pilot voluntary screening program coupled with prenatal diagnosis directed to the prospective prevention of homozygous β-thalassemia (β-thal) in Sardinia. The screening program took two approaches: outreach community testing and hospital testing on request after a period of sensibilization. The outreach testing was very effective as, taking into account the already known number of couples at risk with an affected proband (20), 74% of the couples at risk expected (61) on the basis of the carrier rate were identified. Less effective was the hospital testing in which half of the couples at risk expected were detected (502 with and 199 without an affected proband). After nondirective genetic counseling, approximately 85% of the couples at risk, which had a pregnancy, with no statistically significant difference between those with and those without a proband, requested prenatal testing. This figure showed a steady increase from the beginning in 1977 to 1980. All the pregnancies (42), but two carrying homozygous fetuses, were terminated on parental request. A continuous hospital survey of thal-major admissions in the different hospitals of the counties showed a steady decline in the incidence figure at birth from 1976 (1:213) to 1978 (1:290). These results showed that even in a medium-developed, rural, Catholic population screening coupled with prenatal diagnosis can be successful in the control of a fatal, recessively inherited disorder.

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INTRODUCTION

β-thalassemia (β-thal) in Sardinia meets the prerequisites suggested for a prospective approach for control of recessive genetic disease through heterozygote screening and prenatal diagnosis; that is, if the disease occurs predominantly in a defined population, there are simple and accurate methods for carrier identification and the homozygous state can be detected in utero early in pregnancy [1]. The island of Sardinia, in fact, has a prevalently autochthonous population of 1.4 million inhabitants with a β-thal carrier rate of 13%. The couples at risk are therefore 1:60, and the disease incidence in newborns is 1:238 [2]. In the southern part of this island, where this program was carried out, β^0-thal is the most prevalent β-thal type, as all 401 thal-major patients examined so far but one (a β^+ thal homozygote) were β^0-thal homozygotes [3].

β-thal heterozygotes can be identified by mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) determination combined with Hb A_2 quantitation [4]. Prenatal detection or exclusion of the homozygous state can be achieved through globin-chain synthesis analysis on fetal blood samples, obtained either by placental aspiration or fetoscopy [5–9].

Although there has been great improvement in the management, even under the best conditions with optimal transfusion regimens and appropriate chelation therapy, the homozygous state has a life expectancy of approximately 18–20 years [10].

With these considerations in mind, in September, 1977, we started a pilot, voluntary, genetic screening program directed to the prospective prevention of homozygous β-thal in Southern Sardinia. Here we describe the results obtained in the first 3 years of this ongoing program.

MATERIALS AND METHODS

Screening Program

The screening program, directed to the adult population and primarily to couples at child-bearing age, was carried out in Southern Sardinia (Cagliari and Oristano counties) on a voluntary basis. The program has taken two different approaches: (1) outreach community testing, and (2) hospital testing by individuals requesting the test.

Participants were requested to fill out a questionnaire for purposes of establishing specific baseline personal, health, and attitudinal data. An informed consent by the screenee was not requested, but, prior to testing, an effort was made to inform each person about the nature of the illness, the meaning of "carrier," the implication of being a carrier, and the alternatives available to individuals found to carry the gene. When couples requested testing, only one member was examined and the other was tested if the first member was found to be a carrier.

Outreach Community Testing

This screening, carried out in a few small villages (Pimentel, Samassi, Serrenti, Samatzai, Sarroch, Villacidro, and Settimo S. Pietro), began with a meeting of the community leaders (the mayor, priests, teachers, trade unionists, general practitioners, social workers, etc.) to inform them of the nature of the program, that is, prevention at the population level of this fatal, inherited disorder. Details of counseling, diagnostic procedures, and possible adverse effects were discussed. Posters and information booklets were given for distribution to the adult population, particularly to couples at child-bearing age. Then the informed community leaders held discussion groups for 3 months to better sensibilize the population. At the end of

this 3-month period, a doctor of our team gave a detailed talk, with easy to understand slides, to the general public. This talk included the natural history, actual status of treatment and inheritance pattern of the disease, prevention by carrier identification, and counseling, with particular emphasis on the possible options of people found to be carriers.

After this talk, the first blood sampling was carried out. Thereafter, sampling sessions were held twice weekly at the village community centers (school and medical center), until all the requests had been satisfied. The most convenient time for the population, that is, after 6 P.M. during the week and Sunday morning, was chosen.

Nondirective counseling with a private interview was given at the next session by a physical doctor (biological sciences graduate) to individual carriers or couples. The information provided during the session was directed at giving an informed basis upon which to make reproductive decisions (mate selection, adoption, artificial insemination, and prenatal diagnosis). The very early stage of development of prenatal diagnosis technology was stressed with particular emphasis on details of the fetal blood sampling procedure, including risk to the fetus and failure to obtain sufficient fetal blood for analysis and possible misdiagnosis. Once identified, each carrier was informed of the implications of his carrier status for close relatives, and simple and clearly written educational material was provided for them. Relatives were informed in this way and were given the option to contact the center if they desired further information or wanted to be screened. Before leaving the village, our team provided educational booklets on where and how to be tested to the local marriage registry officer for distribution to soon-to-be-married couples.

Hospital Testing

Before and during this program, the general public was informed and sensibilized about the same topics as in the outreach scheme via local radio, TV stations, newspapers, and talks given by a medical doctor of our team at factories, high schools, large offices, stores, and fairs in the county capitals (Cagliari and Oristano) and suburbs. This campaign was helped by posters and the distribution of information booklets. A more detailed presentation was given to general practitioners, pediatricians, gynecologists, and high school teachers. Again, information booklets were provided to marriage registry offices. Apart from pregnant women, in the first 3 years of this program, all persons had to make a prior appointment for testing, which was carried out in the morning. Counseling sessions were conducted by the same team and with the same criteria as in the outreach program. No division between couples referred by their doctors and those influenced by the education program was possible, since many presented for both reasons. Besides couples identified by population screening, parents of Cooley anemia patients were also counseled.

Diagnostic Schema and Criteria for Carrier Identification

Besides red cell indices, all subjects had hemoglobin (Hb) electrophoresis and Hb A_2 quantitation [11, 12] because in our laboratory with only MCV, MCH, osmotic fragility test (OFT), and Shine and Lal discriminant function [13], 3.5%, 1.5%, 3.5%, and 4.0% carriers, respectively, gave false negatives (fig. 1) [14].

The diagnosis of β-thal carrier state was made on the basis of high Hb A_2 levels associated with reduced MCV and MCH. In our laboratory, Hb A_2 estimation with DE-52 microchromatography in normal subjects gave values from 1.10% to 3.45% (mean = 2.30 $\pm$ 0.43) and in β-thal heterozygotes values from 3.70% to 7.30 (mean = 5.10 $\pm$ 0.50) [12] and our unpublished results, 1980). In a previous study [14], with the exception of one subject, all obligate carriers (638) had elevated Hb A_2 levels. Even in pregnancy, Hb A_2 determination gives a clear-cut distinction between β-thal carriers and normals (our unpublished results, 1980). In our laboratory with DE-52 microchromatography, Hb A_2 levels in iron-deficient carriers were reduced but still in the carrier range [15].

All subjects with low MCV and normal or reduced Hb A_2 levels had iron studies. When serum iron studies gave normal results, Hb H inclusion bodies and, eventually, globin-chain

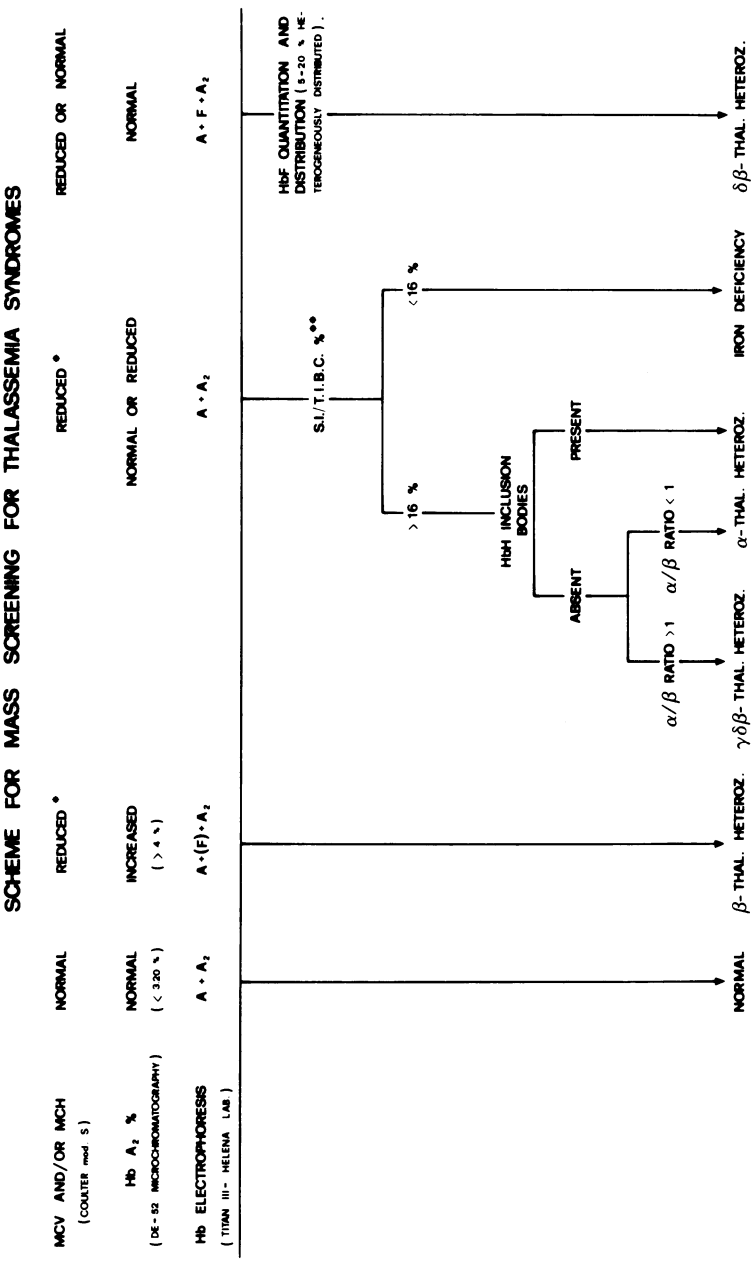

FIG. 1.—Scheme for mass screening for thalassemia syndromes

synthesis analysis were carried out. Subjects with low or normal MCV, normal or reduced Hb A_2 levels, but showing Hb F by electrophoresis, had Hb F quantification and an erythrocyte Hb F distribution study performed.

Cases with microcytosis, low or normal Hb A_2 levels, Hb F levels higher than 5%, heterogeneously distributed in red cells, were considered to have the $\delta\beta^0$-thal trait. Subjects with microcytosis, normal Hb F, normal or reduced Hb A_2, and normal serum iron were diagnosed as having the α-thal trait when the Hb H inclusion bodies test was positive or the α/β globin-chain synthesis ratio was in the α-thal carrier range [16].

Screening Methods

Blood samples were drawn and stored in EDTA. Hematological indices were obtained using the Coulter Counter model ZBI and Coulter Hemoglobinometer or Coulter Counter model "S" (Coulter Electronics, Hileal, Fla.). Hemoglobin electrophoresis was carried out on cellulose acetate plates (Helena Laboratories, Beaumont, Tex.) in Tris-EDTA borate buffer, pH 8.6. Hb A_2 was quantified by DE-52 microchromatography [11], and Hb F was measured by alkali denaturation [17, 18]. Erythrocyte Hb F distribution studies were performed with the Betke-Kleihauer stain [19] using commercial kits (Boehringer-Mannheim, Indianapolis, Ind.) or with immunofluorescence method with anti-γ antiserum (kindly provided by Dr. Bernini, Leiden, Holland). Serum iron and iron-binding capacity were determined by the method of Lauber [20]. Hb H inclusion bodies were obtained by mixing equal volumes of whole blood and 1% brilliant cresyl blue in citrate saline solution and incubating at 37°C for 20 min to 1 hr.

Prenatal Diagnosis

Fetal blood was sampled by placental aspiration with a 20-gauge needle between the 18th and 20th menstrual weeks of pregnancy after placenta localization with ultrasound (Picker Echoview model 80L), as described [6]. Before placental aspiration, venous blood was drawn from the mother for reticulocyte count, fetal hemoglobin stain, and [^{3}H]leucine incubation.

The placental samples obtained were checked immediately for the presence of fetal red cells with a Coulter particle-size analyzer [5]. Smears from the same samples were stained with the Betke-Kleihauer method [19], and the fetal cell percentage determined.

The placental samples containing fetal blood were incubated with [^{3}H]leucine as described [6]. When the fetal blood was less than 80%, fetal red cells were selectively concentrated by differential agglutination with anti-i serum according to Kan et al. [5, 6] or by NH_4 Cl-NH_4 HCO_3 differential lysis of maternal cells [21], as described in [22].

Globin-chain synthesis analysis was carried out by previously described methods [6]. The radioactivity under each globin peak was integrated after subtraction of the residual baseline counts. Then the β/γ-globin-chain synthesis ratio or the absence of β-chain radioactivity was assessed.

Whenever possible, globin-chain synthesis analysis was carried out on blood from aborted fetuses. Infants born after prenatal diagnosis had globin-chain synthesis analysis on cord blood and hematological assessment including Hb F and A_2 determination at 6 months. Diagnosis of the homozygous state was based on a β/γ-globin-chain synthesis ratio of 0. In our laboratory, nonhomozygous fetuses had globin-chain synthesis ratios ranging from 0.28 to 0.142 [23].

RESULTS

Outreach Community Testing

Table 1 summarizes certain demographic characteristics of the population tested and the overall results obtained. The mean percentage of volunteers requesting the test, both single and married, from the total population of subjects at child-bearing age was 32%. Of the individuals tested, 31.3% were male and 68.7% were female.

TABLE 1

OUTREACH COMMUNITY TESTING: DEMOGRAPHIC CHARACTERISTICS AND OVERALL RESULTS

	Pimentel	Samassi	Samatzai	S. Andrea Frius	Sarroch	Serrenti	Settimo S. Pietro	Villacidro	Total
Population	1,160	4,742	1,574	1,763	3,944	4,674	3,583	12,690	34,130
Population at child-bearing age (15–44 yrs)	410	1,683	473	632	1,622	1,653	1,398	4,790	12,661
Population examined	101	1,050	108	191	527	477	442	1,161	4,057
% population examined	24.6	62.4	22.8	30.2	32.5	28.9	31.6	24.2	32.0
Couples at child-bearing age (15–44 yrs)	100	448	156	155	412	508	313	1,227	3,319
Couples at child-bearing age examined	60	301	77	30	215	194	263	885	2,025
% couples at child-bearing age examined	60	67.2	49.4	19.4	52.2	38.2	84	72.1	61.0
β-Thal carrier identified	12	180	20	30	91	76	43	170	622
β-Thal carrier frequency	11.9	17.1	18.1	15.7	17.3	15.9	9.7	14.6	15.3
Adjusted carrier frequency	11.5	14.9	16.3	13.7	14.8	12.9	9.1	13.9	13.6
Couples at risk identified	1	8	2	1	1	1	0	11	25

The mean percentage of couples tested out of the total was 61%. Out of 2,025 couples examined, 25, in which both husband and wife were found to be β-thal carriers and hence at risk for thal major in their offspring, were identified.

Pregnancy was in course in 6.3% of the couples tested. Up to date, there has been no pregnancy in any of the couples at risk identified in the program.

Six hundred twenty-two carriers were identified out of the 4,057 individuals voluntarily tested. The adjusted carrier frequency of 13.6% was calculated on the basis of the number of heterozygotes and nonheterozygotes identified among subjects tested with no known family history of β-thal major or carrier state at the time of testing. The details of the screening results are reported in table 2. As shown, besides β-thal, there are only a few other carrier types ($\delta\beta^0$-thal heterozygotes, sickle cell trait, $\gamma\delta\beta$-thal heterozygotes) whose mating with β-thal carriers could produce thal intermedia or sickle cell disease.

Hospital Testing

The genetic service of the 2nd Pediatric Clinic, where the testing was carried out, takes care of the population of Cagliari and Oristano counties, whose demographic characteristics are summarized in table 3. The villages, where the outreach testing has been carried out, are located in these counties. Of the total single and married population at child-bearing age, 3.7% requested the test, and of these individuals, 34.7% were male and 65.3% female. Of the total population of couples, 8.4% were examined, and out of 6,542 couples examined, 192 at risk were identified.

Pregnancy was in progress in 49.7% and 4% of the couples and nonmarried singles tested, respectively. Until now, 111 couples at risk identified have had a pregnancy (table 4). After counseling, 86 of these elected to have prenatal diagnosis, 19 decided against having it and continued the pregnancy, and six aborted spontaneously during the interval between counseling and the day scheduled for the placental tap.

TABLE 2

DETAILED RESULTS OF THE HOSPITAL AND OUTREACH TESTING PROGRAMS

	Hospital testing	Outreach testing
Normal	8,905	2,988
β-Thal heterozygote	2,398	622
α-Thal heterozygote	1,186	433
$\delta\beta^0$-Thal heterozygote	12	5
Hb S trait	2	4
Hb H disease	6	3
$\gamma\delta\beta$-Thal heterozygote	2	...
β^0-Thal homozygote	2	1
$\delta\beta^0$-β^0 genetic compound	1	...
Hb J Sardegna	6	1
Hb J Oxford	1	...
Hb G Philadelphia	1	...
Hb G San Jose'	1	...
Hb β variants not characterized	2	...
Hereditary spherocytosis	2	...
Total	12,527	4,057

TABLE 3

HOSPITAL TESTING: DEMOGRAPHIC CHARACTERISTICS AND OVERALL RESULTS

	No.
Population	802,888
Population at child-bearing age (15–44 yrs)	339,121
Population examined	12,527 (3.7%)
Total couples at child-bearing age (15–44 yrs)	77,490
Couples at child-bearing age examined	6,542 (8.4%)
Total carriers identified	2,402
Adjusted carriers	1,871
Heterozygote frequency	.168
Couples at risk identified	192

Out of the 12,527 individuals tested, 2,402 carriers were identified. The adjusted carrier frequency, calculated as above, was 16.8%. The details of the screening results are shown in table 2.

Additionally, 502 parents of Cooley anemia patients were counseled and 116 (23.2%) had a pregnancy. After counseling, 91 of these had prenatal diagnosis, 15 decided against prenatal testing and continued the pregnancy, and 10 spontaneously terminated before fetal blood sampling was carried out (table 4). Table 5 shows that there was a steady decline of prenatal testing refusal from 1977 to 1980.

Prenatal Diagnosis

In 91 monitored pregnancies occurring in couples with previous affected offspring, 19 fetuses with no β-chain synthesis were identified (table 6). In all instances but one, which was spontaneously terminated a few days after fetal blood sampling, the parents requested elective termination. There were four fetal losses due to premature labor or fetal hemorrhage. To date, 55 nonhomozygous fetuses have been born. The other pregnancies are still in progress.

In 86 monitored pregnancies occurring in couples with no previous affected offspring, 23 homozygous fetuses were identified (table 6); in all cases but two, the parents requested elective abortions. In the two pregnancies with β-thal homozygous fetuses that continued to term, the respective offspring developed thal major.

TABLE 4

OUTCOME OF THE COUPLES AT RISK IDENTIFIED THAT HAD HAD PREGNANCY

	With previous affected offspring	Without previous affected offspring	Total
Couples at risk identified	502*	192	694
Couples that had had pregnancy	116†	111	227
Couples refusing antenatal diagnosis	15 (12.9%)	19 (17.1%)	34 (15%)
Pregnancies terminated before fetal blood sampling	10 (8.6%)	6 (5.4%)	16 (7%)

* Only couples identified in the hospital testing had had a pregnancy.

† Total no. couples calculated from the heterozygote frequency is ~ 1,300.

TABLE 5

REFUSAL RATE OF ANTENATAL DIAGNOSIS AFTER GENETIC COUNSELING PER YR

	1977	1978	1979	1980
Couples with affected offspring:				
Counseled	32	47	56	34
Refusing the procedure	9 (28.1%)	2 (4.2%)	6 (10.7%)	2 (5.8%)
Couples without affected offspring:				
Counseled	11	42	65	35
Refusing the procedure	3 (27.2%)	9 (21.4%)	9 (13.8%)	3 (8.5%)

There was one fetal loss due to premature labor, and 51 nonhomozygous fetuses have been born at this date. The other pregnancies are still in progress.

A survey of all in- and outpatient admission in the hospitals of the counties showed a steady decline of thal-major incidence at birth from 1976 to 1979 (table 7).

DISCUSSION

In spite of the relatively low number of people tested, the results of this first 3-year program, organized with the ultimate aim of controlling β-thal major in the Southern Sardinian population, are encouraging.

The outreach testing, carried out after a 3-month educational campaign, was very effective, as 32% and 61% of single individuals and couples at child-bearing age, respectively, requested testing and 25 couples at risk with no previous affected children were identified. Since in the villages, where testing was performed, there were 20 homozygotes, the total number of couples at risk will be 45, which is very close to the expected figure of 61 with a carrier rate of 13.6%. Therefore, in these villages, most of the couples at risk were identified and counseled.

In the hospital testing, the percentage of single individuals and couples at child-bearing age requesting testing was very low. The most likely causes were difficulties in informing the inhabitants of the counties, particularly those living far from the city, and the time-lag between request for appointment and actual appointment for

TABLE 6

RESULTS OF ANTENATAL DIAGNOSIS OF THAL MAJOR IN FAMILIES WITH AND WITHOUT PREVIOUS AFFECTED OFFSPRING

	With previous affected offspring	Without previous affected offspring	Total
Pregnancies monitored	91	86	177
Homozygous β-thal fetuses identified	19 (20.9%)	23 (26.7%)	42 (23.7%)
Electively terminated	18*	21†	39
Unaffected offspring born	55	51	106
Complications	4 (4.4%)	1 (1.2%)	5 (2.8%)
In progress	14	11	25

* One was a complication.

† Pregnancies of two homozygous fetuses were continued to term.

TABLE 7

NEWBORN INCIDENCE OF THAL MAJOR IN CAGLIARI AND ORISTANO COUNTIES

Yrs	No. thal major newborns*	No. live births	Incidence
1976	71	15,138	1:213
1977	58	14,850	1:256
1978	49	14,204	1:290
1979	17	13,398	...

* No. patients diagnosed in the different hospitals of the counties up to July, 1980.

screening in the first 2 years of the program. Other possible causes could be fatalism, misunderstanding of the educational message (particularly, lack of comprehension of the different options available), nonacceptance of prenatal testing and elective termination of pregnancy, and fear of lack of confidentiality with possible social consequences of being identified as a carrier. To clarify these points, a long-term prospective controlled study in the villages of the counties is now in progress.

Notwithstanding the low percentage tested (3.7% of the total population and 8.4% of the couples), the number of couples at risk identified (217) was approximately 17% of those expected (1,300) with a carrier rate of 13.0%. Indeed, adding to the number of couples at risk without the proband identified, those (502) known as already having had an affected child, the total number of couples at risk detected results in approximately half of the figure (55%) expected. This highly apparent effectiveness of the screening and counseling program could be due to the fact that many people requesting testing were relatives of homozygotes or heterozygotes or went to the hospital for confirmatory testing but did not reveal prior positive results obtained in previous programs carried out in the schools.

Since people coming for confirmatory testing were probably more prevalent in the hospital than in the outreach testing, the adjusted carrier frequency resulted higher in the former (.189 vs. .136). However, even the heterozygote frequency found in the outreach testing represents a minimum estimate rather than an unbiased one. In fact, in the villages also, there could have been people coming for confirmatory testing without revealing prior positive results and multiple members of the same family could have been tested.

In both hospital and outreach testing, there has been a high prevalence of females requesting testing since they are probably more involved in matters relating to family planning. The high percentage of married couples, particularly in the hospital testing program, probably depends on the educational publicity and counseling that stressed the availability of the prenatal diagnosis service. This may also explain the consistent number of pregnant women requesting testing, particularly in the hospital. The prevalence of married couples and/or pregnant women in the hospital program probably reflects a higher level of motivation than was present in the villages, where the testing was immediately available to all without appointment.

After nondirective genetic counseling, most of the couples at risk (85%) who had a pregnancy requested prenatal testing. Similar attitudes were noted in the Cypriot population living in the United Kingdom, where over 90% of couples at risk are in

favor of antenatal diagnosis as the only available method of ensuring healthy offspring [24]. The absence of statistically significant differences between the number of couples with and without previous affected offspring requesting antenatal testing is again encouraging, since it shows that even the respondent population without the personal dramatic experience of the homozygous state was well informed and accepted antenatal testing as a method to have healthy offspring.

The refusal of antenatal testing in approximately 20% of the couples at risk may be due to fear of complications or to ethical reasons. However, the steady decline of the refusal rate from the beginning in 1977 (27.9%) to the actual figure (7.3%) clearly demonstrates that the fear of complications associated with this new technology was the most prevalent reason against antenatal testing. Between counseling and fetal blood sampling sessions, some pregnancies (7%) were "spontaneously" terminated. This seems a very high figure, even taking into account the anxiety frequently observed after the counseling sessions. Indeed, it is possible that some of these couples had decided to interrupt the pregnancy before attending the counseling session. This could also reflect a misunderstanding of the counseling. All the pregnancies, but two carrying homozygous fetuses, were terminated on parental request. This demonstrates that, even a medium-developed, prevalently rural, Catholic population accepts elective abortion as a method to prevent the birth of a child affected with a fatal recessive disorder. Two couples, in which a homozygous fetus was identified, decided to continue the pregnancy as one member of each was a devout Catholic. These families showed a good adjustment after the development of Cooley anemia in their children. After this experience, the risk to the fetus associated with the sampling procedure was stressed more strongly during counseling to avoid couples having prenatal testing only to establish the status of the fetus.

A continuous hospital survey of thal-major admission in the different hospitals of the counties, including ours, showed a steady decline in the incidence figure at birth from 1976 to 1979. This figure is likely to be extremely accurate as ours is a referral center and all Cooley anemia patients present at our hospital at least once. However, the 1978–79 figure may be slightly underestimated, since in 20%–25%, the initial presentation of Cooley anemia may be delayed until 2–3 years of age, at least in our population [25]. This decline is only partially due to prenatal diagnosis, as the reduction in the incidence of Cooley anemia at birth is higher than the number of homozygous fetuses electively terminated. Other factors determining this reduction could be the rational decision not to have other children by couples already having one or more affected children or identified as carriers in the screening and wider knowledge on family planning techniques. The already mentioned follow-up study should give more precise answers on this point.

In conclusion, our results show that in a medium-developed, rural, Catholic population, screening of an inherited fatal recessive disorder such as thal coupled with prenatal diagnosis is successful. This contrasts with previous experience in Greece concerning sickle cell trait [26] and in Ferrara [27] concerning β-thal, where the effects of genetic screening and counseling, carried out without the option of prenatal diagnosis, were negative, as shown by the lack of difference in the observed and expected (in the case of random mating) figure of mating between carriers.

According to our previous experience [22, 23], fetal blood sampling with placental needling was highly successful with a success rate of 97.7% and the biochemical analysis of fetal blood by globin-chain analysis seems to be very accurate. In fact, in cases in which the nonhomozygous state has been ruled out, no affected children have been born to date and the homozygous incidence figure of 23.7% is very close to that expected for a recessively inherited disorder. The fetal loss rate of 2.8% in this series is much lower than the overall rate of 6.7% noted in over 1,100 cases (including ours) that had been done in several centers until March, 1980 (B. Alter, personal communication, 1980). The 2.8% fetal loss is an acceptable figure, as it is very close to the 3.2% and 3.5% values found in a prospective study concerning normal and amniocentesis midtrimester pregnancies, respectively [28].

This experience in the control of thal major through carrier screening and prenatal diagnosis is promising, but a prospective controlled study, now in progress, is necessary to evaluate the long-term effects of screening and counseling. In fact, previous studies of prospective genetic counseling showed variable effects on the individuals according to the different populations tested and the different screening programs, ranging from significant overall learning and acceptable effect on mood in the programs directed to the prevention of Tay-Sachs disease and β-thal [1, 29] to significant adverse effects such as discrimination, discovery of nonpaternity, etc., in some programs directed to the control of sickle cell anemia [26, 29–39].

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