

PREVALENCE AND GENOTYPES OF α - AND β -THALASSEMIA CARRIERS IN HONG KONG — IMPLICATIONS FOR POPULATION SCREENING

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ABSTRACT

Background The thalassemias are common in southern China. We determined the prevalence of heterozygous carriers of these genetic disorders in Hong Kong and assessed the feasibility of a community-based screening program.

Methods An educational and screening program for the thalassemias was carried out in three high schools with a total of 2420 students. Seventy-five percent of the students agreed to undergo screening, which consisted of blood counts, hemoglobin electrophoresis, serum ferritin measurements, and DNA analyses.

Results Of the 1800 blood samples tested, 150 (8.3 percent) had microcytosis (mean corpuscular volume, $<80 \mu\text{m}^3$). Ninety students (5.0 percent) were carriers of α -thalassemia, of whom 81 (4.5 percent) were carriers of the Southeast Asian type of deletion, in which both α -globin genes on the same chromosome 16 are deleted. Sixty-one students (3.4 percent) were carriers of either β -thalassemia or the mutation coding for hemoglobin E. Six students were carriers of both α - and β -thalassemias. On the basis of these figures, the estimated numbers of pregnancies in Hong Kong in which the fetus is at risk for homozygous α -thalassemia and β -thalassemia major or intermedia are 145 and 80 per year, respectively. In Hong Kong the actual numbers of women referred for prenatal diagnoses of these disorders are approximately 95 and 40 per year, respectively.

Conclusions Despite the availability of hospital-based screening and prenatal diagnosis for many years in Hong Kong, many women carrying fetuses at risk for thalassemia are not referred for genetic counseling. A community-based program of education, screening, and counseling is needed in Hong Kong and southern China. (N Engl J Med 1997;336: 1298-301.)

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THE α - and β -thalassemias are due to mutations of the α - or β -globin genes that markedly decrease or completely prevent the production of α - or β -globin chains.¹ They are the most common inherited single-gene disorders in the world, with the highest prevalence in areas where malaria has been or remains endemic. In Southeast Asia, with a population of approximately 450 million people, the burdens of inherited globin gene disorders in many regions are of such magnitude that they represent a public health concern.²

We assessed the feasibility of a large screening program for thalassemia in Hong Kong, a city of 6 million people, and determined the thalassemia-carrier rates. The results indicate that given the proper information, most high-school students, as well as their parents and teachers, are receptive to a screening program for thalassemia. The data confirm that approximately 8 percent of the population in Hong Kong are heterozygous carriers of α - or β -thalassemia mutations.

METHODS

Subjects

Students from three high schools in separate middle-class or working-class neighborhoods (Hong Kong West, Kowloon City, and Shek Kip Mei) were chosen for this study. In these three schools, there were 2420 students in grades 9 to 13, ranging from 14 to 19 years of age. Ninety-eight percent of these students were ethnic southern Chinese. One of us discussed the study with the three school principals and obtained their approval and cooperation in this investigation.

Educational sessions on the thalassemias given by a pediatrician or a trained nurse were held in school auditoriums for all the students and the teaching staff. Afterward, each student was given an information sheet and a consent form for his or her parents to sign. At a later scheduled session, 5 ml of venous blood, which was anticoagulated with EDTA, was obtained from each participant with a signed parental consent form. Although the study was designed primarily for the students, 72 teachers also asked to be tested.

Each participant received a written report of the results of the screening tests. Those who were found to be carriers of thalassemia mutations were given an opportunity to receive additional information and counseling, initially with a trained nurse. If deemed necessary, counseling with a pediatrician was arranged. Twenty-two students attended these counseling sessions. In addition, all carriers received a card printed with their thalassemia-carrier status for their records and future reference.

Hematologic Studies

Peripheral-blood counts and red-cell indexes were determined according to standard laboratory procedures with the use of Technicon H*1 and H*2 blood analyzers (Technicon, Tarrytown, N.Y.). Hemoglobin H inclusion bodies were detected by incubating peripheral blood with brilliant cresyl blue for 30 minutes at

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37°C.³ Cellulose acetate electrophoresis was used to analyze hemoglobins at a pH of 8.5. Hemoglobin A₂ and fetal hemoglobin were quantitated by the variant hemoglobin testing system (Bio-Rad Laboratories, Hercules, Calif.). We used an immunocytologic technique to detect embryonic ζ -globin chains with a murine monoclonal antibody against ζ -globin chains as previously described.^{3,4} Serum ferritin levels were measured with the IMx Ferritin Assay kit (Abbott Laboratories, Abbott Park, Ill.).

Molecular Analyses

DNA was extracted from peripheral-blood leukocytes. The α -globin genotypes were determined by Southern blotting, hybridized with probes specific for α - and ζ -globin genes, as well as the 3' hypervariable region probe as indicated.⁵ To search for the hemoglobin Constant Spring variant (α^{CS}), which results in the elongation of the α -globin chain, the α 2-globin gene was amplified by the polymerase chain reaction (PCR) and subjected to dot blotting for hybridization with allele-specific-oligonucleotide nonradioactive probes. PCR was also used to identify the Southeast Asian type of deletion (-SEA), in which both α -globin genes on the same chromosome 16 are deleted.⁶

Mutations in the β -globin gene were identified by PCR-based diagnostic strategies, including one using heteroduplexes to detect the deletion of CT TT in codons 41 and 42, dot blotting with allele-specific-oligonucleotide nonradioactive probes to identify the substitution of thymine for cytosine at position 654 in IVS2 as well as the mutation in the β^E allele, and direct nucleotide sequencing to detect all other mutations.⁵ The deletion causing ($^A\gamma\delta\beta$)⁰-thalassemia was detected by Southern blotting with a probe specific for the $^C\gamma$ -globin gene.⁷

RESULTS

Of the 2420 students who attended the informational meetings, 1812 (75 percent) obtained parental consent and agreed to undergo screening. A total of 1800 blood samples were obtained and analyzed (12 students were absent from school on the days on which phlebotomy was scheduled).

One hundred fifty of the 1800 blood samples (8.3 percent) had microcytosis (mean corpuscular volume of less than 80 μm^3). These 150 blood samples were tested for α - and β -thalassemias and iron deficiency. The α -globin genotypes of all 150 blood samples were determined both by Southern analysis and by PCR with primers specific for the (-SEA) type of α -thalassemia deletion. Mutations in the β -globin gene clusters were determined only in blood samples with hemoglobin A₂ concentrations above 3.5 percent.

Mutations related to α -thalassemia were found in 90 of the 1800 samples screened, or 5.0 percent (Table 1). More important, 81 blood samples (4.5 percent) had the (-SEA) deletion. One sample had a deletion of the complete ζ - α -globin gene cluster. Three cases of hemoglobin H disease, involving the deletion of three α -globin genes, were identified. Triplicated α -globin genes were found in five samples. The α^{CS} mutation was not found in any sample.

Sixty-one of the 1800 blood samples screened (3.4 percent) had β -thalassemia mutations, including that coding for hemoglobin E (Table 1). Two thirds of these mutations were accounted for by the deletion of CT TT in codons 41 and 42 and by the substitution of thymine for cytosine at position 654 in IVS2,

in agreement with previous studies.⁸⁻¹⁰ Six persons were carriers of both α - and β -thalassemia mutations. One carrier of probable hemoglobin Queens was identified by hemoglobin electrophoresis.

Five students had iron deficiency, with serum ferritin levels of less than 10 μg per liter.

DISCUSSION

Approximately 2400 high school students attended the educational sessions on the thalassemias, and 75 percent voluntarily entered the screening program by obtaining parental consent and undergoing phlebotomy. This high level of receptivity by students and their families in a properly designed screening program for thalassemia carriers is notable. Recently, Mitchell et al. reported the result of a long-term (begun in 1980) screening program for carriers of thalassemia among high-school students in Montreal.¹¹ They found a high rate of voluntary participation in the programs, a high rate of use of reproductive-counseling options later in life, and a major decline in the incidence of β -thalassemia major in the province of Quebec.¹¹

Thalassemias are common in southern China.¹²⁻¹⁴ In the population we screened, 4.6 percent (95 percent confidence interval, 3.6 to 5.6 percent) were

TABLE 1. RESULTS OF SCREENING OF BLOOD SAMPLES FROM 1800 HIGH-SCHOOL STUDENTS FOR MICROCYTOSIS, THALASSEMIA-CARRIER STATUS, AND IRON DEFICIENCY.

| VARIABLE | NO. OF BLOOD SAMPLES (N=1800) | PERCENT |
|---|-------------------------------|---------|
| Microcytosis (mean corpuscular volume, <80 μm^3) | 150 | 8.3 |
| Deletion associated with α -thalassemia* | 90 | 5.0 |
| (-SEA) Deletion | 81 | 4.5 |
| Deletion of the complete ζ - α -globin gene cluster | 1 | 0.06 |
| (- $\alpha^{3.7}$) Deletion | 6 | 0.3 |
| (- $\alpha^{4.2}$) Deletion | 3 | 0.2 |
| Undetermined | 2 | 0.1 |
| Mutation coding for β -thalassemia* | 55 | 3.1 |
| Deletion of CT TT at codons 41-42 | 22 | 1.2 |
| C→T at position 654 in IVS2 | 18 | 1.0 |
| A→G at nucleotide -28 | 8 | 0.4 |
| A→T at codon 17 | 3 | 0.2 |
| G→T at codon 43 | 2 | 0.1 |
| Insertion of A at codons 71-72 | 1 | 0.06 |
| Deletion causing ($^A\gamma\delta\beta$) ⁰ -thalassemia | 1 | 0.06 |
| Mutation coding for mutant hemoglobin Glu→Lys at codon 26 in the β -globin chain (hemoglobin E) | 7 | 0.4 |
| Probable Leu→Arg at codon 34 in the α -globin chain (hemoglobin Queens) | 6 | 0.3 |
| Iron deficiency | 1 | 0.06 |
| | 5 | 0.3 |

*Six subjects were carriers of both α - and β -thalassemia mutations, three had hemoglobin H disease with deletion of three α -globin genes, and five were carriers of triplicated α -globin genes.

TABLE 2. PREVALENCE OF CLINICALLY SIGNIFICANT α - AND β -THALASSEMIA MUTATIONS.

| VARIABLE | NO. OF SAMPLES (N=1800) | PREVALENCE (95% CI)* |
|---|-------------------------|----------------------|
| percent | | |
| Microcytosis (mean corpuscular volume, <80 μm^3) | 150 | 8.3 (7.1–9.7) |
| α - or β -Thalassemia mutations | 145 | 8.1 (6.8–9.4) |
| (-SEA) Deletion or deletion of the complete ζ - α -globin gene cluster | 82 | 4.6 (3.6–5.6) |
| β -Thalassemia or hemoglobin E mutations | 61 | 3.4 (2.6–4.3) |

*The 95 percent confidence intervals (CI) were computed according to the method of Gardner et al.¹⁵

heterozygous carriers of α -thalassemia involving either the (-SEA) deletion or a deletion of the complete ζ - α -globin gene cluster (Tables 1 and 2) and were therefore at risk of conceiving fetuses with hemoglobin Bart's hydrops fetalis syndrome (absence of all four α -globin genes; commonly, (-SEA/-SEA)). This study confirms that immunocytologic staining for embryonic ζ -globin chains in peripheral-blood smears is a reliable way to identify adult carriers of the (-SEA) deletion (data not shown).^{3–5} We found a low prevalence of deletions involving a single α -globin gene (0.5 percent), but it is likely that many carriers of such mutations have mean corpuscular volumes of more than 80 μm^3 and therefore escaped detection in our screening program.^{16,17}

Fetuses with hemoglobin Bart's hydrops fetalis syndrome usually die in utero during the third trimester or shortly after birth. Serious developmental anomalies, including severe retardation of brain growth, have been reported in these fetuses.^{1,18} Moreover, their mothers have an increased risk of serious complications during pregnancy.¹⁸ In Hong Kong, where there are approximately 70,000 births a year, the number of pregnancies involving a fetus at risk for hemoglobin Bart's hydrops fetalis syndrome would be about 145 per year (95 percent confidence interval, 93 to 221) (Table 3).

Carriers of β -thalassemia mutations including that coding for hemoglobin E accounted for 3.4 percent (95 percent confidence interval, 2.6 to 4.3 percent) of the population screened (Tables 1 and 2). They are at risk of having children with β -thalassemia major and, less frequently, thalassemia intermedia. In addition, a small number of persons are found to be carriers of both α - and β -thalassemia mutations. In the past, children with β -thalassemia major often did not survive to adolescence in developing countries because of the lack of proper health care. This bleak outlook has changed because of the marked economic progress and improved health care in many countries of the Pacific Rim. In Hong Kong, all children with β -thalassemia major currently receive regular blood transfusions and iron-chelation therapy with deferoxamine.^{19,20} With optimal medical care and patient compliance, these children are expected to live to be at least 30 years old.^{21–24} Thirty children with β -thalassemia major in Hong Kong have undergone hematopoietic stem-cell transplantation.²⁵

TABLE 3. PRENATAL DIAGNOSIS OF PREGNANCIES ASSOCIATED WITH A RISK OF α - AND β -THALASSEMIA IN HONG KONG.

| YEAR | PROJECTED NO. OF PREGNANCIES AT RISK (95% CI)* | ACTUAL NO. OF PREGNANCIES WITH REFERRAL FOR PREGNATAL DIAGNOSIS† | NO. OF AFFECTED FETUSES‡ | NO. OF UNAFFECTED FETUSES§ |
|-----------------------|--|--|--------------------------|----------------------------|
| α -Thalassemia | | | | |
| 1993 | 145 (93–221) | 94 | 19 | 75 |
| 1994 | 145 (93–221) | 96 | 28 | 68 |
| 1995 | 145 (93–221) | 95 | 22 | 73 |
| β -Thalassemia | | | | |
| 1993 | 80 (48–131) | 45 | 10 | 35 |
| 1994 | 80 (48–131) | 28 | 5 | 23 |
| 1995 | 80 (48–131) | 46 | 6 | 40 |

*The projected numbers were calculated from the values in Table 2, as follows: for α -thalassemia, 70,000 births per year \times (82 \div 1800); and for β -thalassemia, 70,000 \times (61 \div 1800).² The 95 percent confidence intervals (CI) were calculated from data in Table 2.

†Data are from the Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital, Hong Kong.

‡The fetuses either had hemoglobin Bart's hydrops fetalis syndrome or were homozygous or compound heterozygous for β -thalassemia. All couples with affected pregnancies requested termination of the pregnancies.

§The fetuses were normal or were heterozygous for α - or β -thalassemia.

Despite this progress, β -thalassemia major remains a serious physical, emotional, and financial burden for patients and their families.²⁶ The related health care costs are very high. For example, the annual cost of blood transfusions and deferoxamine treatment for one patient with β -thalassemia major has been reported to be \$30,000 (in U.S. currency).²⁶ On the basis of our results, we estimate that there are 80 pregnancies (95 percent confidence interval, 48 to 131) per year in Hong Kong in which the fetus is at risk for β -thalassemia major or intermedia (Table 3).

During the past three years in Hong Kong, approximately 95 women have been referred annually for prenatal diagnosis of α -thalassemia, and approximately 40 for β -thalassemia (Table 3). These figures suggest that many women in Hong Kong whose pregnancies are at risk of resulting in children with either homozygous α -thalassemia or β -thalassemia major are not identified and referred for genetic counseling. At least 287 patients with transfusion-dependent β -thalassemia major are currently attending pediatric clinics in the eight government-funded hospitals in Hong Kong¹⁹; 110 of these patients are under the age of 10.¹⁹

The success in Sardinia, Italy, and elsewhere in reducing the prevalence of thalassemia major by genetic counseling shows that screening can have a major impact in communities in which the thalassemias are common.^{27,28} Our study demonstrates the feasibility and potential benefits of a community-based screening program in Hong Kong and in the three neighboring provinces of southern China, where 130 million people are at risk for these disorders.

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