PRENATAL DIAGNOSIS
Prenat Diagn 2005; **25**: 163–171.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/pd.1079

Carrier screening for α- and β-thalassemia in pregnancy: the results of an 11-year prospective program in Guangzhou Maternal and Neonatal Hospital

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Objectives To evaluate the first prospective screening program in China for control of α and β-thalassemia in the population of pregnant couples.

Methods During the period between January 1993 and December 2003, a hospital-based preventive program was conducted at the biggest birth center in Guangzhou, with 1/17 of all deliveries in this city referred annually by use of conventional heterozygote screening strategy in combination with the system of regular healthcare examination in pregnancy.

Results The screened records included 49 221 pregnant women, and 4503 husbands of the pregnant women showed positive on the screening test. Of the at-risk couples, there were 198 for α-thal (4.4%) and 83 for β-thal (1.8%), respectively. Genetic counseling was offered to all at-risk couples and a successful prenatal diagnosis was performed for 269 out of 281 (95.7%) for α- or β-thal major, with the remaining 12 couples refusing to accept prenatal diagnosis. Out of 187 pregnancies at risk for homozygous α^0-thal and 82 at risk for β-thal major, 51 hydrops fetalis with Hb Bart's and 18 β-thal major were identified. All pregnancies with affected fetuses were voluntarily terminated, leading to a marked reduction of severe α- and β-thal births at this hospital since the program has been launched.

Conclusions Our hospital-based program proved to be highly effective in reducing severe thals in pregnant populations. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: thalassemias; prenatal screening; genetic counseling; prenatal diagnosis

INTRODUCTION

Thalassemia (thal) syndromes may represent the most appropriate target human inherited disorder that can be controlled by implementation of preventive genetic programs in developing countries (WHO Human Genetics Programme, 1999; Alwan and Modell, 2003). In China, thal is epidemically distributed over the provinces on the south of Changjiang (Yangtse) river. In the mid-1980s, an epidemiological survey of α-thal and β-thal was conducted to a broad extent in 20 provinces of China (National Hemoglobinopathy Cooperative Study Group, 1983; Zeng and Huang, 1987; Wu *et al*., 1988). The highest morbidity was found in Guangdong, Guangxi, and Hainan Provinces. Guangdong is located in the southeastern coast of China with a resident population of 78 million and annual birth rate of one million. Since we have a huge population, the issues relevant to thal will have a major impact on increasing the public health burden. According to our current investigation (Xu *et al*., 2004), the prevalence was 2.54% for β-thal, 4.14% for α-thal caused by southeast Asian type of deletion ($--^{SEA}$), and 4.05% for α-thal caused by a single α-globin gene deletion or mutation. The incidence of the homozygous state in Guangdong is approximately 1 : 1100 for α-thal (including Bart's hydrops fetalis and Hb H disease) and 1 : 6000 for β-thal (Xu *et al*., 2004). Therefore, the universality of thal dramatically produces mental and economic pressure upon our societies and families, which no doubt is a burden on the quality of the newborn population and public health problems in Guangdong as well.

Educational programs, population screening, and genetic counseling for β-thal in populations at risk were well created in Sardinia, the Ferrara district of continental Italy, Greece, and Cyprus. The introduction of these programs has resulted in a marked decline in the number of newborns with thal major from 1 : 250 to 1 : 4000 (Cao *et al*., 1996, 2002). The frequency and severity and the economic and social costs of thals strongly urge a carrier screening and counseling program to be implemented in those developing countries where thals are highly prevalent (WHO Human Genetics Programme, 1999; de Silva *et al*., 2000; Alwan and Modell, 2003). Since 1990s, such similar preventive programs have been initiated in a few developing countries involving Thailand (Jaovisidha *et al*., 2000;

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Received: 25 May 2004
Revised: 13 October 2004
Accepted: 25 October 2004

Tongsong *et al*., 2000), Iran (Ghanei *et al*., 1997; Karimi and Rasekhi, 2002), India (Saxena *et al*., 1998), and Sri Lanka (Perera *et al*., 2000), where the program was carried out at the hospital level. As a developing country, China does not have an adequate healthcare system to prevent thals in rich nations. However, thal, as a 'point of entry' for genetic services into our country, has been an important target for preventive practice of human genetic disorders. In this report, we describe the result of the first hospital-based prospective screening project of controlling severe thal syndrome based on population screening in China. The program has been processed for 11 years in Guangzhou Maternal and Neonatal Hospital (GZMNH), which is the biggest birth size hospital in Guangzhou city. Since January 1993, we have screened 49 221 pregnant women who attended this hospital. Of all the pregnant women in this city, 1/17 referred annually for prenatal diagnosis of thals. We therefore prevented 51 and 18 affected fetuses with α- and β-thal respectively from being born with a disease. Herein we will share our 11-year experience and results of carrier screening for α and β-thal in pregnancy at GZMNH. The practice of such hospital-based program is our first step to extend to the community-based model or city-based model an approach for prevention and control of thals and hemoglobinopathies in southern China.

PATIENTS AND METHODS

Prenatal screening program

This program of prospective carrier screening and genetic counseling was performed in the GZMNH, Guangdong Province, P.R. China, which is the biggest birth size hospital of Guangzhou, with 1/17 of all deliveries in Guangzhou City referred annually by use of conventional heterozygote screening strategy (*J Clin Pathol*, 1994; WHO Hereditary Disease Programme, 1994). All pregnant women, being checked at the hospital by their regular obstetrical healthcare professionals in the first or second trimester, have been screened for both α- and β-thal traits, and other hemoglobinopathies. Within this population, there were approximately 4500 births/year. During the period between January 1993 and December 2003, 49 221 pregnant women underwent this genetic screening program. The husbands of any women with a positive screening test were invited to attend for counseling and testing. In this 11-year program, 4503 husbands of the pregnant women with positive screening test were enrolled to this screening program. A pregnancy in which both members of the couple were carriers was considered a risk and genetic counseling service and prenatal diagnosis were offered. Several accurate methods for prenatal diagnosis are available, such as electrophoretic analysis of umbilical blood or noninvasive ultrasonographic diagnosis for α-thal and DNA tests for α- and β-thal from amniotic fluid or umbilical blood. A detailed description of these optional methods, including operation flow, report time interval, risks of invasive sampling, complications, fetal loss rate, and misdiagnosis rate, were explained clearly and quite adequately by trained counselors for couples at risk in the counseling. The final decision for using which method to perform prenatal diagnosis would be made by couples themselves, after an adequate time for thought. Confirmation studies, using samples isolated from newborns, if possible, were performed. Throughout, informed consent for testing and nondirective counseling for prenatal diagnosis is basically undertaken.

Education programs targeting pregnant women and their partners were considered a part of the preventive project in prenatal screening. All at-risk couples were requested to attend at least two sessions of health education training to gain knowledge about genetics of thals and other relevant aspects in early pregnancy at a pregnancy healthcare school, held by our hospital.

Laboratory methods

The standard guideline of the laboratory diagnosis of hemoglobinopathies (*J Clin Pathol*, 1994; WHO Hereditary Disease Programme, 1994) was used for the diagnosis of α- and β-thal traits and clinical significant hemoglobin variants in pregnant women and their partners. Quality control of a full blood count (FBC) was monitored through participation in the Provincial External Quality Assurance Scheme. The first set of testing for α- and β-thal traits was carried out when the mean cell volume (MCV) was < 80 fL and/or mean cell hemoglobin (MCH) was < 27 pg in combination with the Hb A2 levels: Hb A2 $< 2.5\%$ for α-thal and Hb A2 $> 3.5\%$ for β-thal. The samples with borderline levels of MCV and/or MCH had serum iron measured to assess iron status. And, women with MCV < 80 fL who have reduced or normal Hb A2 levels associated with normal iron status are provisionally diagnosed as having an α-thal trait. Confirmatory testing of positive samples using DNA analysis was not undertaken unless the partner also had a thal trait. Once carrier couples are identified, prenatal diagnosis is accomplished by mutation analysis on polymerase chain reaction (PCR) amplified DNA from amniotic fluid or umbilical blood at 18 to 28 weeks' gestation for β-thal detection, by fetal blood electrophoretic analysis of umbilical blood at 14 to 28 weeks' gestation, or by ultrasonographic diagnosis to measure the fetal cardiothoracic ratio at 14 to 24 weeks' gestation followed by genotyping of α-globin gene for confirmation for severe α-thal using gap-PCR. Non-DNA tests for prenatal diagnosis of α-thal have been performed in our lab since the early 1990s because of their speediness and high accuracy. The couples at risk are quickly informed of the results of their fetus in 1 to 2 h after non-DNA testing. That may be the main reason why these methods are almost always chosen preferentially by couples. Of course, cheaper prices and noninvasive manner (i.e. ultrasonographic diagnosis) are also important factors in method selection. Percutaneous umbilical cord blood sampling (PUBS) was performed by experienced doctors. About 0.5 to 1.5 mL of fetal blood was sampled for electrophoretic analysis.

Coulter JT (Beckman Coulter, Inc, Fullerton, CA) was used to determine peripheral blood counts and red cell indices with standard procedures. High-Speed Automatic Electrophoresis Analytic System (SPIFE, Helena Laboratories, Beaumont, Texas, USA) was applied to assess the concentration of hemoglobins A, A_2, F and any abnormal hemoglobins including Hb Bart's and Hb Constant Spring. Fetal sampling was obtained through amniocentesis or cordocentesis procedure. For DNA analysis, genomic DNA was extracted from amniotic fluid or umbilical blood samples and peripheral blood samples of at-risk couples. The reverse dot blot (RDB) assay (Zhang *et al*., 1994) or the multiplex primer extension/denaturing high performance liquid chromatography (PE/DHPLC) (Wu *et al*., 2003) (Figure 1) was used to define known β-thal mutations in the Chinese population. The fetal blood electrophoretic analysis (Fucharoen *et al*., 1998) and the ultrasound measurement (Lam *et al*., 1999) were both used to predict pregnancies affected by hemoglobin Bart's hydrops fetalis or hemoglobin H (Hb H) disease based on phenotypic features (Panel A in Figure 2). The gap-PCR method (Xiao *et al*., 2000) was used to discriminate heterozygous α-thal from homozygous α-thal, bearing a deletion form of the $--^{SEA}$/allele (Panel B in Figure 2).

Statistic analysis

The performance of prenatal screening was assessed by describing (with 95% confidence intervals) gestation at invasive procedure, the uptake of prenatal diagnosis, and the percentage of identified affected pregnancies that were terminated. Statistical analyses were conducted with an SPSS software program.

RESULTS

Outcome of screening

This study was performed between 1 January 1993 and 31 December 2003. There were 49 221 pregnant women identified for screening. On average, screened sample numbers are 4475 tests/year, in the range of 3902 to

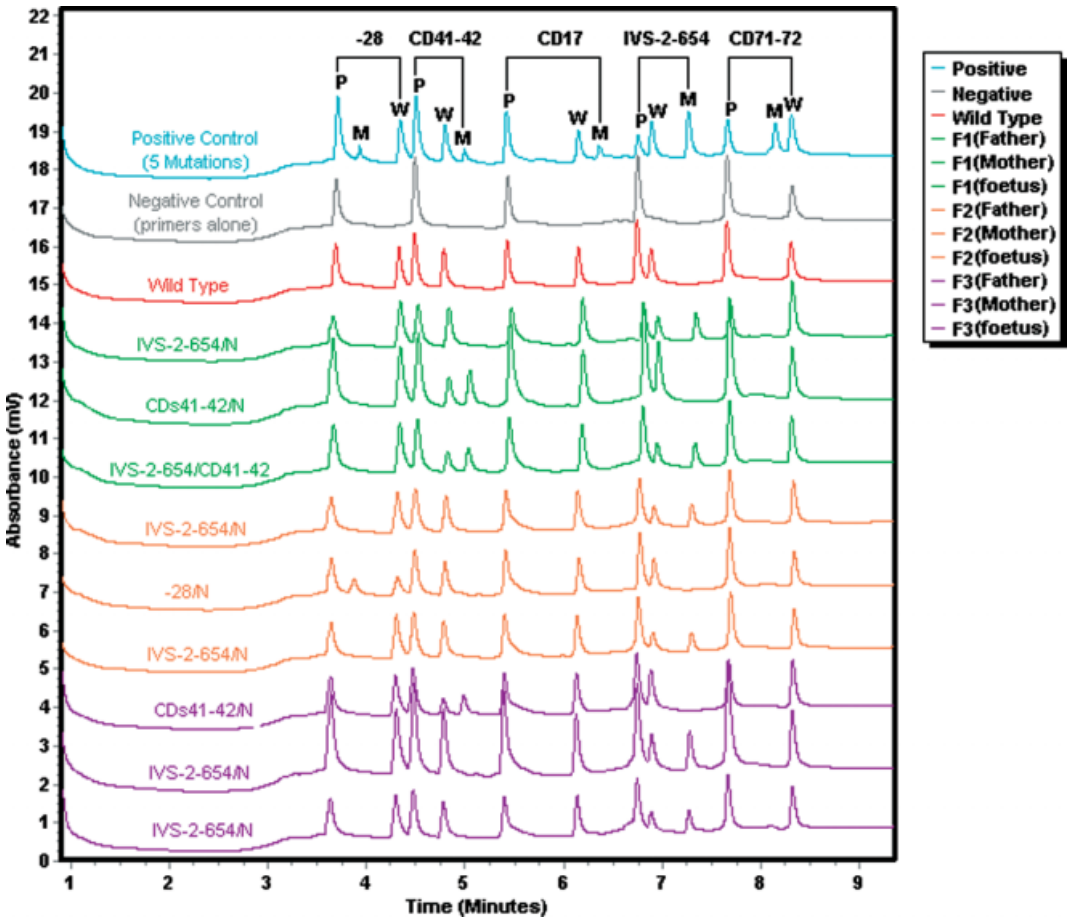

Figure 1—Prenatal diagnosis for β-thalassemia by using PE/DHPLC assay. The representative results of prenatal diagnosis performed in three Chinese families at risk for β-thal major by using PE/DHPLC analysis. P, W, and M stand for primer, wild-type, and mutant respectively. The positive control (cyan) is a mixture of all five mutant β-globin samples as a template for PCR followed by PE. The negative control (gray) illustrates the elution times of the oligonucleotides with no PE products present. Wild-type control (red) is the eluted PE product from a normal individual. Genotypes are labeled to the left of the chromatogram. Family members for each of the three at-risk families (F1 ~ F3) are indicated in the bracket

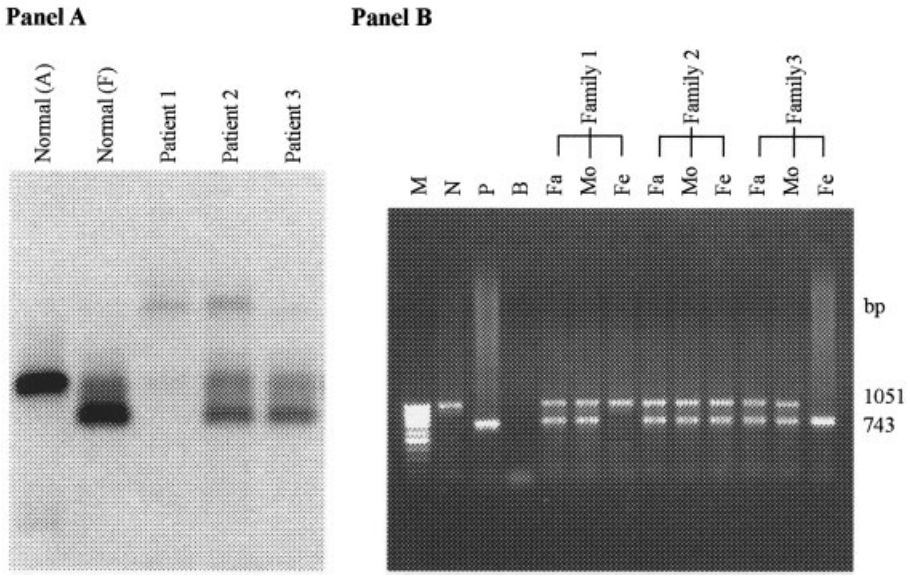

Figure 2—Prenatal diagnosis for α-thalassemia by using fetal blood electrophoretic analysis and gap-PCR amplification. Panel A: the diagrammatic results of fetal blood analysis by electrophoresis. Normal (A) and Normal (F) stand for normal control of adult blood and fetal blood respectively. Patient 1, 2, and 3 were found to be the individuals with Bart's hydrops fetelis syndrome, Hb H disease, and α-thal carrier respectively. Panel B: the representative results of prenatal diagnosis performed in three Chinese families at risk for homozygous α-thal deletion of $--^{SEA}/$. Detection of the $--^{SEA}$/deletion by 1% agarose gel electrophoresis of amplified fragments using gap-PCR. M, molecular size marker of GeneRuler (100 ladder, the highest band 1031 bp, MBI Fermantas Inc., Lithuania). N, P, and B stand for negative (normal), positive (genotype of $--^{SEA}/--^{SEA}$), and blank respectively. Fa = father, Mo = mother, and Fe = fetus. The Arabic numerals indicate the fragment size: the 1051-bp band represents an amplification specific for normal allele and the 743-bp band represents one specific for mutant allele. The results of prenatal diagnosis in the three at-risk families are normal (Family 1), heterozygote (Family 2), and Bart's hydrops fetalis (Family 3) for their fetuses

5016. There were 49 221 complete data sets for analysis: 4587 (9.3%) women tested positive for α- or β-thal trait, with 2804 for α- thal (5.7%) and 1783 for β-thal (3.6%): 139 women and 13 partners of those women with thal trait for abnormal hemoglobin (0.28%), with 8 classes of Hb variants including three clinical significant hemoglobin variants, Hb E of 49, Hb H of 23, and Hb CS of 18. Four thousand five hundred three (98.2%) partners of those women with thal trait underwent testing, 84 (1.8%) did not have their partners tested. The reasons for the failure for the partner to test were as follows: out of city (country), or women were not ready to disclose the details of the partner. A total of 281 (6.2%) were found to be at-risk couples, consisting of 198 for α-thal major (4.4%), and 83 for β-thal major (1.8%) including Eβ-thal. In addition, 131 were diagnosed to be the couples for the discordant thal (α- or β-thal, 2.9%).

Table 1 summarizes the outcome of the screening process from 1993 to 2003, and Table 2 lists the results of Hb variants detected by electrophoresis analysis.

The initial counseling was offered to all 49 221 pregnant women identified for screening. The mean gestation with thal traits was in the range of 12 to 27 weeks when the first interview for thal counseling was attended. The couples with at risk for α- or β-thal major were interviewed one or two weeks after those women for whom thal traits were being tested. There are no significant differences in the gestation at booking between women with α-thal and those with β-thal ($P = 0.677$). There are significant differences in the gestation at performed procedure for α-thal prenatal diagnosis between the invasive one with fetal blood and those that are noninvasive with ultrasound detection ($P < 0.001$).

Uptake of prenatal diagnosis and termination

All 281 at-risk couples were referred to discuss prenatal diagnostic procedures by 12 to 28 weeks' gestation. Of the at-risk couples referred, only 12 couples refused to accept prenatal diagnosis because couples would have opposite thoughts about the reproductive risk they with each other or they did not believe the possibility of having affected fetuses for their offspring. In the past decade, prenatal diagnosis was performed for 269 out of 281 (95.7%) at-risk couples for α- or β-thal major by 12 to 28 weeks' gestation. The mean gestation when carrying out the invasive procedure was 23.07 weeks (95% CI 22.63 to 23.51), compared to 18.12 weeks (95% CI 17.39 to 18.87) for those in whom it was done by noninvasive ultrasound assay. Of the 269 prenatal diagnoses, 208 (77.3%) were performed on amniocentesis or cordocentesis for all of 82 β-thal pregnancies by DNA testing and, for a majority of α-thal ones (126 cases), by fetal blood electrophoretic analysis, and for 61 (22.7%) on direct detection by using ultrasound diagnosis for the rest of α-thal (Table 3).

Out of 187 performed pregnancies at risk for homozygous α^0-thal and 82 at risk for β-thal major, 51 hydrops fetalis with Hb Bart's and 18 β-thal major were identified. Unexpectedly, 3 (1.6%) of 187 pregnancies at risk for hydrops fetalis were diagnosed as fetuses with Hb H

Table 1—Outcomes of screening program in GZMNH from 1993 to 2003

Years	Screened pregnant women	Total pregnant women recorded at Guangzhou	Screened husbands	Women with positive screening test		Couples with positive diagnostic test			Performed prenatal diagnosis	
				α-thal (%)	β-thal (%)	α-thal (%)	β-thal (%)	α/β-thal (%)	α-thal (%)	β-thal (%)
1993	3989	68393	350	232 (5.8)	129 (3.2)	13 (3.7)	8 (2.3)	10 (2.9)	12 (92.3)	7 (87.5)
1994	4564	71617	421	265 (5.8)	164 (3.6)	18 (4.3)	8 (1.9)	9 (2.1)	16 (88.9)	8 (100)
1995	3902	71751	361	227 (5.8)	142 (3.6)	17 (4.7)	7 (1.9)	12 (3.3)	15 (88.2)	7 (100)
1996	4994	74460	464	285 (5.7)	181 (3.6)	19 (4.1)	8 (1.7)	12 (2.6)	17 (89.5)	8 (100)
1997	4392	71989	401	250 (5.7)	158 (3.6)	21 (5.2)	7 (1.7)	10 (2.5)	18 (85.7)	7 (100)
1998	4532	75378	425	263 (5.8)	172 (3.8)	17 (4.0)	8 (1.9)	12 (2.8)	17 (100)	8 (100)[b]
1999	5016	80141	465	285 (5.7)	186 (3.7)	24 (5.2)[a]	8 (1.7)	14 (3.0)	23 (95.8)	8 (100)
2000	4492	81817	418	256 (5.7)	172 (3.8)	21 (5.0)[a]	7 (1.7)	16 (3.8)	21 (100)	7 (100)
2001	4521	80152	411	254 (5.6)	164 (3.6)	17 (4.1)	8 (1.9)	12 (2.9)	17 (100)	8 (100)
2002	4695	80667	415	260 (5.6)	163 (3.4)	16 (3.9)[a]	8 (1.9)	11 (2.7)	16 (100)	8 (100)
2003	4124	86393	372	227 (5.5)	152 (3.6)	15 (4.0)	6 (1.6)	13 (3.5)	15 (100)	6 (100)
total	49221	860758	4503	2804 (5.7)	1783 (3.6)	198 (4.4)	83 (1.8)	131 (2.9)	187 (95.8)	82 (98.8)

[a] Containing four couples contributed by the discordant thal heterozygotes (α-thal in one side and β-thal in her/his partner) through our further investigation.
[b] Actually nine fetuses (including one twin).

disease by fetal blood analysis. In 73 (89.0%) of 82 carrier parents with β-thal, one of the six common Chinese mutations was identified. Seven (8.5%) were detected as one of rare mutations and the remaining two (2.4%) bore unknown mutations in which one of them was identified to be a novel amber mutation in a β^0-thal gene by our further study (Xu *et al*., 1995). So, the prenatal diagnosis in all of 82 at-risk pregnancies can be completely performed, with the final diagnoses of β-globin genotypes for each of fetuses involved in exclusive detection of unknown mutations in one case (Table 4).

All pregnancies with affected fetuses, except for three Hb H disease, were terminated within one week after diagnosis. For each diagnosis, the couple had a one-fourth risk of an affected fetus because two parents was heterozygous, so the expected number of affected fetuses was 67 (25%); the number of actually diagnosed fetuses was 69 (25.6%), including three with hemoglobin H disease. There were three fetal losses (1.11%) that could be related to the prenatal diagnosis procedure. The retesting for confirmation of prenatal diagnosis was conducted in most of PNG (89%). All confirmative detection is in concordance with results of before delivering. This has led to marked reduction of severe thal syndromes in a total of 69 cases since this prospective screening program was launched in the beginning of 1993, thus preventing all of severe α- and β-thal births at this hospital except for the 2 homozygous α^0-thal births that occurred in those 12 couples who refused to accept prenatal diagnosis. Table 5 shows the results in pregnancies where a precise diagnosis was achieved.

DISCUSSION

Beginning in the mid-1980s, we and other groups were the first in China to start the study on epidemiology and molecular basis of α- and β-thal for our local population (Zeng and Huang, 1985; Zhang *et al*., 1988). Supported by Dr Kan YW, we were also dedicated to developing molecular diagnostic techniques to be used in rapid and accurate prenatal diagnosis for at-risk families in southern China (Cai *et al*., 1989; Xu *et al*., 1993a; Xu *et al*., 1993b; Xu *et al*., 1994; Zhang *et al*., 1994; Xiao *et al*., 2000). In the past two decades, the prenatal diagnosis of α- and β-thal in China was dominantly performed by using retrospective program, mainly focused on those Chinese families who have had affected children (Zeng and Huang, 1985; Cai *et al*., 1989; Zhang *et al*., 1990; Xu *et al*., 1993a; Xu *et al*., 1993b; Xu *et al*.,1994; Liao *et al*., 1996). The present study is the first prospective prenatal diagnosis program in China to prevent thals and hemoglobinopathies based on a large-scale population screening, which is actually an extension of the retrospective program and accumulation of our working on thal field for many years. GZMNH is appointed as a center for thal and hemoglobinopathies screening and is the biggest birth size hospital in Guanzhou City. The number of deliveries is up to 4500 per year in this hospital, and is 1/17 of all deliveries in Guangzhou City each year. For 11 years, 49 221 pregnancies were screened at this hospital, over 4500 women were diagnosed as hemoglobinopathies carriers, and 281 were identified as at-risk couples for α- or β-thal major. We therefore prevented 51 and 18 affected fetuses with α- or β-thal respectively from being born with a disease. Up until now, no affected live births have been recorded at our hospital since this screening program, except one affected β-thal birth, as a result of misdiagnosis in the retrospective prenatal diagnosis and two homozygous α^0-thal births in those who refused to accept prenatal diagnosis. This indicates that the prenatal screening program should be conducted in combination with the system of regular healthcare examination in pregnancy previously processed for many years in hospitals at various cities of China. All pregnant women should be routinely screened for both α- and β-thal traits in the first or second trimester, and other hemoglobinopathies when they go to the hospital for their regular obstetrical healthcare examination.

In this article, we described a hospital-based clinic service model to prevent α- and β- thal and hemoglobinopathies in a high-risk area of southern China. This is a more realistic way of reducing birth rate of affected fetuses in our local population because we do not have enough resources to process a prospective screening program in the way of community-based or city-based model. However, China might meet the extensive requirements for the creation of future programs if we promote mass prevention projects in an administrative district (Greengross *et al*., 1999; Modell *et al*., 2001), such as in the whole Guangzhou city, or even in the Guangdong province. In the present program, we have developed a platform applied to clinic service and genetic screening for hemoglobinopathies in population at risk in our city. Our experience suggests, just as mentioned above by Alwan and Modell (2003), that thal as a 'point of entry' for genetic services plays a unique role in promoting the spread of genetic concepts and approaches in Guangzhou. According to our experience gained in both techniques and managements from this program, we would expect to extend this

Table 2—Results of screening for hemoglobin variants by electrophoresis analysis from 49 221 pregnant women's samples and 4503 partners of those women with thal trait in GZMNH from 1993 to 2003

Hemoglobin variants	Number of positive cases			Frequency (‰)
	Pregnant women	Husbands	Total	
Hb E	46	3	49	0.91
Hb H	21	2	23	0.43
Hb CS	15	3	18	0.34
Hb G[a]	14	1	15	0.28
Hb Q[a]	13	2	15	0.28
Hb N[a]	12	1	13	0.24
Hb D[a]	10	1	11	0.20
HB New York	8	0	8	0.15
Total	139	13	152	2.83

[a] represent hemoglobin groups for these variants. Total of 53 724 samples was screened by electrophoresis analysis.

Table 3—The performed prenatal diagnosis and the methods used for prenatal diagnosis of α- and β-thal in the GZMNH from 1993 to 2003

Years	Main diagnostic method	Prenatal diagnosis (n)		Total PNDs
		α-thal	β-thal	
1993–2003	Fetal blood analysis	126	—	126
1999–2003	Ultrasound measurement (DNA: Gap-PCR)	61	—	61
1993.1–1993.12	DNA: ASO hybridization	—	7	7
1994–2002	DNA: Reverse dot blot	—	70	70
2003.1–2003.12	PE/DHPLC	—	6	6

Table 4—Prenatal diagnosis in 83 fetuses at risk for β-thalassemia

Diagnosis	Genotypes	*n*
Normal	—	30
Heterozygote (total)		35
	CDs 41–42 (−TCTT)/N	14
	IVS-2-654 (C → T)/N	8
	−28(A → G)/N	5
	CD17 (A → T)/N	3
	CD26 (G → A)/N	2
	−29(A → G)/N	1
	CDs27–28 (+C)/N	1
	Unknown/N or Normal	1
Homozygote or compound Heterozygote (total)		18
	CDs 41–42 (−TCTT)/IVS-2-654 (C → T)	6
	CDs 41–42 (−TCTT)/ − 28(A → G)	4[a]
	CDs 41–42 (−TCTT)/CD17 (A → T)	2
	IVS-2-654 (C → T)/CD17 (A → T)	2
	CDs 41–42 (−TCTT)/CDs 41–42 (−TCTT)	1
	CDs 41–42 (−TCTT)/CDs 27–28 (+C)	1
	IVS-2-654 (C → T)/CDs 71–72 (+A)	1
	IVS-2-654 (C → T)/CD43 (G → T)	1
Total		83

[a] One twin involved.

strategy to a community-based or city-based approach for control of thals and other hemoglobinopathies in the population of Guangzhou, then of Guangdong province. We believe that we will have the opportunity to establish prenatal diagnostic service at the community or regional level in the developing countries if the allocation of resources based on hospital-based model could be improved in the following aspects: (1) The usable public health education resources should be available. This will help local people become more aware of thals, to get more knowledge about it, and they will know how to choose a genetic service or to make the correct decision after prenatal diagnosis. (2) More local doctors, genetic counselors, and other relevant professional staff should be trained in order to meet the needs of large-scale work demands. (3) The system of tertiary referrals, community register, and quality control for surveillance of thals should be constructed. (4) The local government's functions in organization, and basic construction of relevant policies and laws to support such program should be emphasized.

We found a higher prevalence of Hb H disease (4/10 000) from our over 53 000 samples tested (Table 2), which further confirmed our current results of the higher incidence of silent α-thal defects ($-\alpha^{3.7}$/and $-\alpha^{4.2}$/) in our local population (Xu *et al*., 2004). In this study, because of the ineffective detection of these two silent α-thal defects by the present screening strategy, we could not basically prevent the fetuses at risk for Hb H disease, resulting from $--^{SEA}$/gene in combination with silent α-thal defects ($-\alpha^{3.7}$ or $-\alpha^{4.2}$). The correct diagnosis would allow the affected infants to be properly cared for, and would also raise awareness for the prevention of homozygous α^0-thal or Hb Bart's hydrops fetalis

Table 5—Results in 269 pregnancies where a precise diagnosis was achieved

Fetal diagnosis	α-thalassemia	β-thalassemia	Total (%)
Normal	41	30	71 (26.3)
Trait	92	35	127 (47.0)
Affected	54[a]	18	72 (26.7)
Fetal loss	2	1	3 (1.11)
Misdiagnosis	0	0	0
Total (%)	187 (69.3)	83 (30.7)[b]	270 (100)

[a] Including three patients with Hb H disease.
[b] One twin involved.

syndrome, although prenatal testing for the Hb H disease is usually unwarranted (Lorey *et al*., 2001). Thus, we advise that an effective molecular assay should be added to the prenatal screening in order to characterize the α-thal caused by a single α-globin gene deletion or mutation at region where silent α-thal is commonly found. In 187 at-risk couples with α-thal trait, it is possible that there were homozygous $-\alpha^{3.7}$ ($-\alpha^{3.7}/-\alpha^{3.7}$) or $-\alpha^{4.2}$ ($-\alpha^{4.2}/-\alpha^{4.2}$) involved. It is obvious that the three unexpected cases with Hb H disease among the at-risk couples for homozygous α-thal resulted from one of their parents having genotype of homozygous $-\alpha^{3.7}$ or $-\alpha^{4.2}$. This result is in concordance with the fact that the incidence of homozygous $-\alpha^{3.7}$ or $-\alpha^{4.2}$ is not common in our local population (Xu *et al*., 2004). On the other hand, we should also consider obtaining a biased analysis of the frequency of Hb H disease in a population of our local newborns. Since most patients with Hb H disease will lead normal lives and have treatable late complications, we adopted a prudent policy to provide nondirective counseling for this disease while the at-risk couples attended follow-up counseling for the results of prenatal diagnosis. For our three cases, all of them had the attitude of acceptance of the births of their affected fetuses.

The discordant thal heterozygotes found in couples will probably be a practical issue in genetic counseling. On the one hand, the couples at risk are counseled to inform no risk assessment of affected fetus because the worst results will still be benign even if a fetus inherited from both of his/her parents' mutant genes. On the other hand, their offspring may be at increased risk of having thal major in such parents if any one of the couples is the carrier with coinheritence for α- and β-thal. Furthermore, our current study confirmed that the prevalence of the double heterozygotes for α- and β-thal is 0.26% in our local population (Xu *et al*., 2004). We identified four cases (4/131) in whom one of couples was α-thal carrier; the β-thal partner with borderline HbA_2 values proved to be the carrier with coinheritence of α-thal. These results indicate the existence of at-risk for homozygous α-thal in their pregnancies. The prenatal diagnosis showed Bart's hydrops fetalis in three cases and normal in one case. To prevent from escaping detection of possible concordant heterozygotes of α- or β-thal, as previously reported from β-thal carriers in Hong Kong (Lam *et al*., 1997), we suggest that it should be necessary to screen for α-thal mutations in all β-thal couples in Guangdong area, especially in those couples that have discordant thal. This will allow us to offer, as much as possible, all-sided genetic counseling of the risk assessment of their offspring for the couples regardless of whether the α- or β-thals are the concordant heterozygotes or the discordant ones.

ACKNOWLEDGEMENTS

We thank Dr W. X. Guo for critically reading the manuscript. This work was partially supported by the Fund of National Key Basic Research Developments Programme of the Ministry of Science and Technology, PR China (2001CB510308) and by the National Science Fund for Distinguished Young Scholars (30325037, to Xiang-Min Xu).

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