Detection of α-thalassemia in β-thalassemia carriers and prevention of Hb Bart's hydrops fetalis through prenatal screening

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The aim of this study was to detect α- thalassemia in β-thalassemia carriers during prenatal screening. During a 12-year prenatal screening program, a total of 158 couples (3.2%) were diagnosed to be the discordant α- and β-thalassemia carriers. Of the 158 β-thalassemia partners, seven (4.4%) were found to have co-inheritance of α^0-thalassemia, and three (1.9%) found to have co-inheritance of α^+-thalassemia. Three pregnancies affected with Hb Bart's hydrops fetalis were terminated in the 158 couples. The results showed that molecular analysis must be used for accurate diagnosis of double heterozygotes in couples presumed to be discordant for α- and β-thalassemia on hematologic testing.

Key words: double heterozygotes, thalassemia, screening.

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Thalassemia is one of the most common monogenic diseases in Southern China. Carriers of thalassemia are usually asymptomatic. However, they have a 25% chance of having children with severe outcomes if their spouses are also carriers of the same type of thalassemia. Couples in whom one partner is heterozygous for α- thalassemia and the other is heterozygous for β-thalassemia are often assumed not to be at risk of having offspring with homozygous states of the disease. In our area in the Guangdong province of China, 4.1% of the population are α^0-thalassemia ($--^{SEA}$) heterozygotes and 2.5% β-thalassemia heterozygotes.[1] The chance of finding an individual with co-inheritance of α^0- and β-thalassemia is theoretically 1:1000. In other words, one in every 25 β-thalassemia carriers co-inherits α^0-thalassemia. It is well known that routine screening testing, such as mean cell volume (MCV) and Hb A_2 level, cannot distinguish double heterozygotes for α- and β-thalassemia from the pure β-thalassemia heterozygotes.[2-4] DNA diagnosis is necessary for differentiation. The diagnosis of this double heterozygote state is important for genetic counseling since, unlike the typical β-thalassemia carriers, these individuals will be at risk of having offspring with homozygous α^0-thalassemia if they have a partner who is also an α^0-thalassemia heterozygote. Over the past 12 years, we prospectively screened for the presence of α^0-thalassemia in all β-thalassemia heterozygotes whose partners were α-thalassemia carriers.

Design and Methods

Since the early 1990s, we have been using a hospital-based prenatal screening program for thalassemia.[5] All pregnant women, at their first presentation for prenatal care at our hospital, are screened for both α- and β-thalassemia traits, and other hemoglobinopathies. The husbands of any women with a positive screening test are invited to attend for counseling and testing. Between January 1993 and December 2004, we screened 53,495 pregnant women, of whom 4,976 (9.3%) tested positive for α- or β-thalassemia. We were able to to test 4,890 (98.3%) partners of these women with thalassemia trait. Pregnancies belonging to couples in whom the partners were presumed to be discordant for phenotypes of α- and β-thalassemia on hematologic testing were also studied. Routine screening for thalassemia includes full blood counts and indices, hemoglobin electrophoresis and measurement of Hb A_2 level. The standard diagnostic marker for β- thalassemia is elevation of the Hb A_2 level (>3.5%). Low mean corpuscular volume (MCV) and mean cell hemoglobin (MCH) with a normal HbA_2 indicate an α-thalassemia carrier. Staining for HbH inclusion bodies is also carried out as part of the screening for α thalassemia. When couples were discordant for thalassemia carrier status, molecular investigations were done on the partner with β-thalassemia to exclude the presence of co-existing α^0-thalassemia. Gap-polymerase chain reaction (PCR) was used to determine the three common Chinese α-globin gene deletion mutations ($--^{SEA}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$). Homozygous α^0-thalassemia was diagnosed prenatally in the at-risk pregnancies by

the detection of α-globin gene in the fetal DNA obtained by chorionic villus biopsy or amniocentesis.

Results and Discussion

A total of 158 couples (3.2%; 158/4890) were identified to be discordant thalassemia carriers (Table 1). Of the 158 β-thalassemia partners, seven (4.4%) were found to have co-inheritance of α^0-thalassemia, and three (1.9%) were found to have co-inheritance of α^+-thalassemia. The hematologic features and the α/β-globin genotypes of the ten double heterozygotes are summarized in Table 2. Five partners of the seven individuals who were double α^0- and β-thalassemia heterozygotes were also α^0- thalassemia carriers, making these couples at risk of conceiving fetuses with Hb Bart's hydrops fetalis. Indeed, three pregnancies were affected; two were diagnosed by amniocentesis and the other by chorionic villus biopsy. All affected pregnancies were terminated. The outcomes of the remaining 155 pregnancies were followed up; none of the fetuses was affected by homozygous α^0-thalassemia. In a general strategy of thalassemia screening, elevation of Hb A_2 is the main diagnostic feature of a β- thalassemia carrier. Individuals who have normal Hb A_2 with low MCV and MCH values are considered to be α- thalassemia carriers. Staining for HbH inclusion bodies is also used for screening α-thalassemia. However, the diagnosis of α- thalassemia carriers cannot rely on the detection of HbH inclusion bodies as this is an insensitive and often operator-dependent investigation.[6] Occasionally, carriers with the phenotype of normal Hb A_2, low MCV and low MCH may have an atypical form of β-thalassemia, for example, a mild β-thalassemia mutation, double heterozygosity for δ and β-thalassemia, or deletional β-thalassemia. These carriers need appropriate molecular analysis for correct characterization. Although co-inheritance of α-thalassemia can lead to a reduction in the level of Hb A_2, this does not interfere with the diagnosis of β-thalassemia carriers as the Hb A_2 level in these double α- and β-thalassemia carriers is still higher than the normal level. The present study showed that the presence of an α-thalassemia allele does not suppress the increase in Hb A_2 in β-thalassemia carriers and that staining for HbH inclusion bodies seldom reveals α-thalassemia carriers. Thus a person who is found to be a β-thalassemia carrier by screening may also be an α-thalassemia carrier, but this will be detected only by further DNA diagnosis.

The chance of discovering co-existing α-thalassemia in a β-thalassemia carrier depends on the individual's ethnic background. This chance is, therefore, relatively high in Southeast Asia where 4~20% of the population are α-thalassemia carriers.[7-9] Based on the results of the present study of 53,495 women screened for thalassemia, the incidence of α-thalassemia was 5.7%, which was lower than the general incidence (8.1%) in the whole population of the Guangdong province. This indicates that one in every 18 β-thalassemia carriers will be a double heterozygote. Over the study period, the prevalence of couples with a discordant thalassemia state tended to increase slightly. A possible reason for this is that we changed the α-thalassemia detection test in 1999 to a multiple PCR method which could simulta-

Table 1. Outcomes of the screening program in the present study.

Year	Screened pregnant women	Screened couples*	At-risk couples		
			α-thal (%)	β-thal (%)	α/β-thal (%)
1993	3989	350	13 (3.7)	8 (2.3)	10 (2.9)
1994	4564	421	18 (4.3)	8 (1.9)	9 (2.1)
1995	3902	361	17 (4.7)	7 (1.9)	12 (3.3)
1996	4994	464	19 (4.1)	8 (1.7)	12 (2.6)
1997	4392	401	21 (5.2)	7 (1.7)	10 (2.5)
1998	4532	425	17(4.0)	8 (1.9)	12 (2.8)
1999	5016	465	24 (5.2)	8 (1.7)	18 (3.9)
2000	4492	418	21 (5.0)	7 (1.7)	20 (4.8)
2001	4521	411	17 (4.1)	8 (1.9)	12 (2.9)
2002	4695	415	16 (3.9)	8 (1.9)	15 (3.6)
2003	4124	372	15 (4.0)	6 (1.6)	13 (3.5)
2004	4274	387	16 (4.1)	7 (1.8)	15 (3.9)
Total	**53495**	**4890**	**214 (4.4)**	**90 (1.8)**	**158 (3.2)**

*thal: thalassemia; *the husband was screened only when the woman was screened positive.*

Table 2. Hematologic and genotypic findings in the ten double heterozygotes for α- and β-thalassemia.

Case	Sex	Age (yrs)	Hb (g/dL)	MCV (fL)	MCH (pg)	HbF (%)	HbA_2 (%)	HbH bodies	α-genotype	β-genotype
1	M	30	14.3	71.0	22.9	0.9	5.6	neg	$--^{SEA}/\alpha\alpha$	$\beta^{IVSII654(C\rightarrow T)}/\beta^A$
2	M	23	13.2	69.7	23.2	1.3	5.3	neg	$--^{SEA}/\alpha\alpha$	$\beta^{IVSII654(C\rightarrow T)}/\beta^A$
3	M	28	12.7	66.3	23.4	1.1	4.9	neg	$--^{SEA}/\alpha\alpha$	$\beta^{IVSII654(C\rightarrow T)}/\beta^A$
4	M	26	13.3	67.3	22.6	1.4	5.5	pos	$--^{SEA}/\alpha\alpha$	$\beta^{CDs\ 41/42(-CTTT)}/\beta^A$
5	F	29	11.8	68.4	21.6	1.0	5.3	neg	$--^{SEA}/\alpha\alpha$	$\beta^{CDs\ 41/42(-CTTT)}/\beta^A$
6	F	24	12.5	70.2	21.2	0.5	5.0	neg	$--^{SEA}/\alpha\alpha$	$\beta^{CDs\ 41/42(-CTTT)}/\beta^A$
7	F	27	11.7	69.5	23.6	0.9	5.4	pos	$--^{SEA}/\alpha\alpha$	$\beta^{CD17(A?T)}/\beta^A$
8	F	34	12.2	70.3	21.6	1.1	5.2	neg	$-\alpha^{4.2}/\alpha\alpha$	$\beta^{CDs\ 41/42(-CTTT)}/\beta^A$
9	F	24	12.3	68.2	22.3	1.3	4.9	neg	$-\alpha^{3.7}/\alpha\alpha$	$\beta^{IVSII654(C\rightarrow T)}/\beta^A$
10	F	27	10.1	64.2	23.2	1.0	5.7	neg	$-\alpha^{3.7}/-\alpha^{3.7}$	$\beta^{CDs41\ /42(-CTTT)}/\beta^A$

neously detect the three common Chinese α-globin gene deletions (--^{SEA}, -$\alpha^{3.7}$, and -$\alpha^{4.2}$). In the past, we had given preference to excluding the --^{SEA} deletion in the β-thalassemia carriers. It is imperative to identify a couple's thalassemia status accurately, particularly when they appear to carry discordant forms of thalassemia according to routine hematologic testing. Accurate risk prediction and appropriate genetic counseling are dependent on precise characterization of carrier status. In fact, some rapid PCR-based methods for the simultaneous detection of α/β-globin genotypes are already in use in regions of Southeast Asian with a high prevalence of thalassemia.[10,11]

In our screening strategy, we did not routinely determine the β-thalassemia status in α-thalassemia carriers when the couple was discordant for thalassemia. α^0-thalassemia can be distinguished simply from double α^0- and β-thalassemia by a normal Hb A_2 level. Screening for β-thalassemia in an α^0-thalassemia carrier with a normal level of Hb A_2 is, therefore, unnecessary. Although a normal Hb A_2 β-thalassemia phenotype has been reported to exist, it is a rare entity.[12] For a couple with discordant thalassemias according to hematologic findings, the chance of having a homozygous β-thalassemia child is remote.

DL conceived and designed the study, and wrote the manuscript. The study was performed by DL, JL, XX, YH and HZ under the direction of CL. JL collected and analyzed the data. CL reviewed the manuscript. The authors declare that they have no potential conflicts of interest. Manuscript received November 21, 2005. Accepted March 2, 2006.

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