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ABSTRACT

Hemoglobin (Hb) gene disorders are common inherited diseases in Taiwan. The α- and β-thalassemias are among the well-known Hb diseases in this area. We reviewed abnormal hematological data in 3578 cases, identified between 1998 and 2009, as being at-risk for α-thalassemia (α-thal) (n = 1909; 53.3%), β-thal (n = 743; 20.8%), non-α, β-thal (n = 872; 24.4%), and α-thal combined with β-thal (n = 54; 1.5%), and collected fetal blood samples for prenatal testing. The most common types of α^0- and α^+-thal were the SEA (Southeast Asian) deletion and the $-\alpha^{3.7}$ rightward deletion, with frequencies of 87.79 and 4.85%, respectively. The frequency of the IVS-II-654 (C>T) mutation, the most common β-thal mutation in this region, was 38.6%. Hb E [β26(B8)Glu→Lys, *G*AG>*A*AG] was found to be the most common Hb variant, and it was concluded that Hb Tak [β147 (+AC)], Hb G-Taichung (also known as Hb Q-Thailand) [α74(EF3)Asp→His, *G*AC>*C*AC (α1)], Hb Owari [α121(H4)Val→Met (*G*TG>*A*TG)], and Hb Phnom Penh [α117(GH5)Phe-Ile-α118(H1)Thr (α1)] were very rare. The results of this study provide a primary reference for designing a locally relevant antenatal diagnostic test for controlling the spread of thalassemia.

Keywords Hemoglobin, Genetic disorders, Molecular lesion, Counseling

INTRODUCTION

α-Globin and β-globin gene clusters are commonly associated with hereditary disorders in humans, with approximately 7% of the world population carrying a globin gene mutation that can lead to a hemoglobin (Hb) gene disorder (1). In Taiwan, the Hb gene disorders α-thalassemia

(α-thal), β-thal, and Hb variant-related diseases are common hereditary diseases (2). The α- and β-thalassemias occur at frequencies of 3-5 and 1-3%, respectively (3), whereas Hb variant-related disease is less common. More than 1000 abnormal Hb variants have been identified (http://globin.cse.psu.edu) (4), and some of these variants are clinically silent (5). In general, structural Hb variants (hemoglobinopathies) are due to point mutations in a globin gene that lead to a single amino acid substitution in a globin chain (6). An estimated 150 million people worldwide are carriers of Hb variants (7), and hemoglobinopathies represent a significant health care problem (8). Because several Hb variants are linked to disease, it is clinically important to correctly detect and identify these variants. We describe the establishment of a panel of comprehensive in-house laboratory technology platforms that have been developed over the course of the past decade for diagnosing thalassemia and identifying Hb variants.

MATERIALS AND METHODS

From 1998 to 2009, 3578 specimens were collected from several cohorts of patients or subjects. These included 1) patients with Hb disorders who visited our hospital for anemia treatment; 2) patients referred by other hospitals because of low mean corpuscular volume (MCV), Hb, mean cell Hb (MCH), or abnormal Hb electrophoresis; and 3) subjects for prenatal diagnosis of Hb gene disorders.

Genomic DNA was extracted from peripheral leukocytes using a commercially available kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK). We designed the primer sets using HBA1 (NCBI Reference Sequence: NM_000558.3), HBA2 (NM_000517.3), and HBB DNA sequences (NG_000007.3). α-Thalassemia was detected by 11 pairs of primers using gap-polymerase chain reaction (PCR)-based methods developed by Liu *et al*. (9), and Chang *et al.* (10). This disease was detected at a high frequency in Taiwan and included the $--^{SEA}$, $--^{Phil}$, $--^{Thai}$ deletions, $-\alpha^{3.7}$ (rightward), $-\alpha^{4.2}$ (leftward) deletions, Hb Constant Spring [Hb CS; α142, Term→Gln (*T*AA>*C*AA) (α2)] and Hb Quong Sze [Hb QS; α125(H8)Leu→Pro, C*T*G>C*C*G (α2)]. Common β-thal mutations were detected with seven primer pairs using our own previously described amplification-created restriction site (ACRS)/PCR-RFLP (restriction fragment length polymorphism)-based methods (11-13). β-Thalassemia was detected at a high frequency in Taiwan and included codon 17 (*A*AG>*T*AG), codon 27/28 (+ C), IVS-II-654 (C>T), promoter –28 (A>G), codons 41/42 (–TCTT), codons 71/72 (+A), codon 43 (*G*AG>*T*AG), β-globin deletions [Chinese type, Yunnanese type, and SEA (Southeast Asian) type of hereditary persistence of fetal Hb (HPFH)]. To identify Hb variants, the α1-, α2- and β-globin genes were amplified using the protocols of Chang *et al*. (14,15). Hemoglobin variants were detected at a high frequency in Taiwan and included Hb J-Meinung (also known as Hb J-Bangkok) [β59(E3)Lys→Thr (GGC>GAC)], Hb J-Kaohsiung [β56(D7)Gly→Asp (ACA>ACG)], Hb G-Taichung (also known as Hb Q-Thailand) [a74(EF3)Asp®His, *G*AC>*C*AC (a1)], Hb E [b26(B8)Glu®Lys, *G*AG>*A*AG], and Hb Manitoba [α102(G9)Ser→Arg (AGC>CGC (α2); AGC>AGA (α1)]. The PCR products were purified and directly sequenced as described previously (14,15). Subjects agreed to participate in this study after genetic counseling. Written informed consent was obtained from all pregnant women.

RESULTS

Over the past 10 years, 3578 cases were collected, including 1909 cases of α-thal, 743 cases of β-thal, 54 cases of α-thal combined with β-thal, and 872 cases of non-α or non-β-thal (excluding iron deficiency anemia and other conditions). The frequencies of hemoglobinopathies in Taiwan are listed in Table 1. Table 2 shows the frequencies of α-thal genotypes: α-thal major hydrops, 9.32%; SEA (Z84721.1:g.26264_45564del19301) with the $--^{SEA}/$ type (8.54%); $--^{SEA}/--^{Phil}$ type (Z84721.1:g.11684_43534del31851) (0.6%); and $--^{SEA}/--^{Thai}$ type (Z84721.1:g.10664_44164del33501) (0.16%). There was a high frequency of α-thal carriers: α-thal-1 of the SEA type (71.97%); α-thal-1 of the Philippine type (4.40%); α-thal-2 with the $\alpha^{3.7}$ deletion (Z84721.1:g.34164_37964del3801) (2.93%), and α-thal-1 of the Thailand type (1.68%). Intermediate α-thal cases included the $--^{SEA}/-\alpha^{3.7}$ deletion (1.89%), $--^{SEA}/\alpha^{CS}\alpha$ deletion (Hb CS; HBA2:c.427 T>C) (0.68%), $--^{SEA}/\alpha^{QS}\alpha$ (Hb QS; HBA2:c.377 T>C) (0.68%) and $--^{SEA}/-\alpha^{4.2}$ deletion (0.52%). The α Hb variants included Hb G-Taichung (HBA1:c.223G>C) (0.84%), Hb Owari (HBA2:c.364G>A) (0.26%), and Hb Phnom Penh (HBA1:p.Phe118_Thr119insIle) (0.26%). The most common combinations of α^0-thal with an Hb variant were the $--^{SEA}/\alpha^{Westmead}\alpha$ [Hb Westmead or α122(H5)His→Gln (a2) (HBA2:c.369C>G)] (0.16%) and $--^{SEA}/\alpha^{Phnom\ Penh}\alpha$ (0.10%).

Table 3 shows the frequency of β-thal major IVS-II-654 (HBB:c.316-197C>T) combined with codons 41/42 (HBB:c.125_128delTCTT) (3.5%), codons 41/42 combined with promoter –28 (HBB:c.–78A>G) (1.35%), and IVS-II-654 combined with promoter –28 (1.08%). Frequencies of Hb E/β-thal were 0.67% for Hb E combined with codons 41/42 and 0.67% for Hb E with IVS-II-654. The frequencies of β-thal minor IVS-II-654, codons 41/42, promoter –28, codon 17 (HBB:c.52A>T), Hb E (HBB:c.79G>A), codons 27/28 (HBB:c.84_85insC), and the β-globin gene $--^{SEA}$ deletion of the HPFH type were 31.22, 24.23, 9.96, 7.54, 6.59, 2.15 and 1.62%, respectively. The frequencies of the β Hb variants Hb J-Meinung (HBB:c.170G>A), Hb Kaohsiung (HBB:c.341T>A), and Hb J-Taichung [β129(H7)Ala→Asp (HBB:c.389C>A)] were 1.08, 0.67 and 0.54%, respectively, and the frequency of β-thal combined with an Hb variant (*i.e.,* IVS-II-654 combined with Hb Kaohsiung) was 0.40%.

Table 4 shows frequencies of α-thal combined with β-thal (*i.e.,* the SEA deletion combined with IVS-II-654 (25.93%), codons 41/42 (16.67%), –28 (12.96%) and Hb E (11.11%). The frequency of α-thal combined with β Hb variant (the $-\alpha^{3.7}$ deletion combined with Hb J-Meinung) was 7.41%.

DISCUSSION

In our study, the ($--^{SEA}$) deletion was the most common type of α^0-thal (87.8%) in Taiwan, followed by ($--^{FIL}$) and ($--^{THAI}$) at 5.1 and 1.9%, respectively. The incidence and frequency of these types were similar to those previously reported. Chang *et al*. (16) and Chen *et al*. (17) reported a frequency of the $--^{SEA}$ deletion of over 95%, whereas the frequency of the $--^{THAI}$ deletion was very low (0.5%) (17). These discrepancies may be attributed to the wide range of genetic diversity among Taiwanese individuals, which has arisen from increased numbers of marriages between Taiwanese and immigrants of Southeast Asian descent in recent years. Over 64.1% of these immigrants come from southeast China, 20.8% from Vietnam, 7.8% from Indonesia, 3.6% from Thailand, 1.6% from the Philippines, and 1.3% from Cambodia. The Hb E

trait is the third most common Hb disorder in the world and the most common Hb disorder in Southeast Asia (estimated prevalence, 20-30% *vs*. 0.027% in Taiwan). Although the Hb E trait is not associated with morbidity, thalassemia major (Hb E/β-thal) may develop in the offspring of individuals carrying this variant if the foreign (couples who one of them from Southeast Asia counties) spouse has the β-thal trait and contributes the gene. This combination is the leading cause of transfusion-dependent thalassemia in areas of Southeast Asia. We also correlated genotypes with hematological phenotypes in thalassemia patient groups. It was found that diverse α^0 genotypes had similar hematological profiles. To analyze β-thal, we subdivided the nondeletional form into β^+ and β^0 groups. Levels of Hb, MCV, and MCH were higher in patients with β^+ and β^+ combined with α^0-thal than in patients with β^0 and β^0 combined with α^0-thal. This is consistent with our prior study (18,19).

Given the high prevalence of α^0-thal and β-thal minor in Taiwan, we also identified several cases of β-thal combined with α^0-thal. Compound heterozygote cases of α^0-thal and β-thal in this study shared hematological phenotypes (Hb, MCV, and MCH) with cases of α^0-thal or β-thal minor. Because of the hematological similarity between compound heterozygotes and either the α- or β-thal heterozygote, the likelihood of hydrops fetalis in β-thal minor warrants consideration. Couples in which one spouse has α^0-thal and the other one has β-thal minor, or in which both have β-thal minor, should receive prenatal screening. We view concurrent screening for α- and β-thal as a reasonable prenatal diagnostic approach for such cases in Taiwan.

In cases of α^0-thal combined with an unstable Hb variant, such as Hb CS or Hb QS, the clinical presentation is more severe, and hematological values are markedly changed. In α^0-thal cases combined with a stable or unstable Hb β variant, the change in hematological values is small and resembles that in α^0-thal. In β-thal minor with a stable Hb α variant, hematological values resemble those of pure β-thal minor; however, if the combined Hb α variant is unstable, the hematological values may resemble or differ slightly from those in cases of β-thal minor. Although hematological values in cases of β-thal minor and β-thal minor combined with a stable Hb β variant are similar, if the Hb β variant is unstable, as occurs in cases of Hb Tak (HBB:c.441_442insAC), hematological values may be adversely affected and clinical symptoms may become more severe. We reported the first case of Hb Tak in Taiwan (20). Hb Tak was previously described as being mildly unstable *in vitro*, but no results of stability testing have been reported. Hb Tak is caused by insertion of the dinucleotide AC into codon 146 that removes the normal stop codon at position 147 and leads to a frameshift with elongation of the β chain by 11 amino acids.

Taiwan introduced its national health insurance system to provide universal health care in 1995. β-Thalassemia patients became eligible for comprehensive care. The insurance covers blood transfusions, iron chelation, monitoring of physical development, medication, radiography, surgery, laboratory tests (*e.g.,* Hb electrophoresis), DNA analysis for diagnosis and blood transfusions, and hematopoietic stem cell transplantation (21). Currently, health education, updated clinical diagnostic guidelines, and primary care medical doctors with knowledge of the care of thalassemia are readily accessible to the general population. Despite the availability and success of bone marrow transplantation in curing thalassemia major, transfusions and iron chelation therapy remain the mainstay of treatment in Taiwan. Therefore, lifelong specialized

care and an expensive support system are required. Both impose significant socioeconomic burdens on families and society. Effective antenatal diagnostic service followed by elective abortion may help to decrease the prevalence of this disease in the future.

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REFERENCES

1. Forget BG, Higgs DR, Steinberg M, Nagel RL. *Disorders of Hemoglobin: Genetics, Pathophysiology and Clinical Management.* Cambridge: Cambridge University Press, 2001.
2. Lin HJ, Shih MC, Peng CT, Liu TC, Chen KW, Shih HC, et al. Hematological features and molecular lesions of hemoglobin gene disorders in Taiwanese patients. *Int J Lab Hematol.* 2008 Aug 14. [Epub ahead of print].
3. Ko TM, Xu X. Molecular study and prenatal diagnosis of α- and β-thalassemias in Chinese. *J Formos Med Assoc.* 1998;97(1):5-15. Review.
4. Ko TM, Hsieh FJ, Chen CJ, Hsu PM, Lee TY. Cord blood screening for α-thalassemia in northern Taiwan. *J Formos Med Assoc.* 1998;87(2):146-149.
5. Wild BJ, Bain BJ. Detection and quantitation of normal and variant haemoglobins: an analytical review. *Ann Clin Biochem.* 2004;41(Pt. 5):355-369.
6. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. *Clin Chem.* 2000;46(8, Pt. 2):1284-1290.
7. Shimizu A, Nakanishi T, Miyazaki A. Detection and characterization of variant and modified structures of proteins in blood and tissues by mass spectrometry. *Mass Spectrom Rev.* 2006;25(5):686-712.
8. Daniel YA, Turner C, Haynes RM, Hunt BJ, Dalton RN. Rapid and specific detection of clinically significant haemoglobinopathies using electrospray mass spectrometry-mass spectrometry. *Br J Haematol.* 2005;130(4):635-643.
9. Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of α-thalassemia deletions and ααα globin gene triplication by multiplex PCRs. *Br J Haematol.* 2000;108(2):295-299.
10. Chang JG, Lee LS, Lin CP, Chen PH, Chen CP. Rapid diagnosis of α-thalassemia-1 of Southern Asia type and hydrops fetalis by polymerase chain reaction. *Blood.* 1991;78(3):853-854.
11. Chang JG, Chen PH, Chiou SS, Lee LS, Perng LI, Liu TC. Rapid diagnosis of β-thalassemia mutations in Chinese by naturally and amplified created restriction sites. *Blood.* 1992;80(8):2092-2096.
12. Chiou SS, Liu TC, Tseng WP, Sy WD, Chang JG. Prenatal and molecular diagnosis of β-thalassemia major in Taiwan by naturally and amplified created restriction sites. *Int J Hematol.* 1993;59(1):1-8.
13. Peng CT, Liu SC, Chiou SS, Kuo PL, Shih MC, Chang JY, et al. Molecular characterization of deletional forms of β-thalassemia in Taiwan. *Ann Hematol.* 2003;82(1):33-36.

14. Chang JG, Yang TY, Perng LI, Wang NM, Peng CT, Tsai CH. Hb Siriraj: A G→A substitution at codon 7 of the β-globin chain creates an *Mbo*II cutting site. *Hemoglobin*. 1999;23(2):197-199.
16. Chang JG, Liu TC, Perng LI, Chiou SS, Chen TP, Chen PH, et al. Rapid molecular characterization of Hb H disease in Chinese by polymerase chain reaction. *Ann Hematol.* 1994;68(1):33-37.
17. Chen TP, Liu TC, Chang CS, Chang JG, Tsai HJ, Lin SF. PCR-based analysis of α-thalassemia in Southern Taiwan. *Int J Hematol.* 2002;75(3):277-280.
18. Chang JG, Liu TC, Chiou SS, Chen PH, Lee SS, Chen TP. Molecular basis of β-thalassemia minor in Taiwan. *Int J Hematol.* 1994;59(4):267-272.
19. Peng CT, Wu JY, Tsai CH, Tsai FJ, Chang JG. Molecular diagnosis of patients with β-thalassemia major in central Taiwan by amplified created restriction site analysis. *J Hum Genet.* 1998;43(4):237-241.
20. Shih MC, Wu KH, Liu SC, Chang JG. Hb Tak: a β chain elongation at the end of the β chain, in a Taiwanese. *Hemoglobin.* 2005;29(1):65-67.
21. Peng CT, Chang JS, Wang LY, Chiou SS, Hsiao CC, Wang SC, et al. Update on thalassemia treatment in Taiwan, including bone marrow transplantation, chelation therapy, and cardiomyopathy treatment effects. *Hemoglobin.* 2009;33(5):304-311.

TABLE 1 Distribution of Hemoglobinopathies in Taiwan

Disease	Number of Cases	%
α-thalassemia	1909	53.3
β-thalassemia	743	20.8
α- and β-thalassemia	54	1.5
Non α, β-thalassemia	872	24.4
Total	3578	100.0

TABLE 2 Distribution of α-Thalassemia Genotypes

Disease	Genotype	n	%
α-Thal major (n = 178, 9.32%)	$--^{SEA}/--^{SEA}$ $--^{SEA}/--^{Phil}$ $--^{SEA}/--^{Thai}$	163 12 3	8.54 0.60 0.16
α-Thal carrier (n = 1599, 83.76%)	α-thal-1/$--^{SEA}$ α-thal-1/$--^{Phil}$ α-thal-2/–$\alpha^{3.7}$ α-thal-1/$--^{Thai}$ α-thal-2/$\alpha^{QS}\alpha$ α-thal-1/α^{CS}a $\alpha^{G\text{-}Taichung}\alpha$/–$\alpha^{4.2}$ α-thal-2/–$\alpha^{4.2}$ α2-codon 30 (–GAG) α2-codon 31 (–AG) $\alpha^{CS}\alpha$/–$\alpha^{3.7}$	1374 84 56 32 19 14 12 5 1 1 1	71.97 4.40 2.93 1.68 1.00 0.73 0.63 0.26 0.05 0.05 0.05
α-Thal intermedia (n = 83, 4.35%)	Hb H ($--^{SEA}$/–$\alpha^{3.7}$) Hb H ($--^{SEA}/\alpha^{CS}\alpha$) Hb H ($--^{SEA}/\alpha^{QS}\alpha$) Hb H ($--^{SEA}$/–$\alpha^{4.2}$ Hb GH ($--^{SEA}/\alpha^{G\text{-}Taichung}\alpha$ $\alpha^{G\text{-}Chinese}\alpha/--^{Thai}$	36 13 13 10 9 2	1.89 0.68 0.68 0.52 0.47 0.10
α Hb variants (n = 40, 2.09%)	G-Taichung Owari Phnom Penh Manitoba Ube-2 Woodville G-Chinese <u>Hekinan</u> Westmead Perth	16 5 5 3 3 3 2 1 1 1	0.84 0.26 0.26 0.16 0.16 <u>0.16</u> 0.10 0.05 0.05 0.05
α^0-Thal/Hb variant (n = 9, 0.47%)	$--^{SEA}/\alpha^{Westmead}\alpha$ $--^{SEA}/\alpha^{Phnom\ Penh}\alpha$ $--^{SEA}/\alpha^{Owari}\alpha$ $--^{SEA}/\alpha^{\underline{Hekinan}}\alpha$ $--^{SEA}/\alpha^{Prato}\alpha$ $--^{SEA}/\alpha^{Manitoba}\alpha$	3 2 1 1 1 1	0.16 0.10 0.05 0.05 0.05 0.05

TABLE 3 Distribution of β-Thalassemia Genotypes

Disease	Genotype	n	%
β-Thal major (n = 75, 10.09%)	IVS-II-654/codons 41/42 codons 41/42/promoter –28 IVS-II-654/promoter –28 IVS-II-654/IVS-II-654 promoter –28/ promoter –28 codons 41/42/codons 41/42 IVS-II-654/codons 27/28 codon 17/codons 41/42 codon 17/codon 17 codons 27/28/– –^{Yunanese} codons 27/27/– –^{SEA} (β-HPFH) promoter –28/codon 17 codons 27/28/codons 41/42 codons 41/42/– –^{SEA} (β-HPFH) codon 17/– –^{SEA} (β-HPFH) IVS-II-654/– –^{SEA} (β-HPFH)	26 10 8 6 6 4 2 2 2 2 2 1 1 1 1 1	3.50 1.35 1.08 0.81 0.81 0.54 0.27 0.27 0.27 0.27 0.27 0.13 0.13 0.13 0.13 0.13
Hb E/β-Thal (n = 14, 1.85%)	Hb E/codons 41/42 Hb E/IVS-II-654 Hb E/codon 17 Hb E/promoter –28	5 5 2 2	0.67 0.67 0.27 0.27
β-Thal minor (n = 631, 84.93%)	IVS-II-654 codons 41/42 promoter –28 codon 17 Hb E codons 27/28 – –^{SEA} (β-HPFH) codons 71/72 – –^{Chinese} initiation codon *A*TG>*G*TG Promoter –29 – –^{Yunanese}	232 180 74 56 49 16 12 5 3 2 1 1	31.22 24.23 9.96 7.54 6.59 2.15 1.62 0.67 0.40 0.27 0.13 0.13
β Hb variant (n = 19, 2.56%)	Hb J-Meinung Hb Kaohsiung Hb J -Taichung Hb Tak Hb G-Hsin Chu	8 5 4 1 1	1.08 0.67 0.54 0.13 0.13
β-Thal/Hb variant (n = 4, 0.54%)	IVS-II-654/Hb Kaohsiung IVS-II-654/Hb Tak	3 1	0.40 0.13

TABLE 4 Distribution of α-Thalassemia Combined With β-Thalassemia Genotypes

Disease	Genotype	n	%
α-Thal/β-thal (n = 42, 77.78%)	$--^{SEA}$/IVS-II-654 $--^{SEA}$/codons 41/42 $--^{SEA}$/promoter –28 $--^{SEA}$/Hb E $--^{SEA}$/initiation codon *A*TG>*G*TG $--^{SEA}$/Hb J-Meinung $--^{SEA}$/Hb Kaohsiung $--^{Phil}$/codons 41/42	14 9 7 6 2 2 1 1	25.93 16.67 12.96 11.11 3.70 3.70 1.85 1.85
α-Thal/β Hb variant (n = 6, 11.11%)	$-\alpha^{3.7}$/Hb J-Meinung $-\alpha^{3.7}$/IVS-II-654 $-\alpha^{3.7}$/Hb E	4 1 1	7.41 1.85 1.85
β-Thal/α Hb variant (n = 6, 11.11%)	codons 41/42/HbG-Taichung codons 41/42/$^{J\text{-Meinung}}/_{G\text{-Chinese}}$ codons 41/42/Hb Phnom Penh codon 17/Hb Ottawa IVS-II-654/Hb Ube 2 Hb E/Hb CS	1 1 1 1 1 1	1.85 1.85 1.85 1.85 1.85 1.85