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ORIGINAL ARTICLE

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Prevalence study and molecular characterization of α-thalassemia in Filipinos

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Abstract In order to determine the prevalence and molecular basis of α-thalassemia (thal) among Filipinos, a total of 2954 Filipinos in Taiwan were enrolled in this study. A complete blood count was done for every subject. Those with microcytosis (MCV less than 82.5 fl) were studied with hemoglobin (Hb) high-performance liquid chromatography to determine the levels of Hb A2 and Hb F, and with an enzyme immunoassay to determine plasma ferritin levels. Those who had microcytosis and normal or low levels of Hb A2 and Hb F were further studied with molecular methods for α-globin gene mutations. We used Southern blot hybridization and/or the polymerase chain reaction to detect Southeast Asian deletion, Filipino deletion, rightward and leftward single α-globin gene deletions, and Hb Constant Spring and Hb Quong Sze. Specific amplification and direct DNA sequencing of the α2- and α1-globin genes were carried out in apparent α-thal carriers without any of the above-mentioned mutations. Our results showed that in Filipinos the prevalence of α-thal 1 was 5% (147 carriers) and that of α-thal 2 was 1.7% (49 carriers); two had Hb H disease. Among the α-thal 1 carriers, 89 had the Southeast Asian deletion and 58 had the Filipino deletion. Among the α-thal 2 carriers, 48 had a rightward deletion and one had a leftward deletion. None had Hb Constant Spring or Hb Quong Sze. Specific amplification and DNA sequencing in five apparent α-thal carriers did not reveal mutations in the 2-kb region spanning the α2- and α1-globin genes. The molecular defects of α-thal in Filipinos were different from those in the neighboring ethnic groups. Elucidation of the α-thal mutations in Filipinos is useful in the genetic counseling and prenatal diagnosis of this common disease.

Key words α-Thalassemia · Filipino deletion · Southeast Asian deletion · Filipino

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Introduction

The α-globin gene cluster has been mapped to the telomeric region of the short arm of chromosome 16. The cluster is arranged in the order of 5′-ψζ2-ζ1-ψα-α2-α1-θ1–3′. The whole cluster spans about 50 kb [4, 9]. The two α-globin genes have identical coding sequences and their flanking regions have three stretches of sequence homology. In addition, many *Alu*-repetitive sequences are interspersed within the cluster [4]. These sequence characteristics result in both homologous and illegitimate recombination, which leads to various deletions in the cluster. Illegitimate recombination results in both Southeast Asian deletion (SEA) and Filipino deletion (FIL), both removing the two α-globin genes and leading to α-thal 1 [3, 11, 16, 19, 24, 29]. Homologous recombination between the flanking regions of the two α-globin genes results in rightward or leftward deletion, both removing one α-globin gene and leading to α-thal 2 [9, 11]. In addition to these deletions, nondeletional defects involving the α2-globin gene (Hb Constant Spring [Hb CS] and Hb Quong Sze [Hb QS]) are present in Chinese and Southeast Asians [6, 13, 20].

Homozygous α-thal 1 causes Bart's hemoglobin hydrops fetalis, and various obstetrical complications [23], while compound heterozygous α-thal 1 and α-thal 2 causes Hb H disease. The Philippines is geographically close to Taiwan. Migration of Filipinos within the

ing to previous reports on the ethnic Chinese living in Taiwan, 3.5% are carriers of SEA, 0.17% of FIL, 0.6% of $-\alpha^{3.7}$, and 0.3% of $-\alpha^{4.2}$ [12, 15, 19]. Significant differences in prevalence and molecular defects of α-thal also exist between the Philippines and Melanesia and regions in Southeast Asia [5, 8, 25, 26]. α-Thal 1, which is common in Filipinos, has never been reported in Melanesians. On the other hand, the prevalence of α-thal 2 is generally higher in regions of Melanesia (up to 68%) than in the Philippines [25, 26, 30].

SEA and FIL are common mutations in Southeast Asian countries. Recent studies showed that the average carrier rate of α-thal 1 in Southeast Asians is 6%, a figure comparable to this report [5, 7]. Hb CS is a common nondeletional defect of α-thal in Southeast Asia, especially in the Mekong River basin, in northeastern Thailand and Laos, where its estimated prevalence is 6% [20]. Hb CS and Hb QS are also not uncommon in southern China; a 0.88% prevalence rate for Hb CS and a 0.17% rate for Hb QS were reported in Guangxi province, close to northern Vietnam [13]. However, these two mutations were not found in the Filipinos in this study.

α-Thal is a worldwide problem. Significant differences in its prevalence and molecular defects exist among different ethnic groups. During the past 20 years, more than 1 million refugees from Southeast Asia have entered the United States and Europe [7]. A better understanding of the α-thal characteristics in different ethnic groups is needed for the genetic counseling and prenatal diagnosis of this common disease.

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ORIGINAL ARTICLE

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Prevalence and molecular characterization of β-thalassemia in Filipinos

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Abstract β-Thalassemia (thal) is a common single-gene disease worldwide. However, the prevalence of β-thal and the spectrum of β-globin gene mutations in Filipinos remain unclear. This study sought to answer these two questions. A total of 2954 apparently healthy Filipinos in Taiwan were recruited for a prevalence study. A complete blood count was done in every subject. Those with microcytosis were studied with hemoglobin (Hb) high-performance liquid chromatography to determine the levels of Hb A_2 and Hb F. Twenty-seven subjects had elevated levels of Hb A_2 (>4.0%). These 27 suspected β-thal carriers and another 16 β-thal major patients who were being treated in the Philippines were studied to determine the spectrum of β-globin gene mutations. Gap-PCR was used to detect the Filipino deletion of β-thal, and direct sequencing was used to detect point or small mutations in the β-globin gene. All of the 27 suspected β-thal carriers had one mutation in the β-globin gene, resulting in an overall prevalence of 0.9%. The spectrum of β-thal mutations was similar in the carrier and patient groups. Analysis of the pooled identified seven different mutations in the study population. The Filipino deletion was the most common mutation, accounting for 45.8% (27/59) of the alleles, followed by codon 67 (−TG) (16 alleles), and Hb E (11 alleles). These three mutations accounted for 92% of the Filipino β-thal alleles. Elucidation of the β-thal mutations in Filipinos is useful for the genetic counseling and prenatal diagnosis of this disease.

Key words β-Thalassemia · Filipino deletion of β-globin gene · Filipino · Genetic counseling

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Introduction

β-Thalassemia (thal) is a common single-gene disease worldwide [5, 25]. Except for some long-segment deletions, most mutations resulting in β-thal involve a single or several nucleotides of the β-globin gene [12, 14]. Up to now, about 200 different mutations have been reported worldwide and each ethnic group has its own common mutations [1, 3, 16, 18, 23].

β-Thal homozygotes require life-long blood transfusion and iron chelation therapy for supportive treatment unless bone marrow transplantation is performed, which can result in a permanent cure of the disease. Early prenatal diagnosis using DNA analysis is the most effective way to prevent new cases. National screening programs have been shown to be effective in reducing the incidence in the general population [25]. For such programs to be successful, information about the prevalence and spectrum of the molecular defects in a region or a country is needed [4, 7]. High prevalence rates of β-thal have been reported from many countries in Southeast Asia [1, 9–11, 13, 17]. However, there have been few reports of the prevalence and molecular spectrum of β-thal among Filipinos [8, 21]. This study was designed to determine the prevalence and molecular spectrum of β- thal in Filipinos.

This study showed that Filipino deletion of the β-globin gene was the most common β-thal mutation in Filipinos. As reported previously, the 5′ deletion end point is 4279 bp upstream of the mRNA cap site of the β-globin gene and the 3′ deletion end point lies in a region of the LINE-1 family of repetitive sequences [24]. The deletion spans about 45 kb and the entire β-globin gene is deleted. Recognition of this defect is important because analysis of the other β-globin gene in a β-thal carrier will not reveal any disease-causing mutation. Although the exact deletion breakpoints have not been defined, a rapid gap-PCR diagnosis is available [24]. Since this mutation has a high prevalence in Filipino β-thal, it should be screened for first. As reported by Eng et al. [8], carriers of the Filipino deletion had higher levels of Hb F.

Hb E is a very common hemoglobinopathy in Southeast Asia [9, 13]. In certain regions the prevalence is as high as 40% [1, 20]. Although Hb E heterozygotes and Hb E homozygotes are asymptomatic, compound heterozygotes for Hb E and β-thal usually have severe symptoms and are blood transfusion dependent. Although Hb E is still a common disorder among Filipinos, its prevalence there is much lower than in the neighboring countries [1, 9, 11, 20].

The spectrum of β-thal mutations in Filipinos is unlike that of the neighboring ethnic groups. In Taiwan and southern China, the two most common mutations are codons 41/42 (−TCTT) frameshift and IVS-II-654 (C→T) [16]. In Thailand, Singapore, Malaysia, and Indonesia, the two most common mutations are IVS-I-5 (G→C) and Hb E [1, 9, 10]. In the Filipinos in this study, the IVS-II-654 (C→T), codons 41/42 (−TCTT), and IVS-I-5 (G→C) mutations were rarely found. On the other hand, the common Filipino mutations, i.e., β-globin gene deletion and codon 67 (−TG), were also rarely found in the neighboring countries [6]. In terms of the β-thal mutation spectrum, the Philippines seems quite different from other Southeast Asian countries. Since only three common mutations accounted for 92% of β-thal alleles in Filipinos, carrier screening and prenatal diagnosis of the disease would not be difficult.

During the past 20 years, more than one million Southeast Asians have entered the United States and Europe [2, 11]. A better understanding of the β-thal characteristics in different ethnic groups will improve the quality of genetic counseling and prenatal diagnosis of this common disease.

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