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Common Alpha Globin Genes (*HBA1* and *HBA2*) Mutations in Filipino Patients with Alpha Thalassemia

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Alpha (α) thalassemia results from the absence/reduced synthesis of the α-globin subunit of hemoglobin (Hb). Mutational variants in the *HBA1* and *HBA2*, which code for α-globin, have been reported to cause varying degrees of disease severity. These variants are unique for every population. However, local data on α-globin gene mutations in Filipino α-thalassemics is currently lacking. This study aimed to identify common α-globin gene mutations in Filipino patients suspected with α-thalassemia. Two hundred sixty (260) patients suspected with α-thalassemia underwent deoxyribonucleic acid (DNA) extraction and Alpha Globin StripAssay® mutational analysis. The ($--^{SEA}/\alpha\alpha$), ($-\alpha^{3.7}/--^{SEA}$), ($-\alpha^{3.7}/--^{FIL}$), ($--^{FIL}/\alpha\alpha$), ($\alpha\alpha/\alpha\alpha$), ($-\alpha^{3.7}/\alpha\alpha$), ($-\alpha^{3.7}/-\alpha^{3.7}$), ($-\alpha^{4.2}/--^{SEA}$), ($--^{SEA}/--^{SEA}$), (α2 cd 59/αα), ($-\alpha^{4.2}/-\alpha^{4.2}$), ($-\alpha^{4.2}/--^{FIL}$), ($--^{SEA}/\alpha\alpha^{CS}$), and ($-\alpha^{3.7}/-\alpha^{4.2}$) mutations were found in 30.38%, 24.62%, 20.77%, 12.31%, 6.15%, 1.54%, 1.15%, 0.77%, 0.38%, 0.38%, 0.38%, 0.38%, and 0.38% of the patients, respectively. These results indicate that the ($--^{SEA}/\alpha\alpha$), ($-\alpha^{3.7}/--^{SEA}$) and ($-\alpha^{3.7}/--^{FIL}$) mutations are prevalent in the Filipino patients tested. The high frequencies of ($--^{SEA}$, 28.46%), ($-\alpha^{3.7}$, 24.81%) and ($--^{FIL}$, 16.73%) alleles in this study are important to note as these alleles may increase the risk of HbH and Hb Bart's hydrops fetalis cases in the population.

Keywords: alpha thalassemia, Filipino, *HBA1*, *HBA2*, mutations

INTRODUCTION

Alpha thalassemia is a blood disorder that results from the absence or reduced synthesis of the α-globin subunit of Hb. The clinical manifestation of the disease varies from almost asymptomatic, and very mild microcytic and hypochromic anemia to death due to hemolytic anemia (Borgio 2015; Farashi and Harteveld 2017). The high frequency is reported in tropical and subtropical regions, with reports of approximately 15,000 annual births with the disorder, making it one of the most common human genetic diseases (Williams and Weatherall 2012; Higgs 2013).

Alpha thalassemia results from faulty protein synthesis due to mutations in the α-globin genes, *HBA1* and *HBA2*. The α-globin gene family is located in chromosome 16, with each chromosome copy containing two functional α-globin genes that code for the chains which function as subunits for both fetal and adult Hbs (Galanello and Cao 2011). Mutational variants in the *HBA1* and *HBA2*, which both code for α-globin, have been reported with varying

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degrees of disease severity depending on the number of affected α-globin genes (Chui and Waye 1998; Eng *et al*. 2001; Galanello and Cao 2011). Hb Bart's hydrops fetalis, the most severe form, is due to the deletion of all four α-globin genes, which causes fetal death in utero or shortly after birth (Chui and Waye 1998). HbH disease, on the other hand, involves three affected genes resulting from compound heterozygosity for a double-gene (α^0-thalassemia) deletion and either a single-gene deletion (α^+-thalassemia) or a non-deletional mutation on one of the α-globin genes. Patients with HbH disease have chronic microcytosis and hypochromic hemolytic anemia of varying severity. On the other hand, carriers and traits having only one or two genes affected, respectively, may show relatively benign symptoms but may be at risk of conceiving affected infants if their partners are both carriers (Chui and Waye 1998; Galanello and Cao 2011).

About 90% of α-thalassemia cases are due to deletions of one or both α-globin genes. Among the most common are the ($--^{MED}$) and ($-\alpha^{20.5}$) deletions in Mediterranean countries and the ($--^{SEA}$), ($--^{FIL}$), ($--^{THAI}$), ($-\alpha^{3.7}$) and ($-\alpha^{4.2}$) deletions in the Southeast Asian region (Chong *et al*. 2000; Hung *et al*. 2007; Galanello and Cao 2011). Sequence variants, on the other hand, account for the remaining percentage of α-thalassemia cases. The most common and most severe non-deletional mutations, are the Hb Constant Spring (CS, $\alpha\alpha^{CS}$, α2 codon 142 TAA->CAA), Hb Pakse (α2 codon 142 TAA->TAT), Hb Quong Sze (α2 codon 125 CTG ->CCG), α2 codon 0 Δ1bp (-T), α2 codon 30 Δ3bp (-GAG), α2 codon 35 (TCC->CCC), Hb Suan Dok (α2 codon 109 CTG->CGG), and α2 codon 59 (GGC->GAC) (Eng *et al*. 2001; Wang *et al*. 2003).

Alpha globin gene variants are unique for every population (Cheerva 2013). In the Philippines, however, no study has been done to identify the underlying α-thalassemia mutations. This study aimed to determine the common *HBA1* and *HBA2* mutations in Filipino patients suspected with α-thalassemia. Knowledge of the *HBA1* and *HBA2* variants present in Filipino patients may be useful in the genetic counseling of families of patients detected positive for the disease. Data gathered in this study may aid in the identification of the set of mutations that may be used in the development of a diagnostic kit specific to Filipinos.

MATERIALS AND METHODS

Patient Recruitment

Two hundred sixty (260) unrelated Filipino patients who screened positive for α-thalassemia *via* the VARIANT™ nbs Newborn Hemoglobin Screening System (Bio-Rad, Hercules, CA) through the expanded newborn screening (ENBS) program (n = 164) and patients (n = 96) referred by hematologists – highly suspected with α-thalassemia through the evaluation of CBC results, red cell indices, and upon ruling-out iron deficiency anemia from August 2014 to December 2017 – were recruited in the study (Figure 1). All were unrelated Filipino patients whose ages ranged from 4 d–67 yr old. Patients who were non-Filipinos and were relatives of already recruited patients were excluded. The study was approved by the University of the Philippines Manila Research Ethics Board (UPMREB, 2014 122 01). All patients provided written informed consent and assent.

Sample Preparation

Genomic DNA was extracted from 2-mL peripheral blood of each study participant in ethylenediaminetetraacetic acid (EDTA) tubes using the QIAamp® DNA blood Midi Kit (Qiagen, Valencia, CA), as instructed in the manufacturer's manual. DNA samples were stored at –20 °C prior to mutation analysis.

Mutation Analysis

Detection of *HBA1* and *HBA2* mutations was done using the polymerase chain reaction (PCR) technique and a reverse hybridization assay (Alpha Globin StripAssay®,

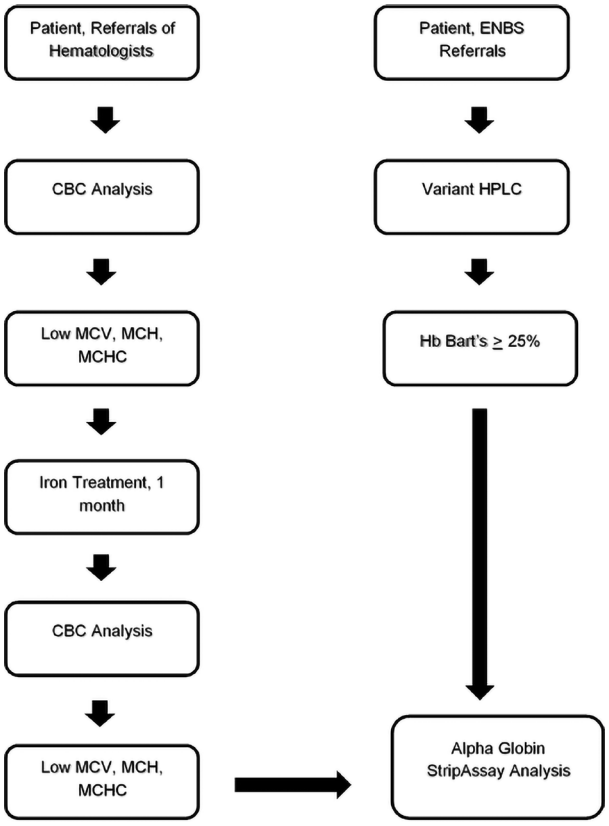

Figure 1. Schematic diagram used in the identification of patients suspected with α-thalassemia in the study.

ViennaLab Diagnostics, GmBH, Vienna, AT) for the rapid and simultaneous detection of 21 α-globin mutations – namely, two single gene deletions ($-\alpha^{3.7}$), ($-\alpha^{4.2}$), five double gene deletions, ($--^{MED}$), ($--^{SEA}$), ($--^{THAI}$), ($--^{FIL}$), ($-\alpha^{20.5}$); ααα(anti-3.7) gene triplication, two-point mutations in the α1 gene (cd 14 G>A; Hb Adana), and 11 point mutations in the α2 gene (initiation cd T>C; cd 19 -G; IVS1 -5nt; cd 59 G>A; Hb Quong Sze; Hb CS; Hb Icaria; Hb Pakse; Hb Koya Dora; polyA-1; polyA-2), using the manufacturer's instructions.

Data Analysis

Genotypic frequencies of detected mutations were calculated by dividing the number of patients with the specific genotype by the total number of patients tested (n = 260). The allelic frequencies of the mutations were calculated by dividing the number of specific alleles detected by the total number of alleles in the population tested (n = 520).

RESULTS

Demographic and Clinical Characteristics of Patients

Two hundred sixty (260) Filipino patients were recruited to participate in the study. The age of the patients ranged from 4 d–67 yr old at the time of interview and recruitment. Majority (55.00%, n = 143) of the patients were female while 45.00% (n = 117) were males. All patients were either initially screened with HbH disease and/or highly suspected with α-thalassemia and were all recommended to have α-globin gene, *HBA1* and *HBA2*, mutation analysis.

Genotypic and Allelic Frequencies of Common *HBA1* and *HBA2* Mutations

The most common genotype detected was ($--^{SEA}/\alpha\alpha$), which was found to be present in 30.38% of the patients tested. This was followed by the three gene deletions ($-\alpha^{3.7}/--^{SEA}$) and ($-\alpha^{3.7}/--^{FIL}$), which were present in 24.62% and 20.77% of the patients, respectively. The cis two-gene deletion, ($--^{FIL}/\alpha\alpha$), single-gene deletion, ($-\alpha^{3.7}/\alpha\alpha$), trans two-gene deletion ($-\alpha^{3.7}/-\alpha^{3.7}$), and three-gene deletion, ($-\alpha^{4.2}/--^{SEA}$) were also detected in 12.31%, 1.54%, 1.15%, and 0.77% of the recruited patients. The four-gene deletion, ($--^{SEA}/--^{SEA}$), the three gene deletions, ($-\alpha^{4.2}/--^{FIL}$), and the trans two gene deletions [($-\alpha^{4.2}/-\alpha^{4.2}$) and ($-\alpha^{3.7}/-\alpha^{4.2}$)] were similarly present in 0.38% of the patients tested. Patients with a heterozygous missense mutation, α2 cd 59, and non-deletional $\alpha\alpha^{CS}$ (HBA2:c:427C>T) mutation compound heterozygous with ($--^{SEA}$), were also seen in the patients tested (0.38%, Table 1). The ($--^{SEA}$), ($-\alpha^{3.7}$), and ($--^{FIL}$) mutations were found to have allelic frequencies of 28.46%, 24.81%, and 16.73%, respectively, in the patients tested (Table 2).

Table 1. Genotypic frequencies of common *HBA1* and *HBA2* mutations in Filipino patients recruited in the study.

***HBA1* and *HBA2* mutations**	**Number of study participants (n = 260)**	**Percentage (%)**
($--^{SEA}/\alpha\alpha$)	79	30.38
($-\alpha^{3.7}/--^{SEA}$)	64	24.62
($-\alpha^{3.7}/--^{FIL}$)	54	20.77
($--^{FIL}/\alpha\alpha$)	32	12.31
(αα/αα)	16	6.15
($-\alpha^{3.7}/\alpha\alpha$)	4	1.54
($-\alpha^{3.7}/-\alpha^{3.7}$)	3	1.15
($-\alpha^{4.2}/--^{SEA}$)	2	0.77
($--^{SEA}/--^{SEA}$)	1	0.38
(α2 cd 59/αα)	1	0.38
($-\alpha^{4.2}/-\alpha^{4.2}$)	1	0.38
($-\alpha^{4.2}/--^{FIL}$)	1	0.38
($--^{SEA}/\alpha\alpha^{CS}$)	1	0.38
($-\alpha^{3.7}/-\alpha^{4.2}$)	1	0.38

Table 2. Allelic frequencies of *HBA1* and *HBA2* mutations in Filipino patients recruited in the study.

***HBA1* and *HBA2* mutations**	**Number of alleles (n = 520)**	**Percentage (%)**
($--^{SEA}$)	148	28.46
($-\alpha^{3.7}$)	129	24.81
(αα)	148	28.46
($--^{FIL}$)	87	16.73
($-\alpha^{4.2}$)	6	1.15
(α2 cd 59)	1	0.19
($\alpha\alpha^{CS}$)	1	0.19

DISCUSSION

Variants of the α-globin genes, *HBA1* and *HBA2*, are unique for every population and have been reported to cause varying degrees of disease severity depending on the number of genes affected (Kountouris *et al*. 2016). The most common genotypes found in this study were the Hb H disease-causing three gene deletions [($-\alpha^{3.7}/--^{FIL}$), ($-\alpha^{3.7}/--^{SEA}$), ($-\alpha^{4.2}/--^{FIL}$), ($-\alpha^{4.2}/--^{SEA}$)] and the compound heterozygous ($--^{SEA}/\alpha\alpha^{CS}$) genotype, which comprised 46.92% of the patients tested. α-thalassemia trait-causing cis two gene deletions [($--^{SEA}/\alpha\alpha$) and ($--^{FIL}/\alpha\alpha$)] and

trans two gene deletions [(-$\alpha^{4.2}$/-$\alpha^{4.2}$), (-$\alpha^{3.7}$/-$\alpha^{3.7}$), and (-$\alpha^{4.2}$/-$\alpha^{3.7}$)] were also determined in 42.69% and 1.92% of the patients, respectively. Interestingly, the clinically significant Hb Bart's hydrops fetalis-causing four gene deletions (--^{SEA}/--^{SEA}) was also detected in one of the subjects. The heterozygous missense mutation, α2 cd 59 (0.38%) was also detected in the study. The (--^{SEA}, 28.46%), (-$\alpha^{3.7}$, 24.81%), and (--^{FIL}, 16.73%) were the most common alleles found.

HbH disease often presents as thalassemia intermedia with moderate anemia; although non-fatal, it is considered clinically significant due to increasing reports that the disease is not as benign as previously thought (Chui *et al*. 2003). In this study, the detected deletional variants that result in HbH were also seen in the study done in Filipinos in Taiwan (Ko *et al*. 1999). However, in addition to mutations identified in the study done by Ko and colleagues, this study also detected one patient with a non-deletional mutation – specifically ($\alpha\alpha^{CS}$) – compound heterozygous with (--^{SEA}). HbH disease that resulted from deletion of the *HBA1* and *HBA2* were reported to be milder than its non-deletional counterpart, which presents with a more severe phenotype. Patients with deletional HbH suffer infection/inflammation-induced hemolysis, silent gallstone, and chronic cholecystitis while patients with non–deletional HbH require blood transfusion, splenectomy, and iron chelation therapy, and present as growth retardation and hydrops fetalis syndrome in some cases (Fucharoen and Viprakasit 2009). Hence, detection of the non-deletional type of mutation in the tested population is important as it may aid in the future development of strategies for medical interventions of the disease.

Alpha thalassemia trait-causing cis two gene deletions [(--^{SEA}/αα) and (--^{FIL}/αα)] in 42.69% of the subjects tested in this study is important to note. These mutations are the most common risk factors for Hb Bart's hydrops fetalis in the Southeast Asian population (Chui and Waye 1998). Parents who are carriers of these mutations have a 25% chance of conceiving a child lacking all α-globin genes (Chui and Waye 1998). The Hb Bart's hydrops fetalis syndrome causes death of affected infants *in utero* or shortly after birth, a condition with detrimental fetal and maternal complications (Chui and Waye 1998). This result suggests that there is a need to improve/implement programs that will address the prevention of the increase in the number of cases in the country through carrier testing of parents. In Ontario, Canada, the prevention of Hb Bart's hydrops fetalis include identifying couples at risk – such as carriers of (--^{SEA}/αα) and (--^{FIL}/αα) – in order to provide them with timely counseling, and prenatal diagnosis during early pregnancy (Chui and Waye 1998).

Hb Bart's hydrops fetalis causing-four gene deletions (--^{SEA}/--^{SEA}) was also detected in one of the patients in the study. Newborns with (--^{FIL}/--^{FIL}) and (--^{SEA}/--^{SEA}) mutations were reported to die early in gestation and during the third trimester or soon after birth, respectively, without intervention (Chui and Waye 1998). The oldest reported newborn to the authors' knowledge with Hb Bart's syndrome was one of the triplets of a Chinese woman. The patient was diagnosed with Hb Barts hydrops fetalis syndrome and survived until 27 mo after birth (Lam *et al*. 1992). However, the patient included in this study with the four-gene deletion was 12 yr old at the time of recruitment and had no intrauterine transfusion nor any transfusions after birth. The nested PCR and sequencing analyses results of the sample of the patient also revealed no amplification of both the *HBA1* and *HBA2* genes. Inherent factors such as the location and type of mutation of the patient and the sequence of the designed primers used may have affected the StripAssay and sequencing analyses results of the patient. Thus, the result of the patient with the four-gene deletion and/or the underlying cause of survival of the patient reported in this study needs further validation and investigation.

The detection of the normal genotype (αα/αα, n = 16, 6.15%) possibly suggests the limitations of Alpha Globin StripAssay® kit used and the referral for genetic testing based on the evaluation of CBC results, red cell indices, and upon ruling-out iron deficiency anemia. The Alpha Globin StripAssay® kit used in this study screens 21 common *HBA1* and *HBA2* mutations with 90–99% coverage mutations only (Puehringer *et al*. 2007). Further mutational analysis of the samples *via* sequencing is highly recommended. It is also important to note that of the 16 patients with a normal genotype, 14 patients were referred by hematologists recruited based on the evaluation of CBC results, red cell indices, and upon ruling-out iron deficiency anemia alone. Improvement or modification of the used inclusion criteria in the study, *i.e.* comprehensive analysis of Hb variants through high-performance liquid chromatography (HPLC) and capillary electrophoresis analysis of the patient's sample, prior to the request for genetic analysis is suggested.

Finally, the high allelic frequencies of (--^{SEA}), (--^{FIL}) and (-$\alpha^{3.7}$) detected in the subjects tested is important to note because these mutations are risk factors for the two clinically severe forms of α-thalassemia, namely HbH and Hb Bart's hydrops fetalis syndrome (Chui and Waye 1998). With these data, national programs that mandate carrier screening of couples with the mentioned mutations prior to marriage and plan of conception should be implemented to address the prevention of the increase in the number of the severe forms of the disorder.

CONCLUSION

The genotypic and allelic frequencies reported in subjects tested in this study provides baseline data on common *HBA1* and *HBA2* mutations in suspected Filipino α-thalassemics. The high prevalence of *HBA1* and *HBA2* variant genotypes and alleles is important to address as these may increase the risk of HbH and Hb Bart's hydrops fetalis cases in the Filipino population. These reported common variants should be included in the development of kits specific to the Filipino population for diagnostic and carrier testing. Moreover, the results of this study emphasize the importance of implementation of guidelines that will focus on early detection of the disease for timely counseling of at-risk couples to prevent an increase of the severe form of the disease in the country.

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