Molecular Characterization of Hb H and AEBart's Diseases in Thai Children: Phramongkutklao Hospital Experiences

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Background: *Alpha-thalassemia is a common genetic disorder in Thailand and is caused by either deletion or non-deletional mutation of one or both α-globin genes. Inactivation of three α-globin genes causes Hb H disease and interaction of Hb H disease with heterozygous Hb E results in AEBart's disease.*
Objective: *The present study aimed to characterize the genotype of α-globin gene in 81 pediatric patients with Hb H and AEBart's diseases in Phramongkutklao Hospital, a tertiary care center for thalassemic patients in central Thailand.*
Material and Method: *Eighty one unrelated pediatric patients including 60 patients with Hb H disease and 21 patients with AEBart's disease were enrolled in our study. Mutation analysis was performed by multiplex gap-PCR, multiplex-ARMS and direct DNA sequencing of both HBA1 and HBA2 genes, respectively.*
Results: *A total of 81 pediatric patients with Hb H and AEBart's diseases who mainly lived in central Thailand were included in the present study. Eight different α-thalassemia mutations interacting to produce seven genotypes of α-globin gene in both Hb H and AEBart's diseases were identified. The number of patients in the non-deletional form was higher than in the deletional form for both Hb H (51.6% VS 48.4%) and AEBart's diseases (52.4% VS 47.6%). The SEA deletion ($--^{SEA}$) was the most common (98.8%) α-thalassemia 1 mutation. While 3.7-kb deletion ($-\alpha^{3.7}$) was the most common (90%) α-thalassemia 2 deletion, Hb CS was the most common (90%) non-deletional a-thalassemia 2. Uncommon non-deletional α-thalassemia 2 mutation identified in our study were Hb QS, Hb PS and initiation codon mutation, respectively.*
Conclusion: *All of the α-thalassemia mutation in our pediatric patients with Hb H and AEBart's diseases have been characterized by the combination of molecular techniques including multiplex gap-PCR, multiplex-ARMS and DNA sequencing of HBA1 and HBA2 genes.*

Keywords: *molecular analysis, α-globin gene, Hb H disease, AEBart's disease, Thai children*

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Thalassemia is the most common inherited hematological disorders in Thailand. The prevalence of thalassemia traits in Thai population were 20–30% for α-thalassemia, 3-9% for β- thalassemia and 20-30% for Hb E[1]. Alpha-thalassemia is one of the major thalassemia types and is caused by mutation in either α_1-globin gene *(HBA1)* or α_2-globin gene *(HBA2)* on chromosome 16. The mutation results in variable decreased or absent α-globin chain. To date, more than 20 different mutations in the α-globin gene causing α-thalassemia have been identified in Thailand[2-11]. Deletion of either one (α-thalassemia 2) or both (α-thalassemia 1) α-globin genes is the most common type of α-globin gene mutation; whereas, non-deletional forms of α-thalassemia 2 are occasionally found. Hb Constant Spring (Hb CS), a termination codon mutation of the *HBA2*, is the most common non-deletion mutation with the prevalence of 1-8%[1].

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The clinical and hematological manifestation of α-thalassemia is variable ranging from silent carrier to fatal Hb Bart's hydrops fetalis syndrome. Interaction of α-thalassemia 1 and α-thalassemia 2 causes hemoglobin H (Hb H) disease and interaction of Hb H disease with heterozygous Hb E results in AEBart's disease[12,13]. Hb H is characterized into two main forms including deletional and non-deletional Hb H diseases. Deletional Hb H disease is caused by a combination of deletion removing both α-globin genes on one chromosome 16 and deletion removing only single α-globin gene on the other chromosome 16. Non-deletional Hb H disease results from a compound heterozygous for deletion removing both α-globin genes on one chromosome 16 and point mutation or small insertion/deletion involving either the *HBA1* or *HBA2* gene on the other chromosome 16.

In the present study, we aimed to characterize the genotype of α-globin gene in 81 pediatric patients with Hb H and AEBart's diseases in Phramongkutklao Hospital, a tertiary care center for thalassemic patients in central Thailand.

Material and Method

Patient selection

Eighty one unrelated pediatric patients including 60 patients with Hb H disease (age range: 3 months-11 year) and 21 patients with AEBart's disease (age range: 6 month- 10 year) who attended the Hematology Clinic at the Department of Pediatrics, Phramongkutklao Hospital, Bangkok, Thailand, from January 2014 to December 2014 were enrolled in our study. Forty males (31 males with Hb H diseases and 9 males with AEBart's disease) and 41 females (30 females with Hb H disease and 11 females with AEBart's disease) were included in the present study. All patients were diagnosed at 18 years of age or less. More than 95% of our patients with Hb H disease and 75% of AEBart's disease patients lived in central Thailand. The remaining patients lived in northern and northeastern Thailand. The study protocol was approved by the Institutional Review Board of Phramongkutklao Hospital, Phramongkutklao College of Medicine, Thailand.

Mutation analysis

After the informed consent was obtained, a total of 81 peripheral blood EDTA samples from all individuals were collected. Genomic DNA was extracted from peripheral blood lymphocytes using commercial available kits according to manufacturer's protocol. The α-globin gene mutations were first characterized using multiplex gap polymerase chain reaction (gap-PCR) to detect common deletions in Chinese and Southeast Asian populations including α-thalassemia 1 [SEA ($--^{SEA}$) and THAI ($--^{THAI}$) deletion] and α-thalassemia 2 [3.7-kb ($-\alpha^{3.7}$) and 4.2-kb ($-\alpha^{4.2}$) deletion] as previously described(14). Second, allele specific PCR or multiplex amplification refractory mutation system (M-ARMS) was performed to detect Hb Constant Spring (Hb CS) and Hb Pakse (Hb PS) as described previously[15]. Unknown non-deletional α-globin gene mutations were further characterized by direct DNA sequencing of all coding regions and exon-intron boundaries of both *HBA1* and *HBA2* genes to detect uncommon point mutations and small rearrangements according to protocols previously described elsewhere[16].

Statistical Analysis

The frequencies were used to describe molecular characteristics of Hb H and AEBart's disease. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp. Armonk, NY).

Results

A total of 81 children were included in our study. Sixty patients were Hb H disease and twenty-one patients were AEBart's disease. All subjects were from unrelated families. More than 70% (60/81) of the patients included in our study were Hb H diseases. Eight different a-thalassemia mutations interacting to produce seven genotypes of α-globin gene in both Hb H and AEBart's disease were identified. Among 60 Hb H disease patients, 29 patients were the combination of α-thalassemia 1 and α-thalassemia 2 deletions including 24 cases of SEA deletion/3.7-kb deletion ($--^{SEA}/-\alpha^{3.7}$), 4 cases of SEA deletion/4.2-kb deletion ($--^{SEA}/-\alpha^{4.2}$) and only 1 case of THAI deletion/3.7-kb deletion ($--^{THAI}/-\alpha^{3.7}$). Thirty one patients were interaction of deletional α-thalassemia 1 and non-deletional α-thalassemia 2 including 29 cases of SEA deletion/Constant Spring ($--^{SEA}/\alpha^{CS}\alpha$) and 1 case each for SEA deletion/Quong Sze ($--^{SEA}/\alpha^{QS}\alpha$) and SEA deletion/initiation codon mutation ($--^{SEA}/\alpha^{int\text{-}TG}\alpha$), respectively. Among 21 patients with AEBart's diseases,

10 individuals were associated with the interaction of heterozygous Hb E and deletional Hb H disease whereas 11 individuals were the combination of Hb E heterozygotes and non-deletional Hb H disease. All 10 cases of the deletional AEBart's diseases were the combination of SEA deletion and 3.7 kb deletion [(--^{SEA}/-$\alpha^{3.7}$),β^{E}/β]. Eleven cases of non-deletional AE-Bart's diseases composed of 9 cases of SEA deletion/ Constant Spring [(--^{SEA}/α^{CS}α),β^{E}/β], 1 case each for both SEA deletion/Pakse [(--^{SEA}/α^{PS}α),β^{E}/β] and SEA deletion/Quong Sze [(--^{SEA}/α^{QS}α),β^{E}/β]. The number of patients in the non-deletional form was higher than in the deletional form for both Hb H (51.6% VS 48.4%) and AEBart's disease (52.4% VS 47.6%) (Table 1).

Among 81 pediatric patients with Hb H and AEBart's disease, the SEA deletion (--^{SEA}) was the most common α-thalassemia 1 mutation and was detected in 80 patients (98.8%) whereas the THAI deletion (--^{THAI}) was identified in only 1 patient (1.2%). Concerning α-thalassemia 2 mutation, Hb CS (α^{CS}α) was the most common which was found in 38 patients (46.9%) followed by 3.7-kb deletion (-$\alpha^{3.7}$) which was identified in 35 patients (43.2%) (Table 2). The 4.2-kb deletion α-thalassemia 2 was identified in 4 patients (4.9%). Other uncommon non-deletional α-thalassemia 2 were identified in 4 patients including 2 patients (2.5%) with Hb QS (α^{QS}α) and 1 patient (1.2%) each for Hb PS (α^{PS}α) and initiation codon mutation (α^{int-TG}α). The 3.7-kb deletion a-thalassemia 2 was detected in the majority (89.7%) of the patients with deletional HbH and AEBart's disease. And also Hb CS was the most common non-deletional α-thalassemia 2 which accounted for 90.4% among non-deletional HbH and AEBart's disease.

All of our 162 α-thalassemia alleles (100%) were characterized by combination of various techniques including multiplex gap-PCR, multiplex amplification refractory mutation system (M-ARMS) and direct DNA sequencing. One hundred percent of α-thalassemia 1 alleles and 48.1% of α-thalassemia

Table 1. The genotypes of α-globin gene in 81 pediatric patients with Hb H and AEBart's disease

Disease	Genotype	n (%)
Hb H disease (n = 60)		
Deletional Hb H disease		29 (48.4)
SEA deletion/3.7-kb deletion	(--^{SEA}/-$\alpha^{3.7}$)	24 (40)
SEA deletion/4.2-kb deletion	(--^{SEA}/-$\alpha^{4.2}$)	4 (6.7)
THAI deletion/3.7-kb deletion	(--^{THAI}/-$\alpha^{3.7}$)	1 (1.7)
Nondeletional Hb H disease		31 (51.6)
SEA deletion/Constant Spring	(--^{SEA}/α^{CS}α)	29 (48.2)
SEA deletion/Quong Sze	(--^{SEA}/α^{QS}α)	1 (1.7)
SEA deletion/initiation codon mutation	(--^{SEA}/α^{int-TG}α)	1 (1.7)
Total		60 (100)
AEBart's disease (n = 21)		
Deletional AEBart's disease		10 (47.6)
SEA deletion/3.7-kb deletion	(--^{SEA}/-$\alpha^{3.7}$) , β^{E}/β	10 (47.6)
Nondeletional AEBart's disease		11 (52.4)
SEA deletion/Constant Spring	(--^{SEA}/α^{CS}α), β^{E}/β	9 (42.8)
SEA deletion/Pakse	(--^{SEA}/α^{PS}α), β^{E}/β	1 (4.8)
SEA deletion/Quong Sze	(--^{SEA}/α^{QS}α), β^{E}/β	1 (4.8)
Total		21 (100)

Table 2. The α-thalassemia 1 and α-thalassemia 2 mutations in 81 pediatric patients with Hb H and AEBart's disease

α-thalassemia allele	n (%)
α-thalassemia 1 (n = 81)	
SEA deletion ($--^{SEA}$)	80 (98.8)
THAI deletion ($--^{THAI}$)	1 (1.2)
Total	81 (100)
α-thalassemia 2 (n = 81)	
Deletion	39
3.7-kb deletion ($-\alpha^{3.7}$)	35 (43.2)
4.2-kb deletion ($-\alpha^{4.2}$)	4 (4.9)
Nondeletion	42
Hb Constant Spring ($\alpha^{CS}\alpha$)	38 (46.9)
Hb Quong Sze ($\alpha^{QS}\alpha$)	2 (2.5)
Hb Pakse ($\alpha^{PS}\alpha$)	1 (1.2)
Initiation codon mutation ($\alpha^{int\text{-}TG}\alpha$)	1 (1.2)
Total	81 (100)

2 alleles were identified by multiplex gap-PCR. Another 48.1% of α-thalassemia 2 alleles were detected by multiplex-ARMS which can detect Hb CS and Hb PS. Finally, direct DNA sequencing of both *HBA1* and *HBA2* gene was used to detect uncommon mutations which were identified in the remaining (3.8%) alleles.

Discussion

Alpha-thalassemia is one of the major thalassemia types worldwide. It's commonly caused by deletion involving one (α-thalassemia 2 or -α) or both (α-thalassemia 1 or --) α-globin genes and less frequently caused by non-deletional mutation ($\alpha^T\alpha$ or $\alpha\alpha^T$). In Thailand, the most common α-thalassemia 1 mutation is SEA deletion ($--^{SEA}$), whereas the most common α-thalassemia 2 mutation is a 3.7 kb or rightward deletion ($-\alpha^{3.7}$), followed by 4.2 kb or leftward deletion ($-\alpha^{4.2}$) and Hb Constant Spring ($\alpha^{CS}\alpha$), a non-deletional form of α-thalassemia 2. The interaction between α-thalassemia 1 and α-thalassemia 2 causes Hb H disease which is found in many parts of the world especially in Southeast Asia because of high prevalence of SEA deletion α-thalassemia 1[12,13]. And also, Hb E is very common in Southeast Asia and especially in Thailand. The interaction of Hb H disease and heterozygous Hb E give rise to broadly known AEBart's disease. Both Hb H disease and AEBart's disease have high prevalence in Thailand[12,13].

In the present study, we aimed to characterize the molecular basis in patients with Hb H and AEBart's diseases in Thai pediatric patients. Of these 60 patients with Hb H disease, non-deletional genotypes (51.6%) were more common than deletional genotypes (48.4%) (Table 1). The genotype of Hb H disease in our study was most commonly (48.2%) caused by the interaction of SEA deletion α-thalassemia 1 and non-deletional Hb CS ($--^{SEA}/\alpha^{CS}\alpha$) and less commonly (40%) from the combination of SEA deletion α-thalassemia 1 and 3.7 kb deletion α-thalassemia 2 ($--^{SEA}/-\alpha^{3.7}$). Among 21 patients with AEBart's disease, the non-deletional forms (52.4%) were also more common than the deletional form (47.6%). All cases of deletional AEBart's disease were the interaction of SEA deletion α-thalassemia 1 and 3.7 kb deletion α-thalassemia 2 [($--^{SEA}/-\alpha^{3.7}$), β^E/β]. The majority of non-deletional AEBart's disease (81.8%) was the combination of SEA deletion α-thalassemia 1 and Hb CS [($--^{SEA}/\alpha^{CS}\alpha$), β^E/β]. The incidence of genetic subtypes of Hb H disease varies in different ethnic groups in which majority of the previous studies revealed higher incidence of deletional than non-deletional Hb H disease[12,17-19]. The α-globin genotypes of Hb H and AEBart's diseases also vary in different regions in Thailand. Our patients who mainly lived in central Thailand have the higher prevalence of non-deletional Hb H and AEBart's disease as in those who lived in northern and northeastern Thailand[15,20].

But the incidence of deletional Hb H disease is higher in the patients from southern Thailand[21]. This may be explained by the different in geographic distribution and age of the patients included in each study. Our study included only pediatric patients with Hb H and AEBart's disease and most of the patients lived in Bangkok and other provinces in this region. Although, the clinical phenotypes of Hb H and AEBart's disease is quite variable, non-deletional forms are often associated with more severe disease than deletional forms as shown in many published literatures[15,17-21]. This finding suggests that deletional Hb H and AEBart's diseases may be asymptomatic and unrecognized in the children and even in the adults.

In Southeast Asia, the SEA deletion ($--^{SEA}$) is the most common α-thalassemia 1 mutation which is caused by a deletion of approximately 19.3 kb in length removing both linked α-globin genes but sparing the embryonic z-globin gene. Another form of α-thalassemia 1 occasionally found in this region is the THAI deletion ($--^{THAI}$) which removes a 33.5 kb-DNA segment including the embryonic z-globin gene[12,13]. Almost all of α-thalassemia 1 (98.8%) in our patients is caused by SEA deletion, while only 1.2% is caused by THAI deletion. The rarity of THAI deletion is concordant with a prevalence ratio of 99:1 of SEA to THAI deletion in the previous study in Thai populations[12, 20]. Among deletional form of α-thalassemia 2, the 3.7-kb deletion ($-\alpha^{3.7}$) is identified in the majority of our patients (90%), followed by 4.2-kb deletion ($-\alpha^{4.2}$) which is detected at the lower frequency (10%). Among non-deletional form of α-thalassemia 2, Hb Constant Spring (Hb CS) was the most common (90%) mutation, followed by uncommon mutations (10%) including Hb Quong Sze (Hb QS), Hb Pakse (Hb PS) and initiation codon mutation, respectively. Both Hb CS (TAA>CAA) and Hb PS (TAA>TAT) are α2-globin gene termination codon mutation leading to unstable mRNA and produces only small amounts of abnormally elongated α-globin chains[9]. Hb PS has the slow migrating pattern on hemoglobin electrophoresis resembling Hb CS. Thus, the differentiation between Hb CS and Hb PS can be performed only by molecular techniques including multiplex-ARMS which we used in our study. Hb QS is caused by missense mutation in codon 125 (CTG>CCG) of the α2-globin gene. This mutation produces highly unstable α-globin chains and results in α-thalassemia phenotype[8]. The ATG>_TG (*HBA2*:c.1delA) mutation is the initiation codon mutation which possibly affects the downstream a-globin gene expression. This mutation results in a-thalassemia 2 phenotype and has been reported in Thai population only once in the recent year[3]. Our patient may be the second case who carries this rare mutation. With our diagnostic strategy, all cases of α-thalassemia 1 and about half of α-thalassemia 2 can be characterized by multiplex gap-PCR. Multiplex-ARMS for Hb CS and Hb PS can identified nearly all cases of nondeletional α-thalassemia 2, and direct DNA sequencing of both *HBA1* and *HBA2* genes is needed in less than 10% of cases in our study.

Since α- and β-thalassemia have high prevalence in Thailand the possibility of concomitant β-thalassemia should be considered when assessing individuals with Hb H disease. Interestingly, two patients in our study were initially diagnosed as heterozygous β-thalassemia due to high Hb A_2 (> 4%). Neither Hb H nor Hb Bart's were detected from the Hb electrophoresis. Hb H inclusion bodies were not presented. These patients showed moderately anemia with markedly hypochromic and microcytic red cells which cannot be explained by only β-thalassemia carrier. Genotype analysis of α-globin genes were performed and revealed interaction of SEA deletion α-thalassemia 1 and 3.7-kb deletion α-thalassemia 2 ($--^{SEA}/-\alpha^{3.7}$) in the patient's DNA indicating concurrent Hb H disease and heterozygous β-thalassemia in both patients. Molecular analysis therefore serves as an important diagnostic tool for the patients who have coinheritance of Hb H disease and β-thalassemia trait which usually lead to more severe anemia than β-thalassemia heterozygote alone, but less than common Hb H disease.

Conclusion

In conclusion, the heterogeneity of molecular defects causing α-thalassemia in Thai children has been demonstrated in the present study. All of the α-thalassemia mutation have been characterized by the combination of various molecular techniques including multiplex gap-PCR, multiplex-ARMS and direct DNA sequencing of *HBA1* and *HBA2* genes. Eight different α-thalassemia mutations interacting to produce seven genotypes of α-globin gene in both Hb H and AEBart's disease were identified in our study. The molecular characterization as performed in the present study is useful not only for diagnostic confirmation, but also for

carrier detection and genotype-phenotype correlation for both α-thalassemia and complex αβ thalassemia syndrome.

What is already known on this topic?

The mutation results in variable decreased or absent α-globin chain in a-thalassemia. At present, more than 20 different mutations in the α-globin gene causing α-thalassemia have been identified in Thailand. Deletion of either one (α-thalassemia 2) or both (α-thalassemia 1) α-globin genes is the most common type of α-globin gene mutation; whereas, non-deletional forms of α-thalassemia 2 are occasionally found. Hb Constant Spring (Hb CS), a termination codon mutation of the *HBA2*, is the most common non-deletion mutation with the prevalence of 1-8%.

Both α- and β-thalassemia have high prevalence in Thailand the possibility of concomitant β-thalassemia should be considered when assessing individuals with Hb H disease.

What this study adds?

More than 95% of our patients with Hb H disease and 75% of AEBart's disease patients were lived in central Thailand. Eight different α-thalassemia mutations interacting to produce seven genotypes of α-globin gene in both Hb H and AEBart's disease were identified in our study. Molecular analysis serves as an important diagnostic tool for the patients who have coinheritance of α-thalassemia and β-thalassemia trait which is useful for diagnostic confirmation, carrier detection, genotype-phenotype correlation both α- and β-thalassemia or complex αβ thalassemia syndrome.

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Potential conflicts of interest

None.

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ลักษณะทางอณูพันธุศาสตร์ของโรค Hb H และ AEBart's ในผู้ป่วยเด็กไทย: ประสบการณ์ในโรงพยาบาลพระมงกุฎเกล้า

บุญชัย บุญวัฒน์, อภิชาติ โพธิอะ, ชาลินี มนต์เสรีนุสรณ์, ปิยะ รุจกิจยานนท์, ชาญชัย ไตรวารี

ภูมิหลัง: *โรคธาลัสสีเมียชนิดอัลฟ่าเป็นโรคพันธุกรรมที่พบบ่อยในประเทศไทย เกิดจากการกลายพันธุ์ชนิดที่มีการขาดหายและไม่มีการขาดหายของจีนอัลฟ่าโกลบิน 1 หรือ 2 จีน การสูญเสียการทำงานของจีนอัลฟ่าโกลบิน 3 จีนทำให้เกิดโรค Hb H และถ้าพบ Hb H ร่วมกับภาวะพาหะของ Hb E ทำให้เกิดโรค AEBart's*

วัตถุประสงค์: *เพื่อศึกษาลักษณะทางพันธุกรรมของจีนอัลฟ่าโกลบินในผู้ป่วยเด็กโรค Hb H และโรค AEBart's จำนวน 81 ราย ในโรงพยาบาลพระมงกุฎเกล้า ที่เป็นศูนย์ให้การรักษาผู้ป่วยธาลัสสีเมียในภาคกลางของประเทศไทย*

วัสดุและวิธีการ: *ผู้ป่วยเด็กจำนวน 81 ราย ประกอบด้วยโรค Hb H 60 รายและโรค AEBart's 21 รายเข้าร่วมในการศึกษาวิเคราะห์หาการกลายพันธุ์ของจีนอัลฟ่าโกลบินด้วยวิธี multiplex gap-PCR, multiplex-ARMS และ DNA sequencing ตามลำดับ*

ผลการศึกษา: *ผู้ป่วยจำนวน 81 ราย ส่วนใหญ่อาศัยอยู่ในภาคกลางของประเทศไทย ตรวจพบมีการกลายพันธุ์ของจีนอัลฟ่าโกลบินทั้งหมด 8 ชนิดประกอบกันเป็นลักษณะทางพันธุกรรมในโรค Hb H และโรค AEBart's ได้ทั้งหมด 7 ชนิด โดยชนิดที่ไม่มีการขาดหายพบได้บ่อยกว่าชนิดที่มีการขาดหายทั้งในโรค Hb H (ร้อยละ 51.6 และร้อยละ 48.4) และโรค AEBart's (ร้อยละ 52.4 และร้อยละ 47.6) การกลายพันธุ์ที่มีการขาดหายชนิด SEA ($--^{SEA}$) พบได้บ่อยที่สุด (ร้อยละ 98.8) ใน α-thalassemia 1 การกลายพันธุ์ที่มีการขาดหายขนาด 3.7 กิโลเบส ($-\alpha^{3.7}$) พบได้บ่อยที่สุด (ร้อยละ 90) ใน α-thalassemia 2 ที่เกิดจากการขาดหายและ Hb CS พบได้บ่อยที่สุด (ร้อยละ 90) ใน α-thalassemia 2 ที่ไม่ได้เกิดจากการขาดหาย ส่วนการกลายพันธุ์ของ α-thalassemia 2 ที่ไม่ได้เกิดจากการขาดหายที่พบไม่บ่อยได้แก่ Hb QS, Hb PS และการกลายพันธุ์ในตำแหน่ง initiation codon ตามลำดับ*

สรุป: *การกลายพันธุ์ของจีนอัลฟ่าโกลบินทั้งหมดในผู้ป่วยเด็กโรค Hb H และโรค AEBart's ในการศึกษานี้สามารถตรวจพบได้ด้วยวิธีการอณูพันธุศาสตร์ที่หลากหลายประกอบด้วย multiplex gap-PCR, multiplex-ARMS และ DNA sequencing ของจีนอัลฟ่าโกลบิน*