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Molecular characteristics of α^+-thalassemia (3.7 kb deletion) in Southeast Asia: Molecular subtypes, haplotypic heterogeneity, multiple founder effects and laboratory diagnostics

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ABSTRACT

Objective: The 3.7 kb deletion ($-\alpha^{3.7}$) is the most common form of α^+-thalassemia found in multiple populations which can be classified into three subtypes. In order not to mis-identify it, the molecular information within each population is required. We have addressed this in northeast Thai and Laos populations.
Methods: Screening for α^+-thalassemia was initially done on 1192 adult Thai subjects. In addition, 77 chromosomes of Thai newborns and 26 chromosomes of Laos with $-\alpha^{3.7}$ α^+-thalassemia were also examined. All subjects were screened for $-\alpha^{3.7}$ α^+-thalassemia and subtyped by PCR-RFLP assay. Exact deletion breakpoint of each $-\alpha^{3.7}$ subtype was determined by DNA sequencing. α-Globin gene haplotypes were determined.
Results: The proportions of $-\alpha^{3.7}$ subtypes found in 216 Thai $-\alpha^{3.7}$ chromosomes were 94.9% for $-\alpha^{3.7I}$, 4.2% for $\alpha^{3.7II}$ and 0.9% for $-\alpha^{3.7III}$. All 26 Laos $-\alpha^{3.7}$ chromosomes were of $-\alpha^{3.7I}$ variety. At least six α-globin gene haplotypes were associated with the $-\alpha^{3.7I}$ α^+-thalassemia.
Conclusion: All $-\alpha^{3.7}$ subtypes were observed among Southeast Asian population. Haplotype analysis indicated a multiple origin of this common disorder in the region. A multiplex PCR assay has been developed for simultaneous detection of all subtypes of $-\alpha^{3.7}$ α^+-thalassemia as well as other α^+-thalassemia found in the region including $-\alpha^{4.2}$ α^+-thalassemia, Hb Constant Spring and Hb Paksé.

1. Introduction

The α-thalassemia is the most common inherited globin gene disorder found worldwide. It can be classified into α^+-thalassemia with decreased and α^0-thalassemia with absent α-globin chain production. The most common form of α^+-thalassemia found in multiple population is the 3.7 kb deletion ($-\alpha^{3.7}$) and the less common one is the 4.2 kb deletion ($-\alpha^{4.2}$) [1,2]. The former is caused by unequal crossing over in the Z blocks of α-globin gene cluster or rightward crossover, leading to a chromosome with one α-globin gene ($-\alpha^{3.7}$) and another with triplicated α-globin gene ($\alpha\alpha\alpha^{anti3.7}$). Based on the deletion breakpoints, this ($-\alpha^{3.7}$) α^+-thalassemia could be divided into three subtypes including $-\alpha^{3.7}$subtype I ($-\alpha^{3.7I}$), $-\alpha^{3.7}$subtype II ($-\alpha^{3.7II}$), and $-\alpha^{3.7}$ subtype III ($-\alpha^{3.7III}$) [3]. Although with different deletion junctions, all of them result in the same phenotype of α^+-thalassemia. Compound heterozygosity of this ($-\alpha^{3.7}$) α^+-thalassemia with α^0-thalassemia leads to the Hb H disease, a thalassemia intermedia syndrome commonly found in many regions of the world [1]. It has been thought that subtype III ($-\alpha^{3.7III}$) is extremely rare in the population. Most of the methods used for identification of this $-\alpha^{3.7}$ allele rely on detection of ($-\alpha^{3.7I}$) and II ($-\alpha^{3.7II}$) [4]. Recently, it has been noted in southern Thai population that the ($-\alpha^{3.7III}$) was existed at about 2% of α^+-thalassemia alleles in the population [5]. Unfortunately, information regarding the prevalence of each $-\alpha^{3.7}$ subtype in northeast Thai and Laos populations is not available. In order not to mis-identify the cases as well as to provide basic information for northeast Thai population, we have examined for the first-time proportions of $-\alpha^{3.7}$ subtypes and determined their associated α-globin gene haplotypes in northeast Thai population. A

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multiplex PCR assay has been developed for rapid identification of all -$\alpha^{3.7}$ subtypes as well as other α^+-thalassemia found in the region including $\alpha^{4.2}$, Hb Constant Spring and Hb Paksé.

2. Materials and methods

2.1. Subjects

Approval of the study protocol was obtained from the Institutional Review Board (IRB) of Khon Kaen University, Thailand (HE612059 and HE542253). Informed consent was obtained prior to blood collection and all data were anonymized with numerical identification throughout the study. Study was done on a total of 1192 northeast Thai encountered at the thalassemia service unit of our referral center at Faculty of Associated Medical Sciences, Khon Kaen University in northeast Thailand during January 2016 to April 2018. They were requested for thalassemia investigation by DNA analysis. In addition, a total of 65 heterozygotes and 6 homozygotes for -$\alpha^{3.7}$ α^+-thalassemia in Thai newborns selectively recruited from our previous study [6] and 26 Laos heterozygotes for -$\alpha^{3.7}$ α^+-thalassemia [2] were also studied.

2.2. DNA analysis

2.2.1. Analysis of -$\alpha^{3.7}$ subtype and α-globin gene haplotyping

To examine the subtypes of the -$\alpha^{3.7}$ α^+-thalassemia, PCR-RFLP was done as shown in Fig. 1. Amplification was done using primers A and Ek (sequences listed in Table 1) and the amplified product was digested to completion with *Apa*I restriction enzyme (5′-GGGCC▼C-3′) (New England Biolabs, Beverly, MA, USA). After complete digestion, the -$\alpha^{3.7}$ subtype I, II and III showed specific fragments of (1471 & 468 bp), 1939 bp and (1675 & 256 bp), respectively. Amplified fragment of each -$\alpha^{3.7}$ subtype was determined to confirm the deletion using direct DNA sequencing (ABI PRISM™ 3730 XL analyzer; Applied Biosystems, Foster City, CA, USA). To determine α-globin gene haplotypes associated with the -$\alpha^{3.7}$ subtypes, 5 polymorphic sites (5'-*Xba*I, *Acc*I, *Rsa*I, α-*Pst*I and θ-*Pst*I-3′) within α-globin gene cluster were determined using PCR-RFLP assay as described [7,8].

Table 1
Sequences and NG_000006.1 locations of oligonucleotide primers used in this study.

Primer	NG_000006.1 location	Sequence (5′ → 3′)
A1	32,755–32,778/5′ α2-globin	AAGTCCACCCCTTCCTTCCTCACC
A1	36,309–36,332/5′ α1-globin	AAGTCCACCCCTTCCTTCCTCACC
A	32,840–32,863/5′ α2-globin	CCCAGAGCCAGGTTTGTTTATCTG
B	38,293–38,315/α1-globin	GAGGCCCAAGGGGCAAGAAGCAT
D	35,941–35,963/5′ α1-globin	TTCTGACTCTGCCCACAGCCTGA
DD	29,851–29,873/ψα1-globin	CCAATGCCGTGGTCCACTTAGAT
Ek	38,562–38,582/3′ α1-globin	AAAGCACTCTAGGGTCCAGCG
αG2	38,253–38,272/α2-globin	GCTGACCTCCAAATACCGTC
αG18	38,252–38,272/α2-globin	ACGGCTACCGAGGCTCCAGCA

2.2.2. Development of a multiplex PCR assay for identification of common forms of α^+-thalassemia

A multiplex PCR assay for rapid identification of 4 common α^+-thalassemia mutations found in Southeast Asia including deletional α^+-thalassemia (-$\alpha^{3.7}$ and -$\alpha^{4.2}$), Hb Constant Spring, and Hb Paksé was developed as shown in Fig. 2 using primers listed in Table 1. As shown in the figure, primer A1 was designated to bind the 5′ regions of both α2 and α1 globin genes. In this system, primers ($A1_{\alpha1}$ + Ek), ($A1_{\alpha2}$ + Ek), (DD + D), ($A1_{\alpha2}$ + αG18) and (αG2 + D) respectively generate specific fragments with 2274, 2024, 1880, 1730 and 1520 bps for internal

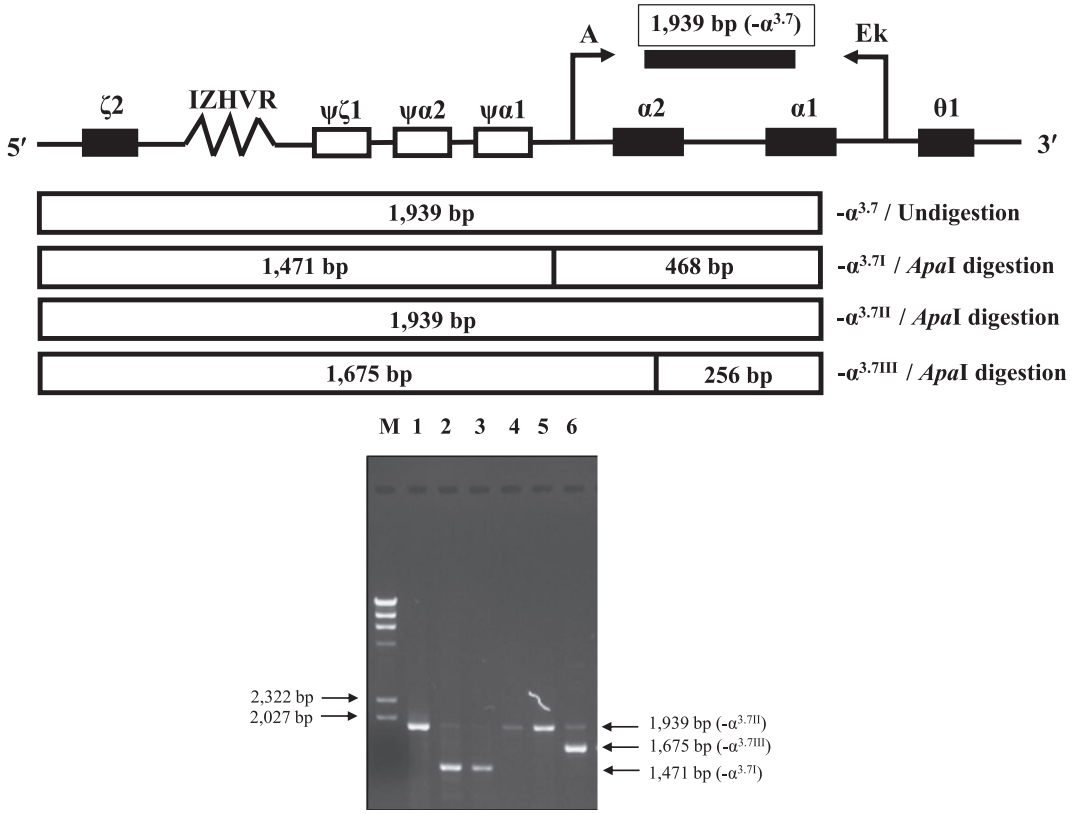

Fig. 1. Subtyping of the -$\alpha^{3.7}$ α^+-thalassemia by PCR-RFLP with *Apa*I restriction digestion. The locations and orientations of primers A and Ek are indicated. The size of amplified DNA (1939 bp) and the *Apa*I-digested fragments of -$\alpha^{3.7I}$ (1471 bp & 468 bp), -$\alpha^{3.7II}$ (1939 bp) and -$\alpha^{3.7III}$ (1675 bp & 256 bp) are depicted. M represents the λ/*Hind*III marker. 1: undigested amplified DNA, 2 & 3: *Apa*I-digested amplified DNA of -$\alpha^{3.7I}$, 4 & 5: *Apa*I-digested amplified DNA of -$\alpha^{3.7II}$ and 6: *Apa*I-digested amplified DNA of -$\alpha^{3.7III}$.

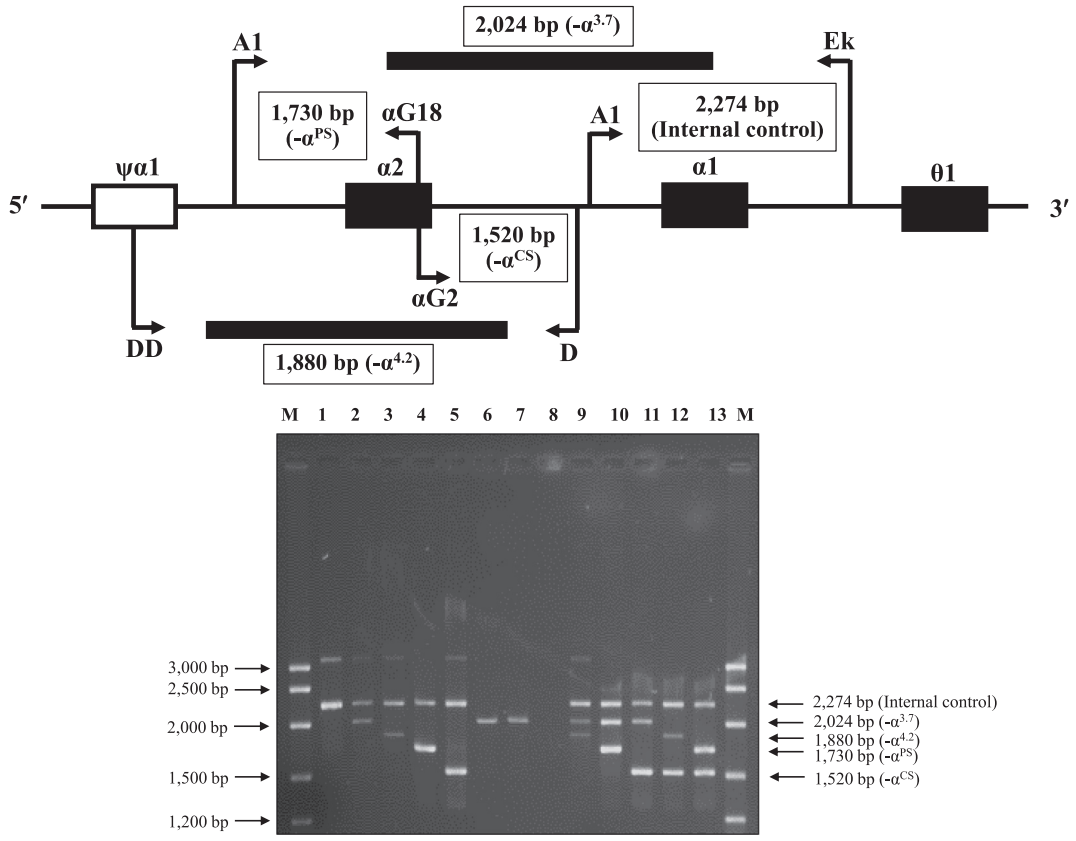

Fig. 2. A multiplex PCR assay for identification of common α^+-thalassemia.
Upper: Locations and orientations of primers used in a multiplex PCR assay and the sizes of each amplified fragments. Primers ($A1_{\alpha1}$ + Ek), ($A1_{\alpha2}$ + Ek), (DD + D), ($A1_{\alpha2}$ + αG18) and (αG2 + D) are used to produce the fragments of 2274 bp, 2024 bp, 1880 bp, 1730 bp and 1520 bp in lengths specific for internal control, $-\alpha^{3.7}$, $-\alpha^{4.2}$, Hb Paksé and Hb Constant Spring genes, respectively.
Lower: A representative agarose gel electrophoresis of PCR products of the above PCR assay. M represents the VC 100 bp plus DNA ladder. 1: Normal control, 2: $-\alpha^{3.7}$ carrier, 3: $-\alpha^{4.2}$ carrier, 4: Hb Paksé carrier, 5: Hb Constant Spring carrier, 6: homozygous for $-\alpha^{3.7}$, 7: compound heterozygous for $-^{SEA}/-\alpha^{3.7}$, 8: homozygous for $-^{SEA}$, 9: compound heterozygous for $-\alpha^{3.7}/-\alpha^{4.2}$, 10: compound heterozygous for $-\alpha^{3.7}/\alpha^{PS}\alpha$, 11: compound heterozygous for $-\alpha^{3.7}/\alpha^{CS}\alpha$, 12: compound heterozygous for $-\alpha^{4.2}/\alpha^{CS}\alpha$ and 13: compound heterozygous for $\alpha^{CS}\alpha/\alpha^{PS}\alpha$.

Table 2
The proportion of $-\alpha^{3.7}$ subtypes among 214 Thai and 26 Laos subjects with the $-\alpha^{3.7}$ α^+-thalassemia.

Subjects	$-\alpha^{3.7}$ α^+-thalassemia subtype (%)			Total
	$-\alpha^{3.7I}$	$-\alpha^{3.7II}$	$-\alpha^{3.7III}$	
Thai	203 (94.9)	9 (4.2)	2 (0.9)	214
Laos	26 (100)	0	0	26

control, $-\alpha^{3.7}$, $-\alpha^{4.2}$, Hb Paksé and Hb Constant Spring mutations. The multiplex PCR reaction mixture (50 μl) contains 50–100 ng DNA, 30 pmol each of primer A1, Ek, DD, D, αG2 and αG18, 200 μM dNTPs, 0.75 M Betaine, 5% DMSO and 2 units *Taq* DNA polymerase (New England Biolabs, Beverly, MA, USA) in 12 mM Tris-HCl buffer pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin. This multiplex PCR was carried out on a Biometra T Personal Thermocycler (Biometra; GmbH Co., Ltd., Göttingen, Germany) using initial heating at 94 °C (3 min), followed by 10 cycles of [94 °C for 30 s, 57 °C for 30 s and 68 °C for 2 min] and 20 cycles of [94 °C for 30 s, 57 °C for 30 s and 68 °C for 2 min (plus 20 s in every cycle)]. PCR product was analyzed on 1.5% agarose gel electrophoresis and visualized under UV light after ethidium bromide staining.

3. Results

3.1. Proportions of $-\alpha^{3.7}$ α^+-thalassemia subtypes

Among 1192 Thai subjects investigated for α^+-thalassemia by DNA analysis, 335 (28.1%) were found to carry $-\alpha^{3.7}$ α^+-thalassemia mutation including 316 heterozygotes and 19 homozygotes. Other α^+-thalassemia identified included carriers of $-\alpha^{4.2}$ α^+-thalassemia (n = 31; 2.7%), Hb Constant Spring (n = 227; 19.5%), and Hb Paksé (n = 33; 2.8%). Of these, 214 subjects with $-\alpha^{3.7}$ α^+-thalassemia as well as 26 known cases of Laos subjects with $-\alpha^{3.7}$ α^+-thalassemia in our archive were subjected to further $-\alpha^{3.7}$ subtyping using *Apa*I restriction digestion in a PCR-RFLP assay shown in Fig. 1. As shown in Table 2, the proportions of $-\alpha^{3.7}$ subtypes in Thai were 203/214 (94.9%) for $-\alpha^{3.7I}$, 9/214 (4.2%) for $-\alpha^{3.7II}$ and 2/214 (0.9%) for $-\alpha^{3.7III}$. In contrast, all 26 Laos subjects were found to carry the $-\alpha^{3.7I}$ chromosomes. Further investigation was also done on another 77 known cases of newborns with $-\alpha^{3.7}$ α^+-thalassemia (including 65 heterozygotes and 6 homozygotes) described in our previous study [6]. It was found that all of them carried $-\alpha^{3.7}$ α^+-thalassemia subtype I ($-\alpha^{3.7I}$), the data indicating of a high prevalence of subtype I ($-\alpha^{3.7I}$) in Thai and Laos populations.

3.2. Determination of the deletion breakpoint of each $-\alpha^{3.7}$ subtype

Deletion breakpoint of each $-\alpha^{3.7}$ α^+-thalassemia was examined by DNA sequencing of the representatives for $-\alpha^{3.7I}$ (n = 4), $-\alpha^{3.7II}$ (n = 3)

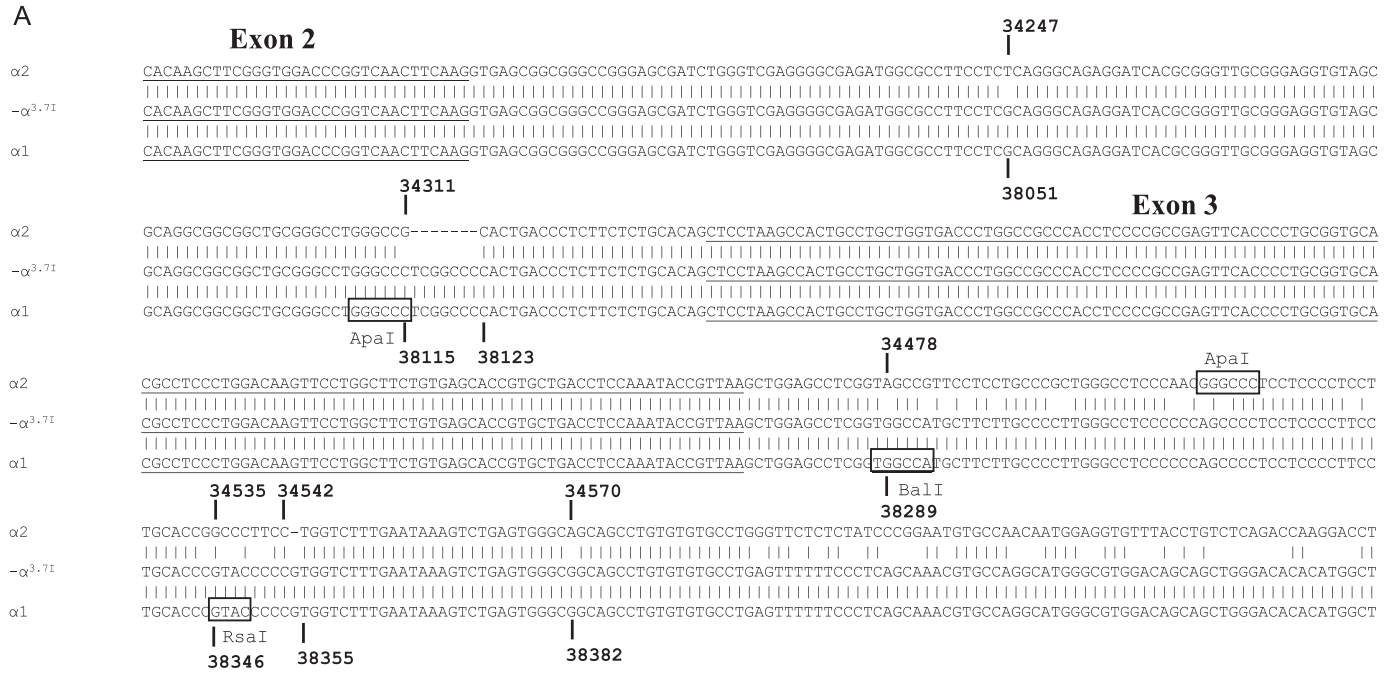

Fig. 3. Sequence comparison across the deletion junctions of the -$\alpha^{3.7I}$ (A), -$\alpha^{3.7II}$ (B) and -$\alpha^{3.7III}$ (C) with normal α1- and α2-globin genes of the NCBI reference sequence: NG_000006.1.

and -$\alpha^{3.7III}$ (n = 2). However, due to the similarity of DNA sequences of α1 and α2 globin genes, the exact deletion breakpoint of each -$\alpha^{3.7}$ subtype could not be defined for certain. As shown in the Fig. 3A, the deletion causing -$\alpha^{3.7I}$ was within the 5′ untranslated region (UTR) of α2-globin gene to IVSII#55 of α1-globin gene. For -$\alpha^{3.7II}$, the deletion started from IVSII#119 of α2-globin gene to 3′UTR + 15 of α1-globin gene (Fig. 3B). In contrast, this was found to be within 3′UTR + 79 of α2-globin gene to 3′UTR + 108 of α1-globin gene for -$\alpha^{3.7III}$ (Fig. 3C). Accordingly, the corresponding HGVS nomenclatures based on the 3'rule of HGVS guideline for these -$\alpha^{3.7}$ subtypes are as follows:

-$\alpha^{3.7I}$: NG_000006.1:g.34247_38050del

-$\alpha^{3.7II}$: NG_000006.1:g.34478_38288del

-$\alpha^{3.7III}$: NG_000006.1:g.34570_38381del

It is noteworthy that the deletions causing these -$\alpha^{3.7}$ α^{+}-thalassemia are in fact 3804–3812 bps or 3.8 kb in length rather than 3.7 kb as noted before by Southern hybridization assay [9,10].

3.3. α-Globin gene haplotypes associated with each -$\alpha^{3.7}$ subtype

α-Globin gene haplotypes based on 5 polymorphic sites mentioned in the Materials & Methods section, linked to each -$\alpha^{3.7}$ subtype in Thai population were examined and haplotypes derived from segregable

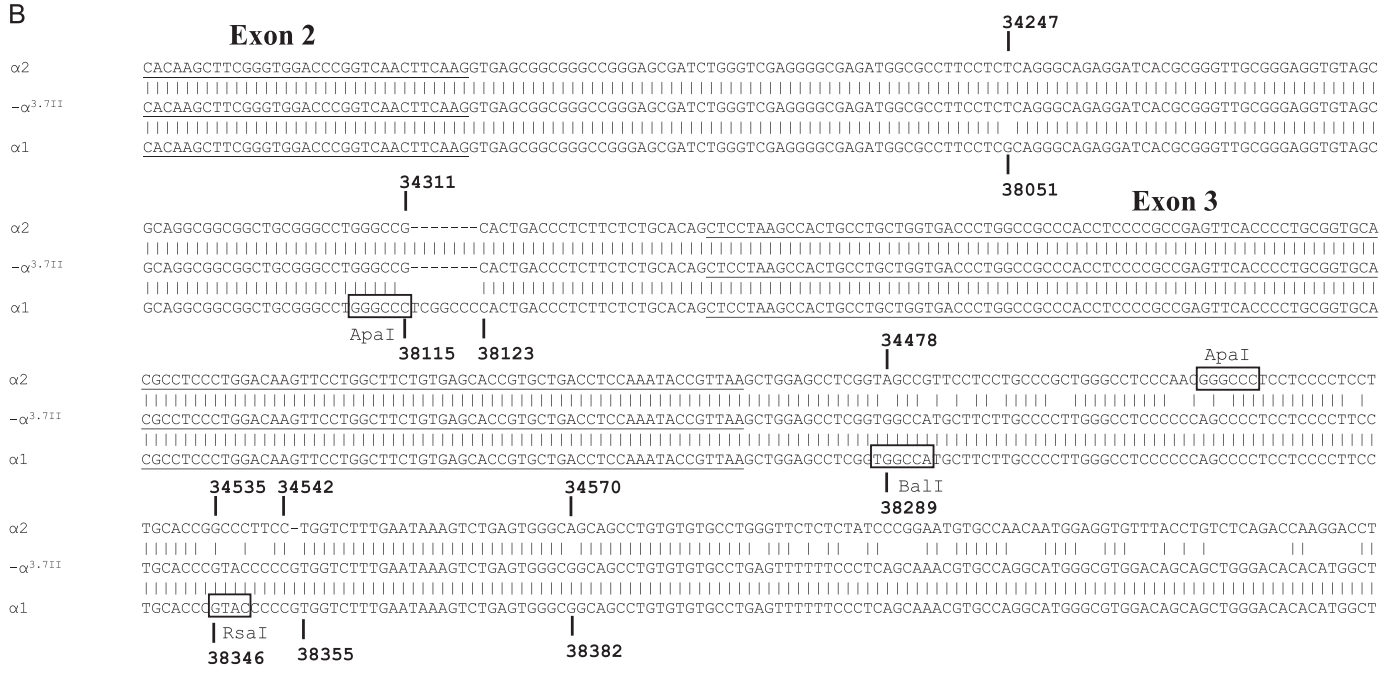

Fig. 3. (*continued*)

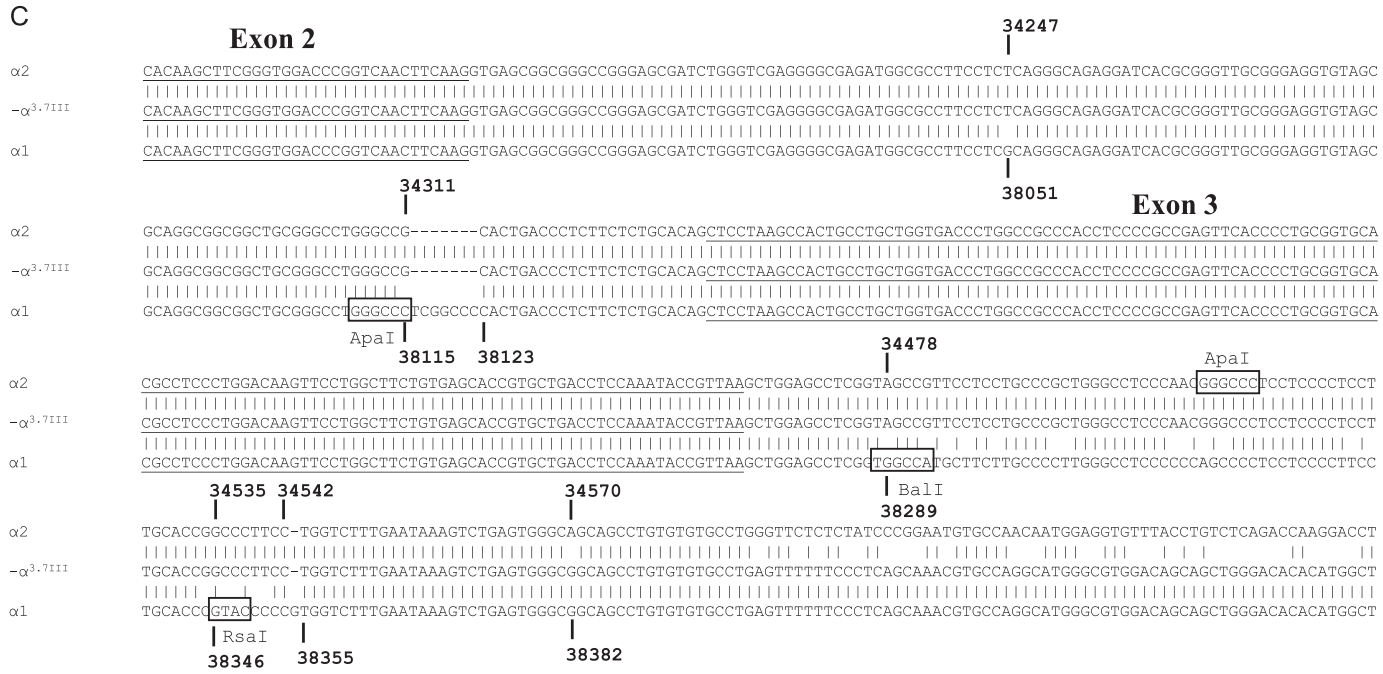

Fig. 3. (*continued*)

Table 3
The α-globin gene haplotypes linked to normal α-globin gene and -α$^{3.7}$ subtypes and among Thai population. n indicates number of chromosomes examined. + and − indicate the presence and absence of polymorphism whereas ± indicates polymorphic site that could not be segregated. 0 = deleted and nd = not done.

-α$^{3.7}$ subtype (no)	Xba I ζ2	ψζ1	Acc I ψα2	Rsa I ψα1	αPst I α2	θPst I α1	θ1	Chromosome (n)	Frequency (%)
Normal	−		+	−	−	−		32	31.7
(101)	+		+	−	−	−		29	28.7
	+		+	+	−	−		19	18.8
	−		+	+	−	−		9	8.9
	−		−	−	−	−		9	8.9
	+		−	−	−	−		2	2.0
	+		+	-	+	+		1	1.0
-α$^{3.7I}$	+		+	−	0	−		25	45.4
(55)	+		+	+	0	−		9	16.4
	+		+	−	0	+		9	16.4
	−		+	+	0	−		8	14.5
	−		+	−	0	−		3	5.5
	−		−	−	0	−		1	1.8
-α$^{3.7II}$	±		±	±	0	−		3	42.9
(7)	±		+	−	0	−		1	14.3
	+		nd	+	0	−		1	14.3
	±		+	−	0	−		1	14.3
	±		+	+	0	−		1	14.3
-α$^{3.7III}$	±		+	±	0	−		1	50.0
(2)	−		+	−	0	−		1	50.0

chromosomes of -α$^{3.7I}$ (n = 55), -α$^{3.7II}$ (n = 7) and -α$^{3.7III}$ (n = 2) as compared to 101 normal chromosomes. Results were summarized in Table 3. Multiple haplotypes were noted especially for the two most common subtypes; -α$^{3.7I}$ and -α$^{3.7II}$, the result indicating of multiple founder effects in Thai population. For the two -α$^{3.7III}$ chromosomes, although accurate haplotype could not be defined in one of them, it is most likely that both -α$^{3.7III}$ were associated with the same haplotype; (- + - 0 -).

3.4. Development of a multiplex PCR system for identification of common α^{+}-thalassemia

A multiplex PCR system for rapid identification of 4 common α^{+}-thalassemia mutations including all subtypes of -α$^{3.7}$ α^{+}-thalassemia, -α$^{4.2}$ α^{+}-thalassemia, Hb Constant Spring, and Hb Paksé was developed as shown in Fig. 2. Validation of this method was done on 102 subjects including normal individuals (n = 50), heterozygous -α$^{3.7}$ (n = 26), Hb Constant Spring carriers (α^{CS}α) (n = 16), heterozygous -α$^{4.2}$ α^{+}-thalassemia (n = 2), homozygous for -α$^{3.7}$/-α$^{3.7}$ (n = 1), and compound

heterozygous for $\alpha^{CS}\alpha/\alpha^{PS}\alpha$ ($n = 3$), $-\alpha^{3.7}/\alpha^{PS}\alpha$ ($n = 2$), $-\alpha^{3.7}/-\alpha^{4.2}$ ($n = 1$) and $-\alpha^{3.7}/\alpha^{CS}\alpha$ ($n = 1$). As compared to the conventional method performed routinely [11], a concordant result was obtained on 101 of 102 cases. In the remaining case with negative for all forms of α^+-thalassemia by conventional methods, a multiplex PCR assay alternatively identified the heterozygosity for α^+-thalassemia with $-\alpha^{3.7}$. Further $-\alpha^{3.7}$ subtyping demonstrated that this case was a carrier of $-\alpha^{3.7III}$, previously undescribed in northeast Thai population. The multiplex PCR system developed could simply differentiate heterozygosity (Fig. 2, lane 2) and homozygosity (Fig. 2, lane 6) of the $-\alpha^{3.7}$ α^+-thalassemia. The system could also be applied for prenatal diagnosis of Hb Bart's hydrops fetalis caused by homozygous α^0-thalassemia in which no amplified fragment was observed (Fig. 2, lane 8).

4. Discussion

The α^+-thalassemia is the most common single gene disorder found worldwide, especially in endemic areas of malaria [12]. Heterozygous or homozygous α^+-thalassemia are not associated with severe thalassemia disease. However, co-inheritance of α^+-thalassemia and α^0-thalassemia results in the Hb H disease with variable clinical severity and interaction of Hb H with Hb E can lead to complex αβ-thalassemia syndromes known as AEBart's and EFBart's diseases, commonly seen among Southeast Asian population [13,14]. Identification of α^+-thalassemia is therefore crucial for genetic counselling of the patients. Among α^+-thalassemia determinants, the approximately 3.7 kb deletion ($-\alpha^{3.7}$) is the most common one which can be found in various populations. We have now demonstrated that the deletion is in fact 3.8 kb in length rather than 3.7 kb defined previously by Southern hybridization and restriction mapping in which the exact deletion breakpoints could not be determined. This $-\alpha^{3.7}$ α^+-thalassemia can be further subdivided based on the deletion breakpoints due to different genetic events into 3 subtypes namely $-\alpha^{3.7I}$, $-\alpha^{3.7II}$ and $-\alpha^{3.7III}$ [9,10]. The sequence data of the $-\alpha^{3.7I}$ from Chinese has been submitted in the GenBank with an Accession no. AF525460.1. We found that the deletion causing the Thai $-\alpha^{3.7I}$ is the same as that reported for the Chinese patient. Surprisingly, the corresponding HGVS names of the $-\alpha^{3.7II}$ and the $-\alpha^{3.7III}$ have not been documented before in the databases. We have therefore updated these data from Thai patients. The sequences of the fragments containing deletion breakpoints of these $-\alpha^{3.7}$ subtypes have been submitted to GenBank (with accession numbers MK600513 for $-\alpha^{3.7I}$, MK600512 for $-\alpha^{3.7II}$ and MK600511 for $-\alpha^{3.7III}$, respectively) (March 2019).

Although α^+-thalassemia is very common in many populations, most of the studies have not subtyped the $-\alpha^{3.7}$ deletions. It is assumable that $-\alpha^{3.7I}$ is the most common one and $-\alpha^{3.7II}$ can be occasionally detected whereas $-\alpha^{3.7III}$ is rare. Study on 174 chromosomes of the $-\alpha^{3.7}$ α^+-thalassemia in Jews and Arab populations of Israel has identified 96% of $-\alpha^{3.7I}$ and 4% of $-\alpha^{3.7II}$. No $-\alpha^{3.7III}$ α^+-thalassemia was detected [15]. The predomination of the $-\alpha^{3.7I}$ were also observed in Papua New Guinean [16], Taiwanese [17] and Kampuchea [18], as well as in an indigenous Senoi Orang Asli in Malaysia [19]. The $-\alpha^{3.7III}$ is the rare subtype and has only been noted with high frequency in Micronesia and Polynesia [20]. We have demonstrated in this study that all $-\alpha^{3.7}$ subtypes could be found among northeast Thai population (Table 2). Although relatively rare, it is worth mentioning that the 2 cases with $-\alpha^{3.7III}$ were discovered for the first time in our population using the multiplex PCR assay developed. These two cases were originally misidentified by a conventional method focusing on $-\alpha^{3.7I}$ and $-\alpha^{3.7II}$ subtypes. This result indicates an inadequacy of our routine identification of the $-\alpha^{3.7}$ thalassemia determinant and points to a need of more appropriate method. A rapid PCR assay for screening of 7 deletions causing α-thalassemia including $-^{SEA}$, $-^{THAI}$, $-^{MED}$, $-^{FIL}$, $-(\alpha)^{20.5}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ has been described [21]. However, a multiplex PCR assay based on a combined gap-PCR and allele specific PCR for simultaneous detection of all common forms of α^+-thalassemia including all subtypes of $-\alpha^{3.7}$, $-\alpha^{4.2}$, Hb Constant Spring and Hb Paksé mutations shown in Fig. 2 should be best for our routine application. The system could identify simultaneously, the zygosity of $-\alpha^{3.7}$ α^+-thalassemia and provide a double-checked system for diagnosis of Hb Bart's hydrops fetalis caused by homozygous α^0-thalassemia in prenatal diagnosis [11].

Of interest is the finding of multiple haplotypes associated with $-\alpha^{3.7}$ α^+-thalassemia in Thai population, likely indicating of a multiple origin (Table 3). At least six different α-globin gene haplotypes were linked to $-\alpha^{3.7I}$ chromosome. The most common one was (*Xba*I +, *Acc*I +, *Rsa*I -, α-*Pst*I 0, θ-*Pst*I -) which is also one of the common haplotypes associated with normal α-globin gene in Thai population. As for $-\alpha^{3.7I}$, the - $\alpha^{3.7II}$ is also linked to more than one haplotype. However, it is not unexpected that the $-\alpha^{3.7III}$ is associated with a single haplotype (*Xba*I -, *Acc*I +, *Rsa*I -, α-*Pst*I 0, θ-*Pst*I -) in Thai population. Many of these haplotypes bearing $-\alpha^{3.7}$ could not easily be derived through genetic recombination events. The remarkably wide geographic distribution of the $-\alpha^{3.7}$, especially $-\alpha^{3.7I}$ and $-\alpha^{3.7II}$, and the haplotypic heterogeneity observed suggest that it probably arose on more than one occasion. In addition, these $-\alpha^{3.7}$ haplotypes observed in Thai population differ from those described in Aboriginal Australians, New Guinea Highlanders and Micronesian [20,22]. It is most likely therefore that the remarkably wide geographic distribution of the $-\alpha^{3.7}$ thalassemia in the population results from multiple founder effects. This is in contrast with the α^0-thalassemia (SEA deletion; $-^{SEA}$) in which a single genetic origin is responsible for the spread and prevalence of the ($-^{SEA}$) in the region [23]. Nonetheless, multiple genetic origins have been documented for other common hemoglobinopathies among Southeast Asian population like Hb Constant Spring [7], Hb Q-Thailand [24] and Hb E [25].

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