Homogeneity of β^0-Thalassemia Codon 17 (A→T) Alleles in Northern Thailand using a Direct DNA Sequencing Method

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The aim of this study was to characterize β-globin gene micro-haplotype polymorphisms (frameworks) associated with a β-thalassemia mutations common in Northern Thailand using a direct DNA sequencing method. A total of 11 β-thalassemia major patients homozygous for the codon 17 (A→T) mutation admitted to Chiang Mai University Hospital were examined. All 22 alleles were found to contain the Asian framework 3A. The homogeneity of the framework associated with the codon 17 (A→T) mutation indicates a relatively recent origin of the codon 17 (A→T) mutation. Similar studies in other East Asian populations may provide information concerning the origin and the migrational spread of this β-thalassemia mutation.

Keywords : *β-thalassemia, Mutation Analysis, Framework, DNA sequencing*

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The β-thalassemias are a group of single gene defects causing reduced or absent β-globin chain synthesis[1]. In Thailand, the prevalence of carriers varies from 3 to 9%[2]. Almost 200 β-thalassemia alleles have currently been characterized[3]. However, in a certain ethnic group a limited subset of mutations is found[4]. In Northern Thailand, the most common mutations are codon 41/42 (-TCTT) and codon 17 (A→T). Homozygosity for codon 17 (A→T) was found in 12.9% of a group of 109 patients with β-thalassemia major and, thus, ranged third in frequency of β-thalassemia genotypes in Northern Thailand[5].

According to Orkin et al[6] and Antonarakis et al[7] the micro-haplotype polymorphism of the β-globin gene designated as 'framework' (FW) is defined by five single nucleotide polymorphisms (SNPs). Four main frameworks are identified in Table 1.

Whereas FW1 and FW2 are common world-wide, FW3 is frequent in Africans, Europeans and West Asians and FW3A, intermediate in structure between FW 2 and FW 3, is characteristic for East Asian populations[7]. The haplotype for many β-thalassemia mutations among the Thais were previously described [8,9] and comparison with Chinese and Burmese had already appeared[10]. However, In previous studies on the fine structure of β-thalassemia chromosomes in East Asia, the framework was usually indirectly diagnosed on the basis of characteristic restriction patterns. To our knowledge, this is the first report on β-globin gene frameworks of β-thalassemia patients in Thailand using direct DNA sequencing.

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Material and Method

Patients and DNA extraction

Eleven unrelated β-thalassemia major patients with known homozygosity for the codon 17 (A→T) mutation attending the hematology clinic

Table 1. Classification of β-globin gene framework[6,7]

	codon 2	IVS-II			
	nt 3	nt 16	nt 74	nt 81	nt 666
FW 1	C	C	G	C	T
FW 2	C	C	T	C	T
FW 3A	T	G	T	C	C
FW 3	T	G	T	T	C

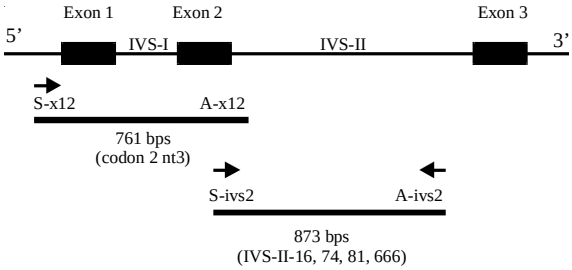
at Chiang Mai University Hospital, Chiang Mai, Thailand, were included in the study after giving informed consent. DNA was extracted from whole blood using a modified version of Chelex-100 extraction method[11] as described elsewhere[5].

Sample characterization

To determine the framework, the β-globin gene region containing the five SNPs were sequenced. Amplicons to identify codon 2 nt 3 were created using primers (S-x12 + A-x12) and PCR conditions previously reported for the detection of β-thalassemia mutations [12]. For the remaining four SNPs located in IVS-II, the amplicons were created using a new set of primer (S-ivs2 + A-ivs2) as described in Fig. 1.

The PCR for the new primers was performed in a 50 μl reaction mixture of 5 μl of genomic DNA, 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.4 μM of each primer, 1x Taq buffer, and one unit of *Taq* DNA polymerase. After the hot start, the condition was 40 cycles of 45 seconds at 94°C, 1 minute at 65°C with a step-down of 0.5°C in each cycle, and 1.30 min at 72°C. The samples were electrophoresed on 2% agarose gel to check size and quality of the PCR product and purified using the QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany) and electrophoresed to estimate the quantity of the PCR product.

The purified PCR product was used as a template for the cycle sequencing reaction. The primer S-X12 was used for codon 2 nt 3. For IVS-II-16, IVS-II-74, and IVS-II-81, the primer was S-IVS2. To obtain a reverse sequence at IVS-II-666, the primer A-IVS2 was used. The reactions were performed using BigDye Terminator Ready Reaction Mix (Applied BioSystems), precipitated with ethanol-sodium acetate, then capillary electrophoresed using an ABI PRISM 310 Genetic Analyzer (Applied BioSystems)



S-x12 5'-aga aga gcc aag gac agg tac g-3' (70399-70420)
A-x12 5'-tgc aat cat tcg tct gtt tcc c-3' (71138-71159)
S-ivs2 5'-tca cct gga caa cct caa g-3' (70955-70973)
A-ivs2 5'-taa tcc agc ctt atc cca ac-3' (71808-71827)

Fig.1 Relative location of PCR primers complementary to the β-globin gene. The primers S-x12 and A-x12 have been previously described[9]. The sequence numbers of primers in parentheses are parted of GenBank (NG_000007.3 GI: 28380636).

as described by the manufacturer. Data were manually compared with the standard sequence of the β-globin gene (GenBank NG_000007.3 GI:28380636).

Result

After three rounds of cycle sequencing for each sample (Fig. 2 and Fig. 3) and reverse base for IVS-II-666 (Fig. 4), all 22 β-thalassemia alleles were found to be associated with framework 3A.

Discussion

In previous studies of β-globin alleles an association of the frameworks with restriction sites for *Ava*II located around IVS-II-16 and for *Bam*HI located 3' to the β-globin gene was determined. The restriction patterns + +, + - and - + indicate FW1, FW2 and FW3, respectively[6,7]. However, FW3 and FW3A cannot be differentiated with this method and gene conversion may give rise to discrepancies[13].

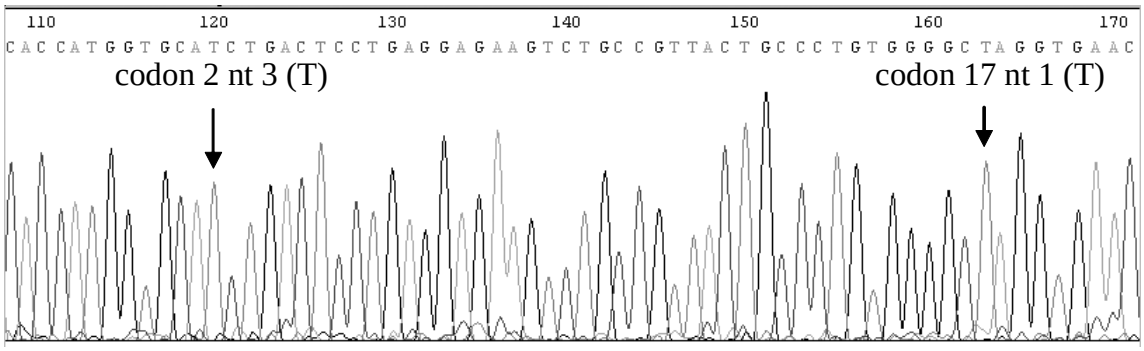


Fig. 2 Pattern of homozygosity of codon 2 nt 3 and β-thalassemia mutation codon 17(A→T) using the sequencing primer S-x12

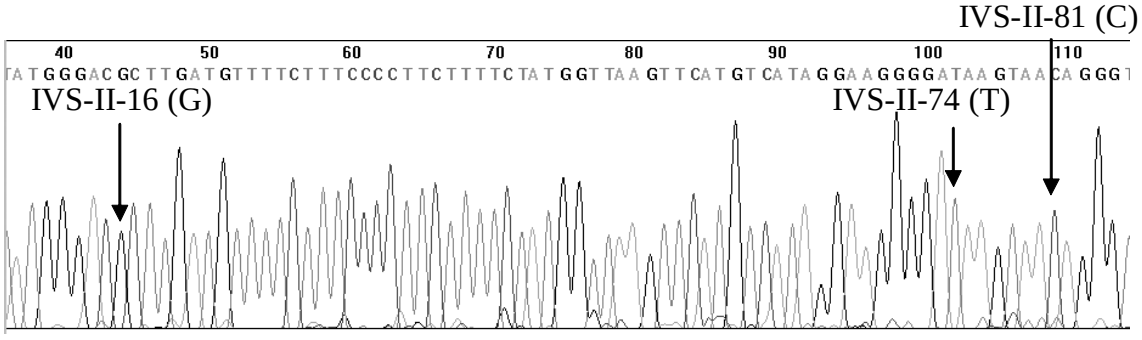


Fig. 3 Pattern of homozygosity of IVS-II-16, IVS-II-74, and IVS-II-81 using the sequencing primer S-ivs2

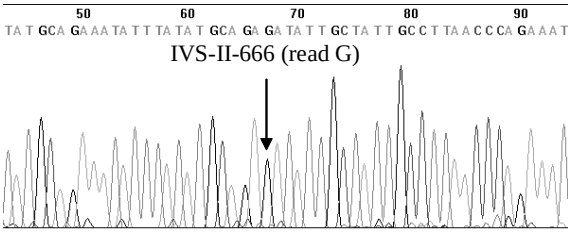


Fig. 4 Reverse sequencing pattern of homozygosity of IVS-II-666 (C) using the primer A-ivs2

Therefore, direct DNA sequencing was employed for the determination of frameworks in the present study. Another advantage of the present study is that homozygotes for the codon 17 (A→T) mutations were selected instead of the more common compound heterozygotes to avoid base errors of cloning from PCR products resulting from reduced fidelity of *Taq* DNA polymerase[14].

The mutation codon 17 (A→T) on 22 β-thalassemia chromosomes in the present study was exclusively associated with framework 3A. This confirms the results of previous reports where limited numbers of probands were examined using the restriction pattern for the diagnosis of the β-globin gene associated framework[8,9,13,15,16]. Apparent homogeneity of the mutation and the associated framework in a vast area of East Asia extending from Korea to Indonesia indicates a relatively recent single origin of the codon 17 (A→T) mutation on a FW3A chromosome. This is in contrast with the more widely distributed β-thalassemia mutation codon 41/42 (-TCTT) which is common in East and South Asian populations[1]. This mutation has been shown to be associated with different frameworks[17], a finding that argues for multiple mutation or more ancient origin with subsequent redistribution by gene conversion or point mutations. Further studies of the frameworks connected with the codon 17 (A→T) mutation employing the DNA sequencing method, especially in Chinese populations, may provide information concerning the origin and the migrational spread of this mutation in East and Southeast Asia.

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*ความเหมือนกันของอัลลีลเบต้าศูนย์ธาลัสซีเมียชนิดโคดอน 17 (A →T) ในภาคเหนือของประเทศไทย จากวิธีหาลำดับเบสโดยตรง**

พันธุ์ชนะ สงวนเสริมศรี, ดาวัลย์ ฉิมภู่, รสริน ว่องวิไลรัตน์, รัตน์ติกา แซ่ตั้ง, ต่อพงศ์ สงวนเสริมศรี

วัตถุประสงค์ของการศึกษาเพื่อหาความหลากหลายของไมโครแฮพโลไทปของเบต้าโกลบินยีน (เค้าโครงในยีน) ที่มีความสัมพันธ์กับการกลายพันธุ์ที่ทำให้เกิดโรคเบต้าธาลัสซีเมียชนิดที่พบมากในภาคเหนือของประเทศไทยโดยการใช้การหาลำดับเบสโดยตรง จากคนไข้โฮโมไซกัสเบต้าธาลัสซีเมียที่มีการกลายพันธุ์ที่โคดอน 17 (A → T) จำนวน 11 คนที่เข้ารับการรักษาที่โรงพยาบาลมหาราชนครเชียงใหม่ พบว่าทั้ง 22 อัลลีลจัดอยู่ในเค้าโครงที่ 3 แบบเอเชีย การพบความเหมือนกันของเค้าโครงที่มีความสัมพันธ์กับการกลายพันธุ์ที่โคดอน 17 (A → T) บ่งชี้ต้นกำเนิดที่ค่อนข้างใหม่ของการกลายพันธุ์ การศึกษาเช่นนี้ในเอเชียตะวันออกอาจให้ข้อมูลเกี่ยวกับกำเนิดและการแพร่กระจายของการกลายพันธุ์ของเบต้าธาลัสซีเมียชนิดนี้