The prevalence of alpha-thalassemia amongst Tai and Mon-Khmer ethnic groups residing in northern Thailand: A population-based study

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Background: Northern Thailand is one of the highest α-thalassemia incidence areas where 30–40% of inhabitants have been reported to carry aberrant α-globin genes. However, all previous α-thalassemia prevalence surveys in northern Thailand have been undertaken without consideration of ethnicity. Here we report the prevalence of α-thalassemia genes in 4 Tai (Yong, Yuan, Khuen, Lue) and 4 Mon-Khmer speaking populations (Blang, Mon, Paluang, Lawa).

Methods: DNA extracted from 141 individuals was genotyped for 4 α-thalassemia deletional types (−SEA, −THAI, −α^3.7, −α^4.2) using MultiplexGap-PCR analysis and 2 non-deletional types (Hb CS, Hb Pakse) using dot-blot hybridization technique.

Results and discussion: A total of 33 α-thalassemia carrying individuals (23.4%) were detected of which 32 were heterozygotes and one was a homozygote. The most common α-thalassemia detected were −α^3.7 (17.7%) and −−SEA (3.5%), while Hb CS was detected in 2.1% of cases. No occurrence of −−THAI, −−α^4.2 and Hb Pakse was observed. The prevalence of α-thalassemia carriers varied between the different ethnic groups, with the Yuan having the highest prevalence of α-thalassemia carriers (50%) while the Lawa had the lowest prevalence (0%). The Paluang had a high prevalence (42%) of a single deletion type (−α^3.7) possibly related to the endogamous marriage traditions of this ethnic group.

Conclusion: The extreme variation of α-thalassemia prevalence among the different ethnic groups highlights the significantly different genetic backgrounds found in these peoples, as consequences of dissimilar cultures. Our study suggests that ethnicity must be considered in any of the disease-causing allele prevalence surveys in this region.

Keywords: Alpha-thalassemia, Northern Thailand, Ethnic group, Multiplex-Gap-PCR, Dot-blot hybridization

Introduction

Alpha (α)-thalassemia is one of the most common hereditary blood disorders worldwide with a high prevalence in sub-tropical and tropical regions including the countries of Southeast Asia. In Thailand, and particularly in the northern part of the country, there is a high incidence of α-thalassemia and the increase in α-thalassemia carriers continues to pose a significant public health concern. Alpha-thalassemia arises from defects of the α-globin genes, located on chromosome 16 which control the production of the α-globin chain, therefore, the reduced amount of α-globin chains resulting from aberrant α-globin genes, leads to a reduction of hemoglobin in red blood cells, anemia and other additional complications.

α-thalassemia is inherited as an autosomal recessive trait with a varying presentation ranging from essentially asymptomatic in heterozygote carriers to the premature death of infants. The degree of severity of presentation depends on the number of α-globin genes affected. Under normal conditions there are two α-globin genes located on each of the chromosome 16. A deletion of one of these four α-globin genes is designated as α-thalassemia^2, while two aberrant α-globin genes is designated as α-thalassemia^1. The most common forms of α-thalassemia^1, reported in Southeast Asia region particularly in Thailand, are the South East Asia (−−SEA) and THAI (−−THAI).
deletions, while the most common α-thalassemia2 deletions are -α3.7 and -α4.2.4,5

Although there are no overt clinical symptom associated with the inheritance of one or two aberrant α-globin genes, three aberrant α-globin genes results in hemoglobin H disease whose symptom can be moderate, and blood transfusion or iron chelation therapy are only occasionally required.3 The most severe form of α-thalassemia is characterized by the deletion of all four α-globin genes which results in Hemoglobin Bart's hydrops fetalis syndrome and affected infants usually die in utero.6 In addition to the deletional α-thalassemias, there are some non-deletional α-thalassemia caused by base substitutions. In Southeast Asian populations, hemoglobin Constant Spring (Hb CS) and hemoglobin Pakse (Hb Pakse) point mutations have been observed. The incidence of those two mutational types varies among populations, however, Hb CS is more common in Thailand than Hb Pakse.4,7

In general, the pathophysiology of deletional α-thalassemia is primarily caused by the relative excess of β-globin chains (β4 tetramer) rather than the under production of α-globin chains. In non-deletional α-thalassemia, although less frequently observed as compared to the deletional type, the pathophysiology might be associated with the instability of α-globin mRNA due to mutations in the stop codon or polyadenylation site leading to the synthesis of unstable α-globin chains. Furthermore, the resulting phenotypes arising from interactions between deletional and non-deletional α-thalassemia for example, Hemoglobin H-Constant Spring, can give rise to more severe phenotypes.8,8

Previous studies have reported that 20–30% of the Thai population carry an α-thalassemia gene. Interestingly, almost one half (40%) live in the northern part of Thailand.9,10 Couples living in such areas have high chances to give birth to an α-thalassemia child, either as a carrier or with overt α-thalassemia disease. The acquisition of accurate data of α-thalassemia prevalence in northern Thailand is therefore necessary for implementing appropriate genetic counseling programs. However, in most of the previous surveys of α-thalassemia incidence, data was derived only from couples participating in hereditary hematologic disease screening, no data on ethnicity was reported.11–13 Recently, clear genetic differences between ethnic populations belong to two major linguistic groups, the Tai and the Mon-Khmer, of northern Thailand had been revealed.14,15 Therefore, pooled sampling population data might not accurately represents the prevalence of α-thalassemia in this area. Thus, this study proposed to comprehensively survey the prevalence of α-thalassemia genes among different ethnicities living in northern Thailand.

Materials and methods

Population study and DNA extraction
One hundred and forty-one samples, belong to eight ethnic populations, were selected as the study population (Table 1). The criteria for population sampling were described elsewhere.16 Subjects were selected from villages with well-documented ethnic history. Volunteers were over 20-years-old, unrelated, healthy, and were members of the majority ethnic group with no admixture from another group for at least three generations. Personal details were collected using form-based oral interviews for ethnicity, cultural aspect, and migration history. Total genomic DNA was extracted from peripheral blood after informed consent, using an inorganic salting out protocol.17 Quality and quantity of extracted genomic DNA were checked by 1% agarose gel electrophoresis and spectrophotometry, respectively. DNA samples were kept at −20°C until use.

Molecular analysis of α-globin gene deletion using MultiplexGap-PCR analysis
Four common deletional types of α-thalassemia i.e.,-α3.7, -α4.2, -SEA and -THAI, were genotyped by

| Linguistic group | Ethnicity | Location (district, province) | No. of samples |
|-----------------|-----------|------------------------------|---------------|
| Tai             | Yong      | Pa Sang, Lamphun             | 20            |
|                 | Yuan      | San Sai, Chiang Mai          | 7             |
|                 |           | Mea Taeng, Chiang Mai        | 9             |
|                 |           | Ban Hong, Lamphun            | 2             |
|                 | Lue       | Doi Sa Kel, Chiang Mai       | 18            |
|                 |           | Pua, Nan                     | 1             |
|                 | Khuen     | Mae Wang, Chiang Mai         | 12            |
|                 |           | San Pa Tong, Chiang Mai      | 6             |
| Mon-Khmer       | Blang     | Mae Chan, Chiang Rai         | 12            |
|                 | Mon       | Mae Sai, Chiang Rai          | 8             |
|                 | Paluang   | Pa Sang, Lamphun             | 9             |
|                 | Lawa      | Mae La Noi, Mae Hong Son     | 18            |
|                 |           | Total                        | 141           |
Multiplex-Gap Polymerase Chain Reaction (PCR) modified from Chong et al.\textsuperscript{18} A total of nine primers, as previously reported, namely α2/3.7-F, 3.7-R, α2-R, 4.2-F, 4.2-R, SEA-F, SEA-R, THAI-F, and THAI-R were used in a single tube reaction.\textsuperscript{18} The amplification was undertaken with an initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 98°C for 45 seconds, then annealing at 60°C for 90 seconds and extension at 72°C for 135 seconds, and another 5 minutes at 72°C for final extension. The PCR products were then analyzed for α-globin gene deletions as compared to positive controls by electrophoresis through 1.5% agarose gels.

**Molecular analysis of α-globin gene mutation using Dot-Blot Hybridization**

Genotypic analysis of two non-deletion types of α-thalassemia, namely Hb CS and Hb Pakse, was performed using dot-blot hybridization following the protocol of Pichanun et al.\textsuperscript{19} Briefly, target PCR products were amplified using primers αF (5’-GCC GCA CTG ACC CTC TTC TCT G-3’) and α2R (5’-GTC CTT GGT CTG AGA CAG GTA A-3’) and following amplification products were denatured, spotted onto nylon membranes and then hybridized with specific normal and mutant probes. Positive controls of normal and previously characterized Hb CS homozygote and Hb Pakse homozygote samples were simultaneously analyzed.\textsuperscript{18-20} A positive signal shows as a blue color spot generated from the enzyme substrate reaction.

**Results**

DNA from a total of 141 individuals belonging to two major linguistic groups, the Tai and the Mon-Khmer of northern Thailand, was genotyped for four α-thalassemia deletional types (--SEA, --THAI, --α3.7, --α4.2) using a MultiplexGap-PCR analysis (Fig. 1) and two α-thalassemia non-deletional types (Hb CS, Hb Pakse) using a dot-blot hybridization methodology (Fig. 2). Among the six types of α-thalassemia surveyed, only three types i.e., --α3.7, --SEA, and Hb CS were observed within eight ethnic groups (Table 2). The most prevalent form detected was --α3.7 (25/33, 75.8%). The deletional --SEA and Hb CS mutation were observed at 15.2% and 9.1%, respectively. The total prevalence of α-thalassemia genes in the surveyed

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**Figure 1** PCR products of α-globin gene deletions determined using a MultiplexGap-PCR methodology, lane 1 = DNA marker, lane 2–5 = positive controls genotypically known as four deletional types of α-thalassemia heterozygotes which are --SEA/αα (1349/1800 bp), --THAI/αα (1153/1800 bp), --α3.7/αα (2022/1800 bp) and --α4.2/αα (1628/1800 bp), respectively, lane 6–9 = unknown samples from the Yong and lane 10–13, 15, 16 = unknown samples from the Yuan. The result showed that 10 of 11 unknown samples were genotyped as normal (αα/αα; 1800/1800 bp) while TY169 (lane 9) was genotyped as heterozygote for SEA type (--SEA/αα; 1349/1800 bp)
populations was 23.4% (33/141) which varied from the highest in the (Tai) Yuan (50%, 9/18) to the lowest in the (Mon-Khmer) Lawa (0%) (Table 2).

Comparison of $\alpha$-thalassemia prevalence between the Tai and Mon-Khmer linguistic groups (Fig. 3) showed that the Tai group (comprising the Yong, the Yuan, the Lue, and the Khuen) showed a higher incidence of $\alpha$-thalassemia (21/75, 28.0%) than the Mon-Khmer (comprising the Blang, the Mon, the Paluang, and the Lawa) group (12/66, 18.2%). Three aberrant $\alpha$-globin alleles, $\alpha^+3.7$, --SEA, and Hb CS, were observed in the Tai, while only $\alpha^+3.7$ and --SEA were found in the Mon-Khmer linguistic group.

### Discussion

This study is the first report of $\alpha$-thalassemia prevalence among the ethnic groups residing in northern Thailand using MultiplexGap-PCR and dot-blot hybridization techniques. The observed prevalence of $\alpha$-thalassemia in this study (23.4%) is lower than previous reports.\textsuperscript{12,21} This is caused by the low incidence of $\alpha$-thalassemia genes in some ethnic groups for instance, the Khuen, the Blang, and the Lawa, leading to a lower overall average as compared to previous studies. The extreme variation of $\alpha$-thalassemia prevalence (from 0 to 50%) between the different ethnic groups of northern Thailand highlights the significantly different genetic backgrounds found in these groups. As a consequence, surveys for $\alpha$-thalassemia undertaken in the population of northern Thailand that do not take into account ethnicity may not be an appropriate methodology for estimating the incidence of disease-causing genes in this region.

The most prevalent $\alpha$-thalassemia gene observed in this study was the $\alpha^+3.7$ deletion. This is consistent with the previous study which reported that $\alpha^+3.7$ and --SEA are the most prevalent forms of aberrant $\alpha$-globin genes found in Chiang Mai, in the upper northern part of Thailand.\textsuperscript{12} However, the prevalence of $\alpha^+3.7$ is usually more common than --SEA.\textsuperscript{4,22} This differential prevalence of the $\alpha^+3.7$ and --SEA alleles observed in this study might be explained by the underlying genetic mutation in the $\alpha$-globin gene found within these ethnic populations. Additionally, the prevalence of non-deletional type of $\alpha$-thalassemia genes Hb CS and Hb Pakse appeared to be lower than the $\alpha^+3.7$ and --SEA deletions (2.1% and 0%, respectively) and the prevalence of these two non-deletional types is even lower as compared to a recent report showing the incidence of Hb CS and Hb Pakse in Phayao population as 10.5 and 0.31%, respectively, again indicating an underlying effect of ethnicity.\textsuperscript{23} Nevertheless, consistent with other studies, a relatively low incidence of Hb Pakse has been reported in the population of northern Thailand.\textsuperscript{24,25}

### Table 2: Type of $\alpha$-thalassemia observed in eight ethnic groups from northern Thailand

| Linguistic group | Ethnicity | Number of samples | Normal |
|------------------|----------|-------------------|--------|
|                  |          |                   | $\alpha^+3.7$ | $\alpha^+4.2$ | --SEA | --THAI | Constant spring | Pakse | %** |
| Tai              | Yong     | 20                | 16       | 2* | 1 | 1 | 1 | 20 |
|                  | Yuan     | 18                | 9        | 6 | 1 | 1 | 2 | 50 |
|                  | Lue      | 19                | 13       | 4 | 2 | 1 | 2 | 32 |
|                  | Khuen    | 18                | 16       | 2 | 1 | 1 | 1 | 11 |
|                   | Blang    | 20                | 18       | 1 | 1 | 1 | 1 | 10 |
| Mon-Khmer        | Mon      | 9                 | 7        | 2 | 1 | 2 | 2 | 10 |
|                  | Paluang  | 19                | 11       | 8 | 1 | 1 | 2 | 22 |
|                  | Lawa     | 18                | 18       | 0 | 0 | 0 | 0 | 10 |
|                  | Total    | 141               | 108      | 25 | 0 | 5 | 0 | 3 | 0 |

* One sample was genotyped as a $\alpha^+3.7$ heterozygote and another was a $\alpha^+3.7$ homozygote.
** The percentage of alpha-thalassemia gene prevalence.
Among the eight ethnic groups studied, the Yuan showed the highest incidence of all six α-globin gene anomalies (50%). The Yuan or Khon Muang is known to be a conglomerate population who tend to marry with different ethnic groups for political and commercial purposes. The Yuan genetic admixture scenario may have led to the various forms of α-thalassemia in the present day population. However, the Palaung who practice endogamous marriage also exhibited a high prevalence of aberrant α-globin genes (42.1%), but only α3.7 alleles were found. It is likely that the α3.7 deletion has accumulated over generations as a consequence of their close intra-relationships, and this has additionally kept other forms of aberrant α-globin genes from being introduced into the Palaung population.

Other forms of deletional α-globin alleles, such as α3.2 and THAI, have been reported in a number of studies in Southeast Asia, but with lower frequencies than the α3.7 and SEA deletions. Although the α3.2 allele has been reported in 0.9% of the northern Thai population it was not observed in our study targeted to particular ethnic groups. The extremely low reported incidence of THAI allele in the northern Thai population is consistent with our current study.

The increase of sampling area involved with other ethnic populations residing in northern Thailand will facilitate a more comprehensive analysis on α-thalassemia gene prevalence and particularly on the complex relationship between α-thalassemia prevalence and ethnicity. Moreover, the data from this study highlight the importance of the consideration of ethnicity when any genetic disorders of interest are to be screened in the population residing in northern Thailand or elsewhere associated with a variety of ethnic populations. This finding also encourages the revision of population screening strategy in northern Thailand for future national population screening for better accuracy.

Disclaimer statements
Contributors PL and JK conceived of the study and coordination, collected samples, generated and analyzed data, and drafted the manuscript. PK and SS participated in laboratory work and data generating. DRS interpreted of data and helped to draft the manuscript. SF and DK involved in revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Funding This work was supported by CMU Junior Research Fellowship program (2013).

Conflicts of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Ethics approval The article has not involved in any clinical research and experiments on volunteer. Ethical Board approval and informed consents are described in the Pan-Asian SNPs consortium’s publication.

Acknowledgments
The authors wish to thank all volunteers and village chiefs for their participation. We are also grateful to Methi Wathikthinnakorn for his technical assistance.

This work was supported by CMU Junior Research Fellowship program.

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