Molecular Analysis of Non-Transfusion Dependent Thalassemia Associated with Hemoglobin E-β-Thalassemia Disease without α-Thalassemia

Paramee Phanrahan1,2, Supawadee Yamsri2, Nattiya Teawtrakul3, Goonnapa Fucharoen2, Kanokwan Sanchaisuriya2 and Supan Fucharoen2.

1 Medical Science Program, Graduate School, Khon Kaen University.
2 Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University.
3 Department of Internal Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

Competing interests: The authors have declared that no competing interests exist.

Abstract. Background: The finding of many Thai Hb E-β0-thalassemia patients with non-transfusion dependent thalassemia (NTDT) phenotype without co-inheritance of α-thalassemia has prompted us to investigate the existence of other genetic modifying factors.

Methods: Study was done on 122 adult Thai patients with NTDT Hb E-β-thalassemia patients without co-inheritance of α-thalassemia. Multiple single-nucleotide polymorphisms (SNPs) associated with γ-globin gene expression including the Gγ-XmnI of HBG2 gene, rs2297339, rs4895441, and rs9399137 of the HBS1L-MYB gene, rs4671393 in the BCL11A gene, and G176AfsX179, T334R, R238H and -154 (C-T) in the KLF1 gene were investigated using PCR and related techniques.

Results: Heterozygous and homozygous for Gγ-XmnI of HBG2 gene were detected at 70.5% and 7.4%, respectively. Further DNA analysis identified the rs2297339 (C-T), rs4895441 (A-G), and rs9399137 (T-C) of HBS1L-MYB gene in 86.9%, 25.4%, and 23.0%, respectively. The rs4671393 (G-A) of the BCL11A gene was found at 31.2%. For the KLF1 gene, only T334R was detected at 9.0%.

Conclusions: It was found that these SNPs, when analyzed in combination, could explain the mild phenotypic expression of all cases. These results underline the importance of these informative SNPs on phenotypic expression of Hb E-β-thalassemia patients.

Keywords: Non-transfusion dependent thalassemia; HBS1L-MYB gene; BCL11A gene; KLF1 gene; Gγ-XmnI polymorphism.

Citation: Phanrahan P., Yamsri S., Teawtrakul N., Fucharoen G., Sanchaisuriya K., Fucharoen S. Molecular analysis of non-transfusion dependent thalassemia associated with hemoglobin E-β-thalassemia disease without α-thalassemia. Mediterr J Hematol Infect Dis 2019, 11(1): e2019038, DOI: http://dx.doi.org/10.4084/MJHID.2019.038

Published: July 1, 2019 Received: February 12, 2019 Accepted: May 17, 2019

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by-nc/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Correspondence to: Dr. Supan Fucharoen. Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand 40002. Tel/Fax +66-43-202083. E-mail: supan@kku.ac.th

Introduction. Thalassemia is one of the most common genetic disorders worldwide, especially in Southeast Asia. Thalassemia results from reduction or absence of globin chain synthesis. Two main types divided by defected globin chains are α-thalassemia and β-thalassemia. On the other hand, it can be divided based on blood transfusion requirement into transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT). The most common thalassemia disease found in northeast Thailand is...
hemoglobin E-β-thalassemia (Hb E-β-thal). It has been shown that clinical severity of this disease is variable, ranging from mild to severe transfusion-dependent thalassemia. Patients with transfusion-dependent Hb E-β-thal disease require lifelong regular blood transfusion for survival, while NTDT patients generally have mild anemia and do not require regular blood transfusion for survival. However, several severe complications in NTDT have been noted including chronic hypoxia, pulmonary hypertension, and thromboembolic events. Understanding of molecular features and accurate prediction of NTDT are therefore essential to reduce the morbidity of the patients. Studies have shown that type of β-thalassemia mutation alone is not enough to predict the clinical phenotype of the patients, and many patients with Hb E-β'-thalassemia are associated with NTDT phenotype. This indicates that other genetic factors might be involved in the clinical expression of the patients. These include a coinheritance of α-thalassemia or the presence of genetic factors associated with increased production of γ-globin chains for Hb F. It has been shown that at least three major loci regulate this level of Hb F: HBG2 gene (C-Xmn1 polymorphism), HBS1L-MYB intergenic region and BCL11A gene. Polymorphisms on these three loci were found to be responsible for Hb F variation in patients with homozygous Hb E, β-thalassemia or sickle cell disease and in healthy Europeans.

Preliminary study on subjects with a mild form of thalassemia encountered among couples at risk of having fetuses with thalassemia diseases in northeast Thailand has been carried out. The result indicated that four informative SNPs, including rs7482144 in HBG2 gene and rs2297339, rs4895441 & rs9399137 of HBS1L-MYB gene were associated with high Hb F levels in the patients. Further studies on homozygous Hb E identified the rs11886868 additionally in the BCL11A gene and 4 SNPs in the Krüppel-like factor 1 (KLF1) gene (G176Asx179, T334R, -154 (C-T) and R328H) to be associated with high Hb F level in homozygous Hb E. It is likely therefore that these informative SNPs might be important genetic modifying factors among NTDT- Hb E-β0-thal patients. However, data on these SNPs among clinically well-defined cases of NTDT with Hb E-β-thal patients in northeast Thailand is relatively limited. It has been known that co-inheritance of α-thalassemia is associated with a mild phenotype of the Hb E-β-thal disease. However, we have demonstrated previously that among Hb E-β0-thal patients associated with NTDT phenotypes, co-inheritance of α-thalassemia could explain the phenotypic expression only in a few cases. We report in this study, the existence of several genetic modifying SNPs in the HBS1L-MYB, BCL11A, and KLF1 genes among 122 clinically well-defined NTDT Hb E-β-thal patients in northeast Thailand.

Materials and Methods.

Specimens. Ethical approval of the study protocol was obtained from the Institutional Review Board of the Khon Kaen University, Khon Kaen, Thailand (HE561018). Archival DNA specimens were obtained from NTDT Hb E-β-thal patients of our previous study. Altogether, specimens of 122 patients with complete hematological data were obtained. All of them enrolled in the project “Epidemiologic study of major complications in adolescence and adult patients with thalassemia in northeast Thailand: the E-SAAN study” conducted at Srinagarind Hospital, Khon Kaen University, Khon Kaen Hospital, Mahasarakham hospital, and Udonthani hospital, all located in northeast Thailand, from October 2012 to June 2014. Inclusion criteria were an age of > 10 years and a diagnosis of thalassemia based on clinical symptoms, e.g., anemia, pallor, hepatosplenomegaly, jaundice, skeleton changes, growth and development deficiency, and a Hb levels of 6.0-10.0 g/dl, Hb and DNA analysis. Cases with abnormal Hb, iron deficiency anemia, and other causes of anemia were excluded.

Hematological and DNA analyses. Hematological parameters were recorded at steady state (no blood transfusion and no fever) using automated blood cell counter (Beckman Coulter Co., Fullerton, California, USA). Hb analysis was done using capillary electrophoresis (Capillary 2; Sebia, Lisses, France) or high-performance liquid chromatography (Variant II, Bio-Rad Laboratories, Hercules, California, USA). Identification of β-thalassemia and the Hb E mutations found in Thailand was performed in our laboratory using allele-specific PCR assays and DNA sequencing. Identification of α'-thalassemia (SEA and THAI deletions), α'-thalassemia (3.7 and 4.2 kb deletions), Hb Constant Spring and Hb Pakse genes are routinely performed in our laboratory using multiplex gap PCR and allele-specific PCR.

SNP Genotyping. Four KLF1 SNPs including G176Asx179, -154 (C-T), T334R and R328H were determined using allele-specific PCR assays and DNA sequencing as described. Representative gel electrophoresis of these SNPs genotyping was shown in Figure 1. The rs4895441 (G-A) and rs9399137 (T-C) of HBS1L-MYB gene and rs4671393 (A-G) of BCL11A gene were determined using high resolution melting (HRM) analysis on an Illumina Eco Real-Time PCR System (Illumina, CA, USA). Primers G166 (5’ CACACACCTCCAGGAGGCACG 3’) and G167 (5’ GGAGGCGGGGAACTCTTAAAT 3’) were used to produce an 84 bp fragment for detection of rs4671393 (A-G) of BCL11A gene. The rs4895441 (G-A) of

www.mjhid.org Mediterra J Hematol Infect Dis 2019; 11; e2019038
Figure 1. Representative agarose gel electrophoresis for identification of four KLF1 SNPs using allele specific PCR assays including the G176AfsX179 (A), -154 (C-T) and T334R (B), and R328H (C).

Figure 2. The temperature shifted curves and difference curves of the three HRM assays for identification of rs4671393 (G-A) in the BCL11A gene and rs4895441 (A-G) & rs9399137 (T-C) of the HBS1L-MYB gene.

HBS1L-MYB intergenic region was determined on a 157 bp fragment generated using primers G156 (5’ GGGGTAAGAGGAACCCAG 3’) and G157 (5’ TCTGAGGGCTTCGAACCTTA 3’). The rs9399137 (T-C) of HBS1L-MYB intergenic region was detected on a 136 bp fragment produced by primers G158 (5’ TCACCTTAAAAGGCGGTATTG 3’) and G159 (5’ TCAGAACTTATCCAAAGATTTAAC 3’). Representative temperature shifted curves, and corresponding difference curves of these HRM assays were demonstrated in Figure 2. Identification of the Gγ-XmnI of HBG2 gene and rs2297339 (C-T) of the HBS1L-MYB gene was done using PCR-restriction fragment length polymorphism (PCR-RFLP) assay as described.8,9

Statistical analysis. The STATA statistical software version 10.0 (StataCorp, Tx, USA.) was used for data analyses. Descriptive statistics, mean and standard deviation, were used to describe all continuous variables, including red blood cell indices and Hb F levels. Multiple regression analysis was applied to demonstrate the effect of various SNPs on Hb F levels. Statistical significance was set at P < 0.05.

Results. Table 1 listed the globin genotypes and associated hematological data of 122 patients studied. Most of them carried β0-thalassemia in trans to the βE globin gene (n = 119). The remaining 3 of them carried the β+-thalassemia mutation with the β-28 mutation. Similar hematological findings between groups with different mutations were observed, but variability in Hb F was noted. Table 2 summarized the frequencies of 9 SNPs of the 4 genes observed among 122 NTDT patients with Hb E-β-thalassemia. These included Gγ-XmnI of the HBG2, G176AfsX179, T334R, -154 (C-T) and R328H of KLF1 gene, rs11886868 of BCL11A gene and rs4895441, rs9399137 and rs2297339 of the HBS1L-MYB. As shown in the table, heterozygosity (+/-) and homozygosity (+/+ for Gγ-XmnI polymorphism of the HBG2 were detected in 86 (70.5%) and 9 (7.4%) cases, respectively.
Table 1 Globin genotypes and associated hematological parameters of 122 NTDT subjects with Hb E-β-thalassemia.

| Globin genotype | No. | RBC (x10¹²) | Hb (g/dL) | Hct (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) | RDW (%) | Hb F (%) |
|-----------------|-----|-------------|-----------|---------|----------|---------|-----------|--------|---------|
| β41/42 / βE     | 69  | 3.2±0.4   | 7.0±1.2  | 23.5±2.9| 73.6±8.0| 22.4±2.4| 30.5±2.0| 29.9±4.4| 27.6±14.3|
| β17 / βE        | 25  | 3.4±0.6   | 7.4±1.0  | 24.2±3.2| 71.7±7.9| 21.8±2.1| 30.6±1.9| 30.1±3.5| 30.0±12.3|
| β71/72 / βE     | 9   | 3.0±0.3   | 6.9±0.8  | 23.6±1.8| 78.5±4.5| 20.4±7.0| 29.3±2.8| 28.6±6.4| 16.1±10.4|
| βIVSII#654 / βE| 6   | 2.9±0.6   | 6.7±1.0  | 21.3±3.8| 75.1±7.5| 23.6±2.6| 31.4±1.8| 26.7±5.0| 23.0±12.6|
| βIVSII#1 / βE  | 5   | 3.7±0.5   | 8.0±0.9  | 26.1±2.7| 72.3±11.5| 22.1±3.4| 30.6±2.7| 30.9±5.4| 29.6±19.1|
| βIVSII#5 / βE  | 4   | 3.2±0.4   | 7.1±1.2  | 23.3±4.2| 74.4±14.9| 22.6±3.6| 30.6±1.7| 31.3±7.9| 10.5±6.3|
| β-28 / βE      | 3   | 4.3±0.7   | 8.4±0.6  | 26.5±1.8| 61.9±7.1| 19.7±2.3| 31.9±0.1| 25.5±0.7| 17.8±6.6|
| β26 / βE       | 1   | 3.1       | 7.8      | 26.5    | 86.1     | 25.4    | 29.5     | 25.6    | 32.8     |

Table 2 The proportions of SNPs in HBG2, KLF1, BCL11A and HBS1L-MYB genes observed among 122 Thai NTDT patients.

| Gene          | SNPs                  | Genotype   | N   | %    |
|---------------|-----------------------|------------|-----|------|
| HBG2 Gγ-XmnI  | /-                    | 27         | 22.1| 70.5 |
|               | +/-                   | 86         | 0   | 0    |
|               | +/+                   | 9          | 7.4 |      |
| G176AfsX179   | Wt/Wt                 | 122        | 100 |      |
|               | Wt/7bp                | 0          | 0   |      |
|               | +7bp/+7bp             | 0          | 0   |      |
| T334R         | Wt/T334R              | 111        | 91.0| 9.0  |
| -154 (C-T)    | Wt/-154 (C-T)         | 122        | 100 |      |
| R328H         | Wt/R328H              | 122        | 100 |      |

BCL11A rs4671393 (G-A)

| Genotype | N   | %    |
|-----------|-----|------|
| GG        | 84  | 68.8 |
| GA        | 35  | 28.7 |
| AA        | 3   | 2.5  |

BCL11A rs4895441 (A-G)

| Genotype | N   | %    |
|-----------|-----|------|
| AA        | 91  | 74.6 |
| AG        | 30  | 24.6 |
| GG        | 1   | 0.8  |

BCL11A rs9399137 (T-C)

| Genotype | N   | %    |
|-----------|-----|------|
| TT        | 94  | 77.0 |
| TC        | 28  | 23.0 |
| CC        | 0   | 0    |

BCL11A rs2297339 (C-T)

| Genotype | N   | %    |
|-----------|-----|------|
| CC        | 16  | 13.1 |
| CT        | 60  | 49.2 |
| TT        | 46  | 37.7 |

Wt: Wild type

Among 4 SNPs of the KLF1 gene examined, including the G176AfsX179, T334R, -154 (C-T) and R238H, only T334R was detected. While no R328H, -154 (C-T) and G176AfsX179 was observed, heterozygosity for the T334R was identified in 11 (9.0%) of 122 cases. In contrast, a relatively higher proportion of the rs4671393 (G-A) of the BCL11A, i.e., GG, GA, and AA varieties were detected in 84 (68.8%), 35 (28.7%) and 3 (2.5%) cases, respectively.

For the HBS1L-MYB gene, the proportions of AA, AG and GG of the rs4895441 (A-G) were identified in 91 (74.6%), 30 (24.6%) and 1 (0.8%) cases, respectively. Heterozygosity for the rs9399137 (T-C) was found in 28 (23.0%) cases. The most common SNP in this HBS1L-MYB gene was found to be the rs2297339 (C-T) including CT and TT which were identified in 60 (49.2%) and 46 (37.7%) cases, respectively.

Multiple regression analysis was applied to demonstrate the effect of these SNPs detected on Hb F levels of 122 subjects with Hb E-β-thal (Table 3). As shown in the table, statistical significance (P < 0.001) was observed only on the homozygosity (+/+) of the Gγ-XmnI polymorphism. However, a low proportion of this Gγ-XmnI (+/+) in this group of Thai patients (9 of 122) makes it unlikely to be the sole factor on phenotypic expression of these cases. In fact, we observed that each patient carried at least one of these
Table 3 Effect of SNPs detected on Hb F levels in 122 Hb E-β-thal patients.

| SNPs                        | Coefficient | 95% CI       | P-value |
|-----------------------------|-------------|--------------|---------|
| +/+                         | 4.62        | -1.31, 10.54 | 0.125   |
| +/−                         | 19.30       | 8.86, 29.75  | < 0.001 |
| rs2297339 (C-T)             |             |              |         |
| CT                          | -4.93       | -12.61, 2.75 | 0.206   |
| TT                          | -3.09       | -11.12, 4.94 | 0.447   |
| T334R (GA & AA)             | 3.17        | -5.35, 11.68 | 0.463   |
| rs4671393 (C-T)             | 3.98        | -1.34, 9.29  | 0.141   |
| rs4895441 (AG & GG)         | -1.07       | -11.43, 9.29 | 0.838   |
| rs9399137 (TC)              | 9.01        | -1.47, 19.50 | 0.091   |

Table 4 Proportions of patients according to number of carrying SNPs (1-5) observed among 122 Thai NTDT patients with Hb E-β-thalassemia disease.

| Number of SNPs | SNPs                          | N  | %  |
|----------------|-------------------------------|----|----|
| 1              | XmnI                          | 3  | 2.5|
|                | rs2297339 (C-T)               | 8  | 6.6|
|                | rs4671393 (A-G)               | 1  | 0.8|
| 2              | XmnI and T334R                | 1  | 0.8|
|                | XmnI and rs2297339 (C-T)      | 45 | 36.9|
|                | XmnI and rs4671393 (A-G)      | 5  | 4.1|
|                | XmnI and rs4895441 (G-A)      | 1  | 0.8|
|                | T334R and rs2297339 (C-T)     | 2  | 1.6|
|                | rs2297339 (C-T) and rs4671393 (A-G) | 5 | 4.1 |
| 3              | XmnI, T334R and rs2297339 (C-T) | 3 | 2.5 |
|                | XmnI, rs2297339 (C-T) and rs4671393 (A-G) | 16 | 13.1 |
|                | XmnI, rs2297339 (C-T) and rs4895441 (G-A) | 1 | 0.8 |
|                | XmnI, rs4895441 (G-A) and rs9399137 (T-C) | 1 | 0.8 |
|                | rs2297339 (C-T), rs4671393 (A-G) and rs4895441 (G-A) | 2 | 1.6 |
|                | rs2297339 (C-T), rs4895441 (G-A) and rs9399137 (T-C) | 6 | 4.9 |
| 4              | XmnI, T334R, rs2297339 (C-T) and rs4895441 (G-A) | 1 | 0.8 |
|                | XmnI, T334R, rs4895441 (G-A) and rs9399137 (T-C) | 1 | 0.8 |
|                | XmnI, rs2297339 (C-T), rs4671393 (A-G) and rs9399137 (T-C) | 2 | 1.6 |
|                | XmnI, rs2297339 (C-T), rs4895441 (G-A) and rs9399137 (T-C) | 10 | 8.2 |
|                | XmnI, rs4671393 (A-G) and rs4895441 (G-A) and rs9399137 (T-C) | 2 | 1.6 |
|                | T334R, rs2297339 (C-T), rs4895441 (G-A) and rs9399137 (T-C) | 1 | 0.8 |
|                | rs2297339 (C-T), rs4671393 (A-G), rs4895441 (G-A) and rs9399137 (T-C) | 1 | 0.8 |
| 5              | XmnI, T334R, rs4671393 (A-G), rs4895441 (G-A) and rs9399137 (T-C) | 1 | 0.8 |
|                | XmnI, T334R, rs4671393 (A-G), rs4895441 (G-A) and rs9399137 (T-C) | 1 | 0.8 |
|                | XmnI, rs2297339 (C-T), rs4671393 (A-G), rs4895441 (G-A) and rs9399137 (T-C) | 2 | 1.6 |

Summary 122 100

Figure 3. Proportions of subjects with 1-5 SNPs among 122 Thai NTDT patients with Hb E-β-thalassemia disease.

SNPs. Table 4 listed number of patients carrying 1-5 SNPs observed, and Figure 3 plots the proportions of subjects in correspondence with the number of conferring SNPs in this study. As shown in the figure, while only 12 of 122 cases carried single SNP, the remaining subjects had 2-5 SNPs at different genes, possibly indicating of interaction between these SNPs in the phenotypic modification of the cases.

Discussion. NTDT refers to as thalassemia phenotype that does not require blood transfusions for survival. Most of the patients have mild anemia, with baseline
Hb levels ranging from 7.0-9.0 g/dl and have a higher life expectancy. However, they may still suffer from many complications if not properly managed, including pulmonary hypertension and subsequent thrombotic events. Diagnosis and understanding of the basis for NTDT are therefore important.\textsuperscript{7,20,21}

It has been known that major genetic modifying factor in β-thalassemia disease is a coincidence of α-thalassemia as this leads to a more balanced in α- and non-α- globin chains ratio. However, this could not explain the phenotypic expression of all cases. Multiple single nucleotide polymorphisms (SNPs) associated with high Hb F expression have been identified in many populations on genes such as the HBG2, BCL11A, HBS1L-MYB, and KLF1 genes.\textsuperscript{22-25} The results from our study of 122 Thai NTDT Hb E-β-thalassemia patients without α-thalassemia revealed that all of them carried at least one SNPs in these modifying genes (Table 4). While the majority of them (59 of 122) had two SNPs, the remaining carried one (12 of 122), four (18 of 122) or five (4 of 122) SNPs as shown in Figure 3. These 9 genetic modifying SNPs on the \(G^\gamma\)-XmnI, HBS1L-MYB, BCL11A, and KLF1 genes are known to play important roles in modifying disease severity. Among them, the \(G^\gamma\)-XmnI polymorphism was the most common SNP observed in our patients, i.e., 70.5% in heterozygous and 7.4% in homozygous states. Study in Thai homozygous Hb E has indicated a strong association between this polymorphism and increased Hb F level. We also observed that the \(G^\gamma\)-XmnI (+/+ ) has a significant effect on the Hb F in Thai NTDT Hb E-β-thalassemia patients, as shown in Table 3. However, the finding of only 9 of 122 cases with homozygotic form (+/+ ) of this polymorphism (Table 2) might underscore the importance of this SNP in Thai population and point possibly to interaction with other genetic modifiers.

We have previously documented in Thai subjects with homozygous Hb E that four KLF1 SNPs including G176AfsX179, T334R, -154C, and T allele of rs2297339 (C-T) of the HBS1L-MYB intergenic region were observed among our Thai NTDT patients. This data is consistent with a previous finding for Thai homozygous Hb E.\textsuperscript{15} Study on the Mediterranean β-thalassemia intermediate patients has indicated a minor effect of the rs4671393 (G-A) of the BCL11A and the rs4895441 (A-G) & rs9399137 (T-C) of HBS1L-MYB intergenic region on phenotypic expression of the patients.\textsuperscript{28}

Conclusions. Considering all the results, we found that among 122 Thai NTDT patients investigated, a total of 6 SNPs including \(G^\gamma\)-XmnI of HBG2 gene, T334R of KLF1 gene, A allele of rs4671393 in BCL11A gene and T allele of rs2297339, G allele of rs4895441 and C allele of rs9399137 in HBS1L-MYB intergenic region, alone or in combination with others could be used to explain the mild phenotypic expression of all cases. Further study on NTDT subjects of other populations would be required to prove that screening of these informative SNPs in the NTDT patients is useful for clinical prediction and improving genetic counseling of the patients.

Acknowledgment. This work was supported by Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, Thailand.

References:

1. Weatherall DJ, Clegg JB. The thalassemia syndromes. 4th ed.; Oxford: Blackwell Science; 2001. https://doi.org/10.1002/9780470696705
2. Yamsri S, Sanchaisuriya K, Fucharoen G, Sae-Ung N, Ratanasiri T, Fucharoen S. Prevention of severe thalassemia in northeast Thailand: 16 years of experience at a single university center. Prenat Diagn 2010;30:540-546. https://doi.org/10.1002/pd.2514 PMid:20509153
3. Italia K, Dabke P, Sawant P, Naidkami A, Ghosh K, Colah RB. Hb E-β-thalassemia in five Indian states. Hemoglobin 2016;40:310-315. https://doi.org/10.1080/03630260.2016.1201487 PMid:27623935
4. George E, Wong HB. Hb E beta+–thalassaemia in West Malaysia: clinical features in the most common beta-thalassaemia mutation of the Malays [IVS 1-5 (G→C)], Singapore Med J 1993;34:500-503.
5. Winichagoon P, Fucharoen S, Chen P, Wasi P. Genetic factors affecting clinical severity in beta-thalassaemia syndromes. J Pediatr Hematol Oncol 2000;22:573-580. https://doi.org/10.1097/00043426-200011000-00026 PMid:11132233
6. Fucharoen S, Ketvichit P, Pootrakul P, Siritanaratkul N, Piankijagum A, Wasi P. Clinical manifestation of beta-thalassaemia/hemoglobin E disease. J Pediatr Hematol Oncol 2000;22:552-557.
Taher AT. Non-transfusion dependent thalassemia: an update on complications and management. Int J Mol Sci 2018;19:182.

13. Prayalw P, Teawtrakul N, Jetsrisuraparb A, Pongudom S, G. Fucharoen S. Non-transfusion-dependent thalassemia in northeast Thailand. Acta Haematol 2016;135:15-20.

14. Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion-dependent thalassemias. Haematologica 2013;98:833-844.

15. Chaouch L, Moumni I, Ouragini H, et al. rs1188686 and rs4671393 of the HBS1L-MYB intergenic region in southeast Asian populations associated with elevated Hb F levels in sickle cell disease. Blood Cells Mol Dis 2009;42:32-35.

16. Chaouch L, Moumni I, Ouragini H, et al. rs1188686 and rs4671393 of the HBS1L-MYB intergenic region in southeast Asian populations associated with elevated Hb F levels in sickle cell disease. Blood Cells Mol Dis 2009;42:32-35.

17. Prayalw P, Teawtrakul N, Jetsrisuraparb A, Pongudom S, G. Fucharoen S. Non-transfusion-dependent thalassemia in northeast Thailand. Acta Haematol 2016;135:15-20.

18. Prayalw P, Teawtrakul N, Jetsrisuraparb A, Pongudom S, G. Fucharoen S. Non-transfusion-dependent thalassemia in northeast Thailand. Acta Haematol 2016;135:15-20.