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Clinical and hematologic features of β^0-thalassemia (frameshift 41/42 mutation) in Thai patients

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Background and Objectives. Frameshift 41/42 mutation is the most common mutation of β-thalassemia found in Thailand. We studied clinical and hematologic features in 84 patients and relatives with frameshift 41/42 to determine whether it is possible to predict phenotypic severity from genetic factors.

Design and Methods. The clinical phenotypes and hematologic data of Thai patients with frameshift 41/42 were studied. α thalassemia, Hb Constant Spring (HbCS) genes and the presence of XmnI-$^G\gamma$ polymorphism were studied in patients who had mild symptoms.

Results. Homozygotes for frameshift 41/42 and compound heterozygotes for frameshift 41/42 and β^0-thalassemia produced severe symptoms and have a thalassemia major phenotype. Combination of frameshift 41/42 and β^0-thalassemia or Hb E produced mild to moderate symptoms with thalassemia intermedia phenotype and severe symptoms with thalassemia major phenotype. The co-inheritance of α-thalassemia or HbCS gene or the presence of XmnI-$^G\gamma$ polymorphism was not associated with mild disease in patients with frameshift 41/42 and HbE.

Interpretation and Conclusions. The clinical phenotype of homozygotes for frameshift 41/42 and compound heterozygotes for frameshift 41/42 and β^0-thalassemia could be used to predict a severe phenotype with thalassemia major. However, the clinical phenotype of compound heterozygotes of frameshift 41/42 and β^0-thalassemia or Hb E were variable and could not be accurately predicted. Associations between concomitant α-thalassemia or HbCS of the presence of XmnI-$^G\gamma$ polymorphism and a mild clinical phenotype are not apparent, indicating the involvement of other ameliorating determinants or genetic modifications.

Key words: β^0-thalassemia, frameshift 41/42 mutation, genotype, phenotype

Thalassemia represents a major public health problem in Thailand. β-thalassemia and hemoglobin E (Hb E) are extremely common; the incidence of β-thalassemia trait varies from 3 to 9%, and that of Hb E trait varies from 8 to 54%.[1] Hb E is a mild β^+-thalassemia.[2] Over 170 mutations affecting the β-globin gene lead to a reduction (β^+) or absence (β^0) of β-globin gene production resulting in β-thalassemia.[3] There are three main clinical phenotypes of β-thalassemia: thalassemia major (TM), thalassemia trait (TT) and thalassemia intermedia (TI).[4] TM results from the inheritance of two β-thalassemia alleles (homozygous or compound heterozygous) and requires regular blood transfusion from infancy for survival. The thalassemia trait (TT) due to a single β-thalassemia allele (β^0 or β^+) is usually asymptomatic. Thalassemia intermedia (TI) is more severe than the asymptomatic TT but milder than the transfusion-dependent TM.

Analyses of the underlying molecular defects of β-thalassemia in Thailand[5-9] have provided a valuable database for the prenatal diagnosis of β-thalassemia. At least 24 mutations affecting the β-globin gene were characterized in Thailand.[9] Four base pairs deletion at codons 41/42 or frameshift 41/42 mutation leading to absence of β-globin gene production resulting in β^0-thalassemia[10] was the most common mutation found in Thailand.[9] In this report, we describe the clinical phenotypes of Thai patients who are homozygotes for frameshift 41/42 or compound heterozygotes for frameshift 41/42 and other β^0- or β^+-thalassemia mutations.

Design and Methods

Eighty-eight patients and relatives with frameshift 41/42 (β^0-thalassemia) were studied. All patients attended the Pediatric Hematology and Oncology Clinic of Songklanagarind Hospital, Faculty of Medicine, Prince of Songkla University. All patients had been followed for many years, ever since their diagnosis was made in the first years of their life or later. Disease severity was assessed according to the following variables: age at presentation and age at time of review;

hemoglobin (Hb) concentration at presentation and steady-state Hb level; if transfused, age of first transfusion and frequency of transfusion; presence of splenomegaly/hepatomegaly and presence of bone changes, growth deficiency and general clinical status. Iron deficiency and other causes of anemia and splenomegaly were excluded. Based on criteria modified from Ho *et al.*,[11] a broad classification of phenotypic clinical severity could be derived: mild if a Hb level of > 7 g/dL could be maintained without transfusion or transfusion frequency was less than once every 2 years or less than 6-monthly if transfusion was started after the age of 10 years, no thalassemic facies or mild thalassemic facies recognized by an experienced observer and minimal bone changes and no growth deficiency; severe, if transfusion requirements began at age 4 years or above and needed to be given at an interval of between 6 weeks and 4 months, or 4 months or less if transfusion requirements commenced before the age of 4 years, and classical physical appearance of thalassemic disease. Those with transfusion requirements between the two groups were classified as moderately affected. Splenectomy was done in patients who had hypersplenism (massive splenomegaly associated with either increasing transfusion requirement or pancytopenia).[4]

Hematologic and hemoglobin analyses were carried out using the standard laboratory techniques described in a previous report.[12] Full blood count and red blood cell indices were obtained by a Coulter electronic counter. Hemoglobin electrophoresis was carried out on starch-gel electrophoresis at pH 8.6. Hemoglobins A_2 and E were quantified by elution following cellulose acetate electrophoresis and Hb F by alkali denaturation.

DNA analysis

DNA was extracted from peripheral blood leukocytes of the patients using a standard method. Amplified DNA by polymerase chain reaction (PCR) was analyzed for known β-thalassemia mutations found in Thailand using dot-blot hybridization as previously described.[6] The C-T base substitution at position-158 upsteam of the Gγ-globin gene as referred to the XmnI-$^{G}\gamma$ cleavage site was screened by Southern blot hybridization of XmnI-digested genomic DNA[13] in patients with frameshift 41/42 and Hb E who had mild clinical symptoms. The Southeast Asia (SEA) or α-thalassemia 1 gene deletion and hemoglobin Constant Spring (Hb CS) genes were screened for by a rapid and simultaneous non-radioactive method based on the PCR using allele-specific primers[14] and α-thalassemia 2 gene deletions (3.7 and 4.2 kb deletions) were also screened for by allele-specific primers on the PCR amplified DNA[15] in patients with frameshift 41/42 and HbE who had mild clinical symptoms.

Results

The results are shown in Tables 1 and 2 for 6 homozygotes, 34 heterozygotes and 48 cases that were compound heterozygotes for this mutation and other β-thalassemia mutations. Homozygosity of frameshift 41/42 produced severe clinical symptoms of thalassemic disease with onset of anemia during the first 6 months of life. Compound heterozygosity of frameshift 41/42 and β⁰-thalassemia (codon 17, A-T or IVS-1 nt 1, G-T) produced severe symptoms with onset of anemia during the first year of life. Compound heterozygosity of frameshift 41/42 and uncharacterized mutation of β-thalassemia also produced severe symptoms. The clinical severity of patients with compound heterozygosity of frameshift 41/42 and β⁺-thalassemia [-28 ATA, A-G or IVS-1 nt 5, G-C or Hb E (codon 26, G-A)] varied from mild to severe degree (Table 2). Patients with compound heterozygosity of frameshift 41/42 and - 28 ATA, A-G had moderate or severe symptoms. A boy with compound heterozygosity of frameshift 41/42 and IVS-1 nt 5, G-C had severe symptoms. In patients with compound heterozygosity of frameshift 41/42 and Hb E, the clinical severity ranged from mild to severe (Table 3). The onset of anemia in patients with severe symptoms was

Table 1. Hematologic and clinical features of patients with homozygous frameshift 41/42 and compound heterozygosity of frameshift 41/42 and β⁰-thalassemia.

	Homozygous frameshift 41/42 (n = 6)	*Frameshift 41/42 and codon 17, A-T (n=4)*	*Frameshift 41/42 and IVS-1nt1, G-T (n=1)*	*Frameshift 41/42 and uncharacterized (n=5)*
Age at onset of anemia (months)	4.00±2.19	5.75±4.19	10	5.0±3.08
Grading of severity	severe	severe	severe	severe
Splenectomy (no. of patients)	3	2	1	3
Hb (g/dL)	4.73±1.82	4.55±1.14	4.2	5.85±1.12
Hct (%)	14.22±5.37	13.50±4.43	14	17.3±3.3
RBC x 10^{12}/L	2.47±0.60	1.67±0.33	N.D.	2.51±0.43
MCV (fL)	57.33±1.15	N.D.	N.D.	57.33±3.79
MCH (pg)	21.05±3.00	29.25±2.05	N.D.	20.3±0.35
Hb A_2 (%)	2.17±0.92	3.5	1.86	2.65±0.69
Hb F (%)	97.63±0.75	96.5	98.14	83.31±24.61
Hb A (%)	–	–	–	42.1

Data shown as mean±S.D., N.D. = not determined.

Table 2. Hematologic and clinical features for frameshift 41/42 heterozygosity and compound heterozygosity of frameshift 41/42 and β⁺-thalassemia or Hb E.

	Frameshift 41/42 and Hb E (n = 32)	*Frameshift 41/42 and -28 ATA, A-G (n=5)*	*Frameshift 41/42 and IVS-1 nt 5, G-C (n=1)*	*Frameshift 41/42 heterozygosity (n=34)*
Age at onset of anemia (months)	37.97±25.78	11.60±4.0	5	N.A.
Grading of severity	mild - severe	moderate - severe	severe	asymptomatic
Splenectomy (no. of patients)	3	3	1	-
Hb (g/dL)	6.66±1.73	7.54±1.52	7.1	11.60±1.28
Hct (%)	20.58±5.39	22.80±3.56	20	36.37±4.63
RBC x 10^{12}/L	3.38±0.88	3.21±0.70	N.D.	5.59±0.61
MCV (fL)	60.52±5.33	69.00±1.73	N.D.	63.90±4.20
MCH (pg)	20.19±2.49	22.97±1.31	N.D.	20.46±1.16
Hb A_2 (%)	–	3.29±1.48	2.2	5.78±1.19
Hb E (+ A_2) (%)	51.57±10.38	–	–	–
Hb F (%)	47.89±10.90	41.82±12.29	80.0	–
Hb A (%)	–	54.88±11.91	17.8	94.20±1.19

Data shown as mean±S.D., N.D. = not determined; N.A. = not applicable.

significantly earlier than in those with mild symptoms ($p = 0.003$).

α-thalassemia, Hb Constant Spring (Hb CS) genes and the presence of XmnI-$^{G}\gamma$ polymorphism in both or either alleles (XmnI +/+ or XmnI +/-) were not detected in 11 of 13 patients with frameshift 41/42 and Hb E who had mild symptoms.

Discussion

The clinical severity of β-thalassemia can be ameliorated by at least 3 factors.[11] These are the type of β-thalassemia mutation, the co-inheritance of α-thalassemia and the inheritance of a genetic determinant(s) for enhanced γ-globin chain production which can compensate for the lack of β-chains. Homozygous frameshift 41/42 or homozygous β⁰-thalassemia produces severe clinical symptoms leading to thalassemia major (TM). Compound heterozygosity of frameshift 41/42 and other β⁰-thalassemia mutations also produces severe symptoms and a TM phenotype. Patients with compound heterozygosity of frameshift 41/42 and uncharacterized mutation of β-thalassemia have a TM phenotype. A combination of frameshift 41/42 and -28 ATA, A-G (β⁰-thalassemia and mild β⁺-thalassemia) has produced moderate clinical symptoms and a TI phenotype in two patients but three patients with this compound heterozygosity have severe transfusion-dependent TM. A small number of patients with compound heterozygosity of frameshift 41/42 and IVS-1 nt 5, G-C (β⁰-thalassemia and severe β⁺-thalassemia) have been reported with severe transfusion-dependent TM.[11] One of our patients with this compound heterozygosity has severe symptoms. There are reports of compound heterozygotes for frameshift 41/42 and Hb E producing a TI phenotype with variable clinical severity ranging from mild to severe.[16] About two-thirds of our patients with com-

Table 3. Patients with compound heterozygosity of frameshift 41/42 and Hb E according to clinical phenotype.

	Clinical severity			*p value**	
Parameter	*mild (n = 13)*	*moderate (n = 8)*	*severe (n = 11)*	*mild vs severe*	*mild vs moderate*
Age at onset of anemia (months)	53.08±29.48	31.0±19.95	21.18±16.3	0.003	0.055
Splenectomy (no. of patients)	–	1	2	–	–
Hb (g/dL)	8.14±0.98	6.08±0.91	5.33±1.58	0.0001	0.0001
Hct (%)	25.38±2.90	18.38±3.54	16.50±4.36	0.0000	0.0004
RBC x 10^{12}/L	4.09±0.80	2.85±0.81	3.03±0.52	0.013	0.027
MCV (fL)	60.75±7.42	62.0±4.95	59.38±3.02	0.64	0.73
MCH (pg)	19.68±2.84	21.51±3.2	19.77±1.35	0.94	0.34
Hb E (%)	50.44±14.34	50.23±8.12	53.86±5.59	0.46	0.97
Hb F (%)	49.56±14.34	47.51±10.95	46.14±5.59	0.46	0.73
XmnI - $^{G}\gamma$ polymorphism	-/- (11)+	ND	ND	–	–
α-thalassemia$_1$, SEA type	neg (11)+	ND	ND	–	–
α-thalassemia$_2$ (3.7 and 4.2 kb del)	neg (11)+	ND	ND	–	–
Hb CS gene	neg (11)+	ND	ND	–	–

*Data shown as mean±S.D., neg = negative, ND = not determined, *p value from a non-parametric Kruskal-Wallis test, p < 0.05 = statistically significant.±number of patients studied.*

pound heterozygosity of frameshift 41/42 and HbE have mild and moderate symptoms and have a TI phenotype. One-third, however, of patients with this compound heterozygosity have severe transfusion-dependent TM. The co-inheritance of α-thalassemia was not associated with mild disease in 11 patients with compound heterozygosity of frameshift 41/42 and Hb E.[16] The association between α-thalassemia or Hb CS genes and mild disease was not evident in 11 of our 13 patients with frameshift 41/42 and Hb E.

The presence of XmnI-$^{G}\gamma$ cleavage site or XmnI +/+ genotype was associated with mild symptoms in one patient with compound heterozygosity of frameshift 41/42 and Hb E),[16] but no association between XmnI +/+ or XmnI +/- genotype and milder disease was evident in 11 of our 13 patients with this compound heterozygosity. All patients with frameshift 41/42 heterozygosity had TT phenotype and were asymptomatic.

Conclusions

Our observations indicate that the clinical phenotype of homozygous frameshift 41/42 and compound heterozygosity of frameshift 41/42 and β⁰-thalassemia could be used to predict a severe phenotype with TM. However, the clinical phenotypes of compound heterozygosity of frameshift 41/42 and β⁺-thalassemia were variable and could not be accurately predicted based only on the type of β-thalassemia mutation, the co-inheritance of α-thalassemia or Hb CS gene or the presence of XmnI-$^{G}\gamma$ polymorphism. Further study to define the nature of the genetic modification which interacts with β-thalassemia and can ameliorate the clinical severity will provide a basis for more accurate prediction and enable better understanding of the process of the disease. These observations may be useful for genetic counselling, prenatal diagnosis and therapeutic planning for patients with frameshift 41/42 mutation of β-thalassemia.

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VL: conception and design of the study, interpretation of the data and preparation of the manuscript. MW: collection of clinical data. BS: laboratory experiments and statistical analyses. AW: collection of clinical data. SF: laboratory experiments for the co-inheritance of alpha-thalassemia, HbCS gene and the presence of XmnI-$^{G}\gamma$ polymorphism. We thank Mrs. Tawil Rukwong for preparation of the manuscript and Dr. Alan F Geater for editing the manuscript. Many thanks to our patients and their parents who co-operated in this study and thanks to the Pediatric residents and staff and nurses who helped in taking care of our patients.

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Potential implications for clinical practice

These observations may be useful for genetic counselling, prenatal diagnosis and therapeutic planning for patients with frameshift 41/42 mutation.

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