

HEMATOLOGICAL AND MOLECULAR CHARACTERIZATION OF BETA-THALASSEMIA/HB TAK COMPOUND HETEROZYGOTE

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Abstract. We report a case of β -thalassemia/Hb Tak compound heterozygote. The 7 year-old Thai boy presented with plethora since birth. Hemoglobin electrophoresis showed a major band between Hb A₂ and Hb F and absent Hb A. DNA sequencing study demonstrated an AC insertion at the terminal codon of the β -globin gene. The clinical feature of polycythemia reflected a high oxygen affinity of Hb Tak.

INTRODUCTION

Thalassemia is the most common hereditary anemia in Thailand and other parts of Southeast Asia (Wasi, 1978; Weatherall and Clegg, 2001). The condition is caused by a decrease or absence of globin chain production resulting in chronic hemolytic anemia (Weatherall and Clegg, 2001). Thalassemic patients manifest with varying degree of anemia, bony changes, enlarged liver or spleen and delayed growth. Thalassemia is classified into two major subtypes; alpha(α)- and beta(β)- thalassemia which are results of mutations of the α - and β -globin genes respectively. The β -globin gene cluster is made up of non α -globin genes arranged in the order of 5'- ϵ - γ - δ - $\psi\beta$ - β -3' on chromosome 11 (Weatherall, 2001). Each individual has 2 β -globin genes which code for β -globin chains. Mutation affecting one β -globin allele causes thalassemia minor phenotype characterized by normal hemoglobin level, low mean corpuscular volume (MCV) and increased hemoglobin A₂ (Hb A₂) level. Thalassemia major or intermedia phenotype characterized by anemia, signs of extramedullary hematopoiesis, anisopoikilocytotic erythrocytes and absent or decreased Hb A along with increased production of

Hb F occurs when both β -globin alleles are affected (Weatherall and Clegg, 2001).

Other than causing thalassemia, mutations of globin genes may result in hemoglobinopathies or abnormal hemoglobins. The variant β -globin allele usually synthesizes β -globin peptides at the same rate as the normal β -globin allele but the products are abnormal in structure. Hemoglobinopathies are classified clinically into 3 groups: 1) structural mutants that result in a thalassemic phenotype, 2) unstable hemoglobins, and 3) mutants with abnormal oxygen affinity. The most common β -globin mutant in Thailand is Hb E. Heterozygotes of β -globin mutants are mostly asymptomatic although compound heterozygotes of β -globin mutants and β -thalassemia usually result in a thalassemic phenotype (Bunn, 2001).

Hemoglobin Tak (Hb Tak) is a mutant hemoglobin with high oxygen affinity resulting in erythrocytotic phenotype. It was first reported in a Thai patient in 1971 by Flatz *et al.* The variant is a result of β -globin chain elongation from the C-terminus by 11 amino acid residues (Lehmann *et al.*, 1975; Sanguansermisri *et al.*, 1983; Fucharoen *et al.*, 1997; Hoyer *et al.*, 1998). Molecular study further identifies an AC (or CA) insertion at the terminal codon of β -globin gene causing an interruption of the stop codon and thus the chain elongation (Sanguansermisri *et al.*, 1983; Fucharoen *et al.*, 1997; Hoyer *et al.*, 1998). Hb Tak migrates between Hb A₂ and Hb F at alkaline pH (Lehmann *et al.*, 1975; Sanguansermisri *et al.*, 1983; Fucharoen *et al.*, 1997; Hoyer *et al.*, 1998).

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Hb Tak heterozygotes are clinically asymptomatic and are usually identified incidentally by a population screen or a study in a family with a known affected patient. Hb Tak heterozygotes have 30-40% Hb Tak with normal or slightly high Hb level between 14-17 g/dl (Flatz *et al*, 1971; Sanguanserm Sri *et al*, 1983; Fucharoen *et al*, 1997). Compound heterozygotes between β -thalassemia and Hb Tak, however, with only Hb Tak and Hb F, are characteristically polycythemic which may be explained by the high oxygen affinity of the mutant hemoglobin (Imai *et al*, 1975; 2001). Patients are usually brought to physicians because of looking plethoric or 'cyanotic' suspected of other more prevalent polycythemic or cyanotic conditions such as congenital cyanotic heart diseases.

There have been sporadic reports of Hb Tak in Thais and patients of other Southeast Asian background suggesting that this Hb variant may be a prevalent hemoglobinopathy in this geographic area in addition to Hb E and Hb Constant Spring (Hb CS). The understanding of this abnormal hemoglobin is therefore important. The characteristic clinical finding of polycythemia without other identifiable cause provides a clue to a prompt recognition of the condition. We now report clinical and hematologic findings in a case of β -thalassemia/Hb Tak compound heterozygote (β -thal/Hb Tak). Detailed molecular investigation will be discussed.

MATERIALS AND METHOD

A case report

A 7 year-old Thai boy was referred for a cardiology consultation with a history of 'looking red' since infancy. Apart from occasional dyspnea on exertion, he was otherwise well and had normal growth and development. He was the second offspring of non-consanguineous parents. Both parents and the elder brother were healthy. There was no history of blood diseases or hereditary conditions in the family.

Physical examination revealed a boy with hyperemic conjunctiva and plethoric skin. No dysmorphic features were present. Oxygen saturation was within the normal range. Cardiovas-

cular examination was non-contributory. Liver and spleen were enlarged. Chest radiograph showed mild cardiomegaly and increased pulmonary blood flow. Echocardiology was performed to exclude any underlying cyanotic cardiac condition that may cause polycythemia. No structural heart defect was detected. Good biventricular function was demonstrated.

Because of polycythemia and hepatosplenomegaly without identifiable cardiac cause, blood was collected from the patient and family members for investigation of abnormal hematologic condition.

Hematological studies

Complete blood counts were performed using a Cell Dyn 4000 blood cell counter (Abbott laboratories). Hb electrophoresis was performed at alkaline pH on cellulose acetate gel (Helena Laboratories, Beaumont, Texas). Quantitation of Hb A₂, Hb F and abnormal Hb from the patient was done by the automated High Performance Liquid Chromatography (VARIANT Hemoglobin Testing System; Bio-Rad Laboratories) using the β -Thalassemia Short Program.

Molecular analysis

Genomic DNA was extracted from peripheral blood leukocytes. To detect mutation, regions of the β -globin gene (exon 1,2 and 3) were amplified using polymerase chain reaction (PCR), and the mutation was identified by direct nucleotide sequencing of the PCR product using the ABI PRISM Dye Terminator Cycle Sequencing kit and an ABI 310 automated sequencer (Perkin-Elmer Corporation).

Ethics

The review was approved by the institution's Research Ethics Committee.

RESULTS

Table 1 summarizes the results of hematologic findings of the subjects. Hemoglobin electrophoresis of the patient, brother and mother showed an abnormal band between Hb A₂ and Hb F (Fig 1). HPLC demonstrated an abnormal Hb eluted after Hb A₂ (Fig 2). The amount of the

Table 1
Hematologic profiles of the patient and family members.

Parameters	Patient	Brother	Father	Mother
Hb g/dl	20	13.4	14.4	14.3
Hct %	65	43.9	47.0	44.5
MCV fl	N/A	75	66	78
Hb typing	F,Tak, A ₂	A, Tak, A ₂	A, A ₂	A, Tak, A ₂
Hb A ₂ /E %	1.7	2.8	4.8	3.2
Hb F %	4.3	0.7	1.5	0.8
Abnormal Hb %	89.4	26.9	-	26.9

N/A : not available

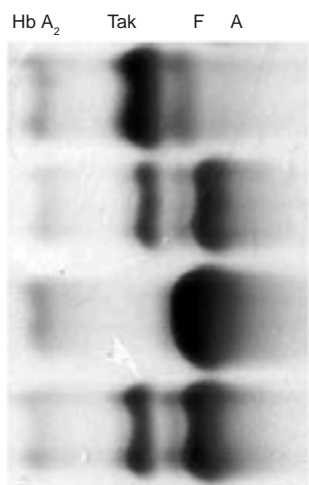


Fig 1–Hemoglobin electrophoresis of the patient, brother and mother showed an abnormal band between Hb A₂ and Hb F.

abnormal Hb was 89.4%. The abnormal hemoglobin from the patient was defined later by DNA sequencing technic as Hb Tak (codon 147^{+AC}) (Fig 3). The study of the DNA sequence showed that the patient was a compound heterozygote of Hb Tak and codon41/42-4bp deletion (codon41/42-CTTT).

DISCUSSION

The patient had no functioning normal β-globin gene. He was found to be plethoric since birth. The diagnosis of β-thalassemia/Hb Tak

ANALYTE ID	%	TIME	AREA
F	4.3	1.16	138842
P3	0.7	1.76	22047
A0	3.6	2.19	116756
Unknown 1	0.2	2.60	6837
A2	1.7	3.55	48112
D-WINDOW	89.4	4.21	2877327
TOTAL AREA			3209921
F	4.3%	A ₂	1.7%

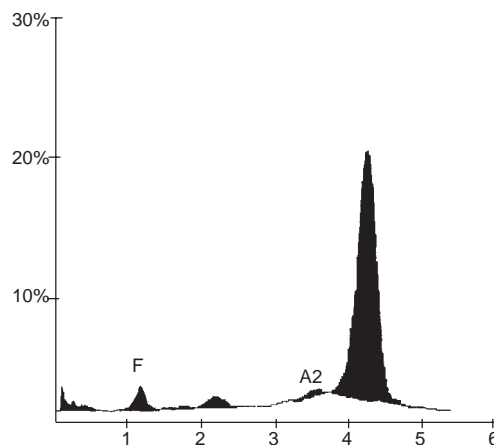


Fig 2–HPLC demonstrated an abnormal Hb eluted after Hb A₂.

compound heterozygote (β-thal/Hb Tak) was made by hemoglobin typing and molecular analysis.

The AC inserion at the terminal codon of the β-globin gene causes a frameshift mutation which results in a substitution of the C-terminal histidine residue by threonine and an elongation of β-globin peptides. Because the C-terminal his-

A A G T A T C A C N C T A N T C N C T C T
 80 145 146 147390 40

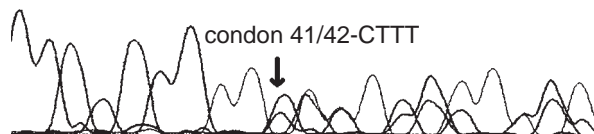
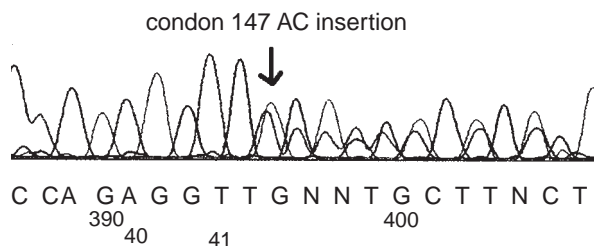


Fig 3—DNA sequencing of β -globin gene from the patient showed an AC insertion at the terminal codon characteristic of Hb Tak and a CTTT deletion at the codon 41/42.

tidine residue is involved in the conformation of deoxy-hemoglobin (Perutz, 1970), the elongated Hb Tak is predicted to be unstable in de-oxygenated form. It has been shown to have a very low partial pressure of oxygen at 50% of saturation (P_{50}) value indicating an increased oxygen affinity (Imai *et al*, 1975; 2001). Hematologic findings in our case were consistent with the previously reported two cases of β -thal/Hb Tak and two cases of Hb Tak/Hb E compound heterozygote, all of whom demonstrated polycythemic phenotype reflecting the increased oxygen affinity of Hb Tak. Only one of the previous reports of a β -thal/Hb Tak compound heterozygote showed a girl with thalassemic phenotype. Because of unavailable paternal hematologic data, it was postulated that other factors such as concurrent α -thalassemia, not the Hb Tak itself, may be responsible for the clinical manifestations (Lehmann *et al*, 1975; Hoyer *et al*, 1998). Previously reported cases of β -thal/Hb Tak and Hb Tak/Hb E compound heterozygotes are summarized in Table 2.

The Hb electrophoresis in our patient showed a predicted phenotype of β -thal/Hb Tak compound heterozygote with absent Hb A, increased Hb F and a large band between Hb A₂ and Hb F. Study of DNA sequence showed an AC insertion at the

Table 2
 Reviewed cases of β -thal/Hb Tak and Hb Tak/Hb E compound heterozygotes.

Case no.	Age, sex	Nationality	Presentation	Hb (g/dl)	Hb typing	Study method	Reference
1	9y, F	Thai	Severe anemia, hepato-splenomegaly, mongoloid faces, retarded growth	6.3	F,Tak,A ₂	Peptide chromatography	Lehmann <i>et al</i> , 1975
2	18m, M	Thai	Incidental finding of plethora, splenomegaly	15-18	F,Tak,A ₂	Peptide chromatography	Sanguanserm Sri <i>et al</i> , 1983
3	14y, M	Thai	Splenomegaly, recurrent headache and chest pain, plethora	19.9	F,Tak,A ₂	Peptide chromatography	Sanguanserm Sri <i>et al</i> , 1983
4	Adult, M	Thai	Healthy, detected on regular health check-up	15.1	E, Tak	DNA sequencing	Fucharoen <i>et al</i> , 1997
5	37y, M	Cambodian	Asymptomatic	17.8	E, Tak	DNA sequencing	Hoyer <i>et al</i> , 1998
6	7y, M	Thai	Plethora, hepato-splenomegaly,	20.0	F,Tak,A ₂	DNA sequencing	Present study

C-terminal codon of the β -globin gene on one allele and a 4 base-pair deletion at codon 41/42 on the other. This represented the pathophysiology of Hb Tak at the molecular level.

The mother and elder brother were carriers of the Hb Tak. They both had normal hemoglobin levels and were clinically asymptomatic. In these two subjects, hemoglobin study which lead to a diagnosis of Hb Tak heterozygote was indicated by the family history alone. With normal or slightly high hemoglobin level (14-16 g/dl), Hb Tak heterozygotes are usually asymptomatic although severe hyperbilirubinemia secondary to polycythemia has been reported in a neonate (Lie-Injo *et al*, 1977). This may be explained by a high level of Hb F in the neonatal period. An increased oxygen affinity of both Hb F and Hb Tak leads to a higher demand for hemoglobin production and increased bilirubin production as a consequence.

In summary, an investigation for polycythemia without other identifiable cause in the Thais and probably the wider Southeast Asian ethnic group should include hemoglobin analysis. The DNA sequencing is a rapid, non-radioactive assay to provide the definitive diagnosis.

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