β^0 -Thalassemia Trait (IVS-I-1 G→T) in a Japanese Family

Akihiro YOKOYAMA, Tsuyoshi NAKAMAKI, Kazunari YAMADA, Michiaki KOIKE,
Shigeru TOMOYASU, Noriyasu HIRAYAMA*, Nobuyoshi TSURUOKA and Teruo HARANO**

Three subjects in a family with microcytic and hypochromic anemia were studied; red blood cell morphology indicated aniso-poikilocytosis and hypochromasia. Target and tear-drop cells were also noted. In all three cases evaluated, there was an increase in HbA₂ levels and a decline in the β/α synthesis ratio. Direct cloning and DNA sequencing identified a point mutation (G→T) at position 1 of intervening sequence I. The resulting reduction of β -globin chain synthesis is considered to give rise to β^0 -thalassemia phenotype. This point mutation is to our knowledge, the first case in Japan.

(Internal Medicine 32: 865–868, 1993)

Key words: β -thalassemia, intron, point mutation, abnormal splicing

Introduction

Thalassemia (1, 2) was first described in 1925 by Cooley as a syndrome presented as serious anemia, splenomegaly and bony deformities. Subsequent research disclosed that the thalassemia as a group resulted from defective synthesis of one or more of the polypeptide chains of hemoglobin (Hb). Recent advances in molecular biology have identified various mutations of globin gene which are responsible for thalassemia syndromes (3). We describe here a Japanese family with β^0 -thalassemia trait caused by a single base substitution at position 1 of intervening sequence (IVS)-I in the β -globin gene.

Case Report

A 51-year-old Japanese woman (II-4) was admitted to our hospital because of microcytic, hypochromic anemia (Fig. 1). Iron supplements was not effective 22 or 23 years ago. Her son (II-1) and daughter (II-2) also had microcytic and hypochromic anemia in spite of normal serum levels of iron. Her elder brother (II-1) was diagnosed as having anemia, but no detailed information has been available.

The patient was slightly anemic but otherwise not remarkable on physical examination. The spleen was not enlarged on abdominal sonography.

Laboratory data on admission indicated microcytic anemia with a mean corpuscular Hb concentration (MCHC), near the lower normal limit (Table 1). Red blood cell morphology

showed slight aniso-poikilocytosis, target and tear-drop cells and hypochromasia (Fig. 2). There was slight erythroid hyperplasia in the bone marrow (nucleated cell count $12.0 \times 10^4/\mu\text{l}$, M/E ratio 1.9). A siderogram revealed 98% for type 0 and 2% for type I.

The serum iron level was 39 $\mu\text{g/dl}$, total iron binding capacity (TIBC) 216 $\mu\text{g/dl}$ and ferritin 133 ng/ml. Results of other biochemical and serological tests were unremarkable.

Fig. 1. Pedigree of the family with β^0 -thalassemia heterozygotes. $\square/\square$ Deceased, $\square/\square$ No history and not examined, $\blacksquare/\bullet$ Affected on history, $\blacksquare/\bullet$ Affected and examined.

From the Department of Hematology, Showa University School of Medicine, Tokyo, *the Department of Internal Medicine, Ohta Hospital, Tokyo and **the Department of Biochemistry, Kawasaki Medical School, Okayama

Received for publication March 31, 1993; Accepted for publication October 20, 1993

Reprint requests should be addressed to Dr. Akihiro Yokoyama, the Department of Hematology, Showa University School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142

Table 1. Laboratory Data on Admission

Peripheral blood		Serological test	
WBC	5,900/ μ l	CRP	<0.1mg/dl
RBC	443×10^4 / μ l	Coombs test	
Hb	8.9 g/dl	direct	(-)
Ht	27.1%	indirect	(-)
MCV	61.2fl	haptoglobin	124mg/dl
MCH	19.5pg	hemopexin	83.0mg/dl
MCHC	31.8%	transferrin	224mg/dl
Ret	11‰	Ferrokinetics (^{59}Fe -citrate)	
anisocytosis	(+)	Fe	39 μ g/dl
poikilocytosis	(+)	PID T1/2	52.80 min
hypochromasia	(+)	PIT	0.320mg/kg/day
target cell	(+)	%RCU	95.70%
tear-drop cell	(+)	RIT	0.306mg/kg/day
Plt	31.8×10^4 / μ l		
Bone marrow		Red cell life span ($^{51}\text{CrNaO}_4$)	
NCC	12.0×10^4 / μ l	T1/2	26.59 days
Mgk	44/ μ l	Osmotic fragility test	
M/E ratio	1.9	Giffin-Sanford method	
siderogram		MIN	0.38%
0 type	98%	MAX	<0.28%
I type	2%		
Blood Chemistry		Coil planet centrifuge	
TP	6.2g/dl	HEP	52 mOsm
Alb	3.5g/dl	HSP	100 mOsm
BUN	18.0mg/dl	Heinz body test	
Crn	1.1mg/dl	Pt	17.0%
UA	4.7mg/dl	Control	18.0%
T-Bil	0.5mg/dl		
D-Bil	0.3mg/dl		
GOT	10 IU/l		
GPT	9 IU/l		
LDH	290 IU/l		
Fe	39 μ g/dl		
TIBC	216 μ g/dl		
Ferritin	133ng/ml

Fig. 2. Light micrograph of patient's red blood cells (peripheral blood, Wright-Giemsa stain, $\times 250$).

Table 2. Peripheral Blood Data of the Patient (II-4), Her Son (III-1) and Daughter (III-2), and Results of Hemoglobin Analysis

	II-4	III-1	III-2
Peripheral blood values			
RBC $\times 10^4$ / μ l	501	626	512
Hb g/dl	9.5	12.3	10.7
Ht %	30.0	39.3	33.6
MCV fl	59.9	62.8	65.6
MCH pg	19.0	19.6	20.9
MCHC %	31.7	31.3	31.8
Ret %	0.8	1.4	1.1
Hemoglobin analysis			
Hb F (0.1–1.2)%	0.93	0.89	2.28
Hb A ₂ (2.2–3.2)%	5.17	5.33	5.29
Abn Hb	(-)	(-)	(-)
Instability	(-)	(-)	(-)
β/α ratio(0.9–1.2)	0.38	0.44	0.60

Ferrokinetics indicated a slight decrease in the plasma iron disappearance rate (PID) with a T1/2 of 52.80 min. However, red cell iron utilization (%RCU) was normal. The red cell life span measured by ^{51}Cr -RBC was slightly decreased with a T1/2 of 26.59 days. Osmotic fragility tests using the Giffin-Sanford and coil planet centrifuge methods, indicated increased erythrocyte osmotic resistance. The results of the Heinz body test (4), isopropanol test (5) and isoelectric focusing (6) did not reveal any qualitative abnormalities of Hb. Hb A₂ was increased to 5.17% and the β/α globin synthesis ratio (7) was reduced to 0.38.

A son and a daughter of the patient also had similar microcytic anemia (Table 2). High Hb A₂ levels and decreased β/α synthesis ratios were common to the three individuals. Hb F levels were increased in the daughter. These data were consistent with β -thalassemia minor.

Southern blot analysis did not indicate a large deletion or duplication in the α -globin gene and the β -globin gene cluster in all three cases. The β -globin gene was amplified by the

polymerase chain reaction (PCR). The DNA fragments were directly cloned using phage vector M13mp19 (8), and subjected to sequence analysis by the dideoxy method (9) from the 5' promoter region (containing CACCC, CCAAT and ATAAA sequences) to the 3' end (containing the polyadenylation site). One-point mutation was found at the same position in all three cases: a guanine (G) to a thymine (T) transversion at the first base of the first intron of the β -globin gene (Fig. 3). Intragenic sequence polymorphisms, including a nucleotide at the 3rd position of codon 2 (C, Hgi AI polymorphism: +) and nucleotides at 42(G) of the IVS-I, and 16 (C, Ava II polymorphism: +), 74(T) and 81(C) of the IVS-II of the β -globin gene were observed in the thalassemic gene.

Discussion

This appears to be the first report in Japan of a point mutation (G→T) at position 1 of IVS-I of the β -globin gene. This

Fig. 3. Top: Autoradiogram of sequencing ladders of directly cloned β -globin genes. Shown is the G $\rightarrow$ T substitution at position 1 of IVS-I heterozygotes ($\blacktriangle$). Bottom: β -globin gene mutation at splice junction.

mutation is one of the most common causes of β^0 -thalassemia among Asian Indians, but it is rare in Chinese (10–12).

In eukaryote cells, intron (IVS) is removed after transcription of the genomic DNA during normal processing of the mRNA precursor (13). GT is indispensable at the 5' end of pre-

mRNA (the donor site in splicing), while AG is indispensable at the 3' end (the acceptor site) (14, 15). To date, 12 different mutations in either the GT or AG consensus site have been reported for the β -globin gene. All were associated with β^0 -thalassemia phenotype (10). In the family described in this

report, the point mutation precludes the production of the normal mRNA, resulting in the absence of effective β -globin synthesis. The mechanism would be the same for some other mutations in the identical site (16).

References

- 1) Weatherall DJ. The thalassemias. in: Hematology 3rd ed., Williams WJ, et al. Ed. McGraw-Hill Co., New York, p.493, 1983.
- 2) Bunn HF, Forget BG. Hemoglobin, Molecular, Genetic and Clinical Aspects. WB Saunders Co., Philadelphia, p.22, 1986.
- 3) Harano T. Molecular analysis of thalassemia. IGAKU NO AYUMI. (J Clin Exp Med) **162**(9): 593, 1992 (in Japanese).
- 4) Dacie JV, Lewis SM. Laboratory methods used in the investigation of the haemolytic anaemias. in: Practical Haematology 4th ed reprint, J. & A. Churchill Ltd., London, p.179, 1970.
- 5) Carrell RW, Kay R. A simple method for detection of unstable haemoglobins. Br J Haematol **23**: 615, 1972.
- 6) Harano T, Harano K, Koide T, Okada M, Ueda S, Shibata S. Mass screening method of abnormal hemoglobin by isoelectric focusing. Jpn J Clin Pathol **28**: 149, 1980 (in Japanese).
- 7) Harano T, Ueda S. Biosynthetic and analytical method of hemoglobin – a new technique for the diagnosis of thalassemia. Jpn J Clin Pathol **29**: 670, 1981 (in Japanese).
- 8) Messing J. New M13 vectors for cloning. in: Methods in Enzymology vol. 101, Wu R, Grossman L, Moldave K, Ed. Academic Press, New York, p.20, 1983.
- 9) Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA **74**: 5463, 1977.
- 10) Kazazian HH Jr. The thalassemia syndromes: Molecular basis and prenatal diagnosis in 1990. Semin Hematol **27**(3): 209, 1990.
- 11) Varamalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of β -thalassaemia mutations on the Indian subcontinent: the basis for prenatal diagnosis. Br J Haematol **78**: 242, 1991.
- 12) Kazazian HH Jr, Orkin SH, Antonarakis SE, et al. Molecular characterization of seven β -thalassemia mutations in Asian Indians. EMBO J **3**: 593, 1984.
- 13) Breitbart RE, Andreadis A, Nadal-Ginard B. Alternative Splicing: A ubiquitous mechanism for the generation of multiple protein isoforms from single genes. Ann Rev Biochem **56**: 467, 1987.
- 14) Efstratiadis A, Posakony JW, Maniatis T, et al. The structure and evolution of the human β -globin gene family. Cell **21**: 653, 1980.
- 15) Breathnach R, Benoist C, O'Hare K, Gannon F, Chambon P. Ovalbumin gene: Evidence for a leader sequence in mRNA and DNA sequences at the exon-intron boundaries. Proc Natl Acad Sci USA **75**: 4853, 1978.
- 16) Treisman RA, Proudfoot NJ, Shander M, Maniatis T. A single-base change at a splice site in a β^0 -thalassemic gene causes abnormal RNA splicing. Cell **29**: 903, 1982.