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A Case of Glanzmann's Thrombasthenia with β_3 Subunit Missense Mutation

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≡ Abstract ≡

Glanzmann's thrombasthenia is an autosomal recessively inherited hemorrhagic disorder that results from quantitative and qualitative abnormalities in platelet membrane glycoprotein IIb-IIIa, also known as $\alpha_{IIb}\beta_3$ integrin which is an adhesion receptor for fibrinogen and von Willebrand factor. We describe here a 4-year-old girl who had Glanzmann's thrombasthenia with the β_3 subunit missense mutation.

Key words : Glanzmann's thrombasthenia, $\alpha_{IIb}\beta_{3\alpha}$ integrin, missense mutation

Introduction

In 1918, Glanzmann, a Swiss pediatrician, described a somewhat heterogenous group of disorders that he termed "thrombasthenie" ("weak platelets"), and these disorders were characterized by normal platelet counts and platelet morphology, but the patients had prolonged bleeding times[1]. Braunsteiner and Pakesch in 1956 added to our understanding of what is now termed Glanzmann's thrombasthenia by noting that the platelets

from these patients failed to spread onto a surface[2]. Glanzmann's thrombasthenia is an autosomal recessive hemorrhagic disorder that is the result of the quantitative and qualitative abnormalities in the platelet membrane glycoprotein IIb-IIIa(also known as $\alpha_{IIb}\beta_3$) which is an adhesion receptor for fibrinogen and von Willebrand factor(vWF)[4]. Integrin $\alpha_{IIb}\beta_3$ is a calcium-dependent heterodimer that is exclusively expressed in megakaryocytes and

Table 1. Mutations within the α_{IIb} β-Propeller Domain

Patient	Genotype	Mutation*	Mutation phenotype	Amino acid† substitution
FLD	Homozygote	818G>A	Missense	G242D
Japanese-1	Compound	959T>C	Missense	F289S
	heterozygote	unknown	unknown	unknown
FL	Homozygote	1063G>A	Missense	E324K
Swiss	Compound	1063G>A	Missense	E324K
	heterozygote	1787T>C	Missense	I565T
Japanese-2	Compound	1063G>A	Missense	E324K
	heterozygote	unknown	unknown	unknown
KJ	Homozygote	1073G>A	Missense	R327H
Mila-1	Homozygote	1073G>A	Missense	R327H
LM	Homozygote	1346G>A	Missense	G418D
LeM	Compound	1366-1371del	Del:In frame	V425D426del
	heterozygote	unknown	unknown	unknown
Frankfurt	Homozygote	620C>T	Missense	T176I
LW	Homozygote	641T>C	Missense	L183P
Mennonite	Homozygote	526C>G	Missense	P145A
JF	Compound	526C>G	Missense	P145A
	heterozygote	2929C>T	Missense	R946X
Chinese-14	Compound	527C>T	Missense	P145L
	heterozygote	IVS15(-1)Gdel	unknown	unknown

*The nomenclature is based on recommendations by (14). The cDNA nucleotide begins with the A nucleotiede of the ATG start codon as +1[15]. Nucleotide substiturion: the CDNA nucleotide number is followed by the nucleotide > nucleotide substitution. Abbreviation: del-deletion, inv-inversion, IVS-intervening sequence.

†The amino acid numbering begins with methionine of the ATG start codon[15] and the amino acid codon number excluding the leader sequence is in parentheses. Amino acid substitutions are designated by amino acid-codon number-amino acid. Single letter amino acid code: C = cysteine, N = asparagine, P = proline, Q = glutamine, R = arginine, S = serine, V = valine, W = tryptophan, Y = tyrosine, X = nonsense mutation.

Table 2. Mutations within the β_3 MIDAS Domain

Patient	Genotype	Mutation	Mutation phenotype	Amino acid substitution
Cam	Homozygote	433G>T	Missense	D119Y
NR	Homozygote	433G>A	Missense	D119N
Strasbourg I	Homozygote	718C>T	Missense	R214W
CM	Homozygote	718C>T	Missense	R214W
ET	Homozygote	719G>A	Missense	R214Q
SH	Homozygote	725G>A	Missense	R216Q
MK	Homozygote	428T>G	Missense	L177W
BL	Homozygote	563C>T	Missense	S162L
LD	Compound	847delGC	Del: Out of	Premature
	heterozygote	863T>C	frame missense	termination L262P

Table 3. Mutations within the β_3 Cytoplasmic Domain

Patient	Genotype	Mutation	Mutation phenotype	Amino acid substitution
RM	Compound heterozygote	1791delT	Del: Out of	Premature
		2248C>T	frame missense	termination R724X
Paris I	Compound heterozygote	2332T>C	Missense	S752P
		Unknown	Unknown	No transcript

platelets, so that the absence of platelet aggregation results in a markedly prolonged bleeding time[5]. Glanzmann's thrombasthenia is subclassified into type Ⅰ(severely quantitative; surface expression of $\alpha_{IIb}\beta_3$ < 5% of the nornal value), type Ⅱ(mildly quantitative; α_{IIb} β_3 5-20% of the normal value) and variant(qualitative), although patients with both quantitative and qualitative abnormalities have been recently reported[6].

Several mutations in the α_{IIb} and β_3 genes have recently been identified in patients with Glanzmann's thrombasthenia. These include minor or major deletions, insertions, inversions, and mostly point mutations located throughout both genes[7, 8].

We report here on a case of a 4-year old girl with Glanzmann's thrombasthenia who had a β_3 subunit missense mutation.

Case

Patient: OO Chang, F/4 years

Chief complaint: Epistaxis for 8 hours

Past and Family history: The girl was born

as the second child after 40 weeks of gestation via a normal transvaginal delivery. The Apgar score was 8 at 1 minute and 9 at 5 minutes, and the birth weight was 2,770 g. She had suffered from a bleeding tendency including frequent epistaxis since early childhood and she had been admitted to the hospital four times for this reason. To the best of our knowledge, her parents were unrelated and her brother and her other relatives had no bleeding diathesis.

Present illness: She had bled from the nose for 8 hours, but she had no active bleeding when she was in the hospital.

Physical examination: She looked pale and acutely ill. Her body temperature was 36.8 ℃, the heart rate was 168 beats per minute, and the blood pressure was 100/60 ㎜Hg. Upon auscultation, her lung sounds were clear and her heart beat displayed regular tachycardia without any audible murmur.

Laboratory data: Hypochromic microcytic anemia (hemoglobin 5.6 g/dℓ, hematocrit 17.2%, mean corpuscular volume 67 fL), and a normal platelet count (308,000/㎣) were noted. The bleeding time was over 15 minutes (normal 1-4 minutes). The activated partial thromboplastin time (PTT), the prothrombin time (PT) and clot retraction were all within the normal range. The platelet morphology and size were normal on the peripheral blood smear. Aggregation studies revealed the absent of platelet aggregation in response to agonists such as adenosine diphosphate (ADP), collagen, arachidonic acid and thrombin, but there was normal platelet agglutination in response to ristocetin. The surface expression of the $\alpha_{IIb}\beta_3$ on her platelets was quantitated by flow cytometry. The level of complex expression was 10 %. She was diagnosed as having type II Glanzmann's thrombasthenia. An informed consent to collect the patient's subsequent blood samples for genetic analysis was obtained from her and her parents.

To analyze the genetic defect, the nucleotide sequences of the α_{IIb} and β_3 coding regions were determined by direct sequencing of the RT-PCR products from the platelet RNA. The sequences in the coding regions were identical to those of the published sequences of α_{IIb}[9] and β_3[10], except for a single nucleotide substitution in β_3. The patient had a heterozygous A to C substitution at position 937 in exon 5 of the β_3 cDNA (the cDNA numbering was based on (10))(Fig. 1).

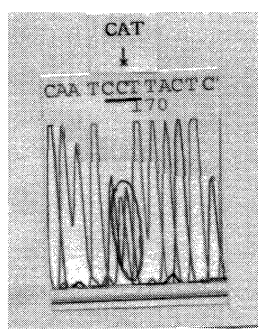

Fig. 1. Genomic analysis of this patient's β3 subunit gene. This shows a heterozygous A to C substitution at position 937 in exon 5 of the β3 cDNA.

This substitution results in a histidine-306 to proline amino acid substitution (H280P).

Progress and treatment: The patient received transfusion of packed red blood cells to relieve her symptomatic anemia that was due to the acute blood loss on the admission day. Her hemoglobin level was increased to 9.5 g/dL and she discharged on hospital day 3.

Discussion

The identification of the precise genetic defect underlying platelet hemorrhagic disorders may be of value to provide insight into the protein structure-function relationships of these disorders, to improve the differential diagnosis, to allow for early genetic testing for at-risk relatives, to unravel the correlation (if any) between the genotype and the clinical phenotype, and to assist in the development of new specific treatments, including gene therapy[11]. The main obstacle to studying Glanzmann's thrombasthenia is the large size of the α_{IIb} and β_3 genes and the intron/exon boundaries. A variety of mutations has been previously reported on(Table 1, 2, 3)[7, 12].

We have identified a missense mutation, CAT to CCT at position 937 in exon 5 of the integrin β_3 cDNA of Glanzmann's thrombasthenia in this case. In 1998, the same mutation of Glanzmann's thrombasthenia was identified for the first time in a Japanese patient. Ambo et al demonstrated that this mutation resulted in the deficient expression of the β_3 in the Glanzmann's thrombasthenia patients' platelets by performing a transfection experiment using Chinese hamster ovary cells[13]. To the best of our knowledge, there have been no reports of the same missense mutation of Glanzmann's thrombasthenia in Koreans. But, in this case, the transfection experiment using cells was omitted and the evidence that this missense mutation is the pathogenic genetic mutation for Glanzmann's thrombasthenia was replaced by Ambo et al's experiment.

국문 요약

황자영 · 서세영 · 한승훈 · 김소영 · 김현희 · 이원배
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Glanzmann's thrombasthenia는 혈소판 표면의 fibrinogen과 von Willebrand factor(vWF)의 수용체인 당단백 $\alpha_{IIb}\beta_3$의 결함으로 인해 ristocetin을 제외한 모든 agonist들에 대해 응집 이상을 보여 혈소판 수와 형태는 정상이면서 심한 출혈 시간의 연장을 가져오는 상염색체 열성 유전 질환이다. 저자들은 II형 Glanzmann's thrombasthenia로 진단된 4세 여아에서 β_3 유전자의 이상 중 보고되지 않은 부위의 이상을 최초로 밝혔기에 문헌 고찰과 함께 보고하는 바이다.

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