

Recurrent Bleeding Symptoms in an Infant with Heterozygous Mutation of Gamma Glutamyl Carboxylase Gene

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Abstract

Background: Congenital combined deficiency of the vitamin K-dependent coagulation factors (VKCFD) is a rare bleeding disorder. The mutations of γ -glutamyl carboxylase (GGCX), named VKCFD1, and the vitamin K epoxide reductase (VKOR) complex, named VKCFD2, have been reported.

Methods: The levels of prothrombin induced by vitamin K absence II (PIVKA II), in a female infant patient and her parents, were measured by enzyme immunoassay (Diagnostica Stago). The genetics of the *VKORC* and *GGCX* genes of all exons were identified using polymerase chain reaction technique (PCR) and submitted for sequencing.

Findings: A female infant was presented with recurrent bleeding. Her coagulogram revealed prolonged APTT and PT. The elevated PIVKAI suggested a defect in the vitamin K pathway. Genetic studies demonstrated a heterozygous mutation in the *GGCX* gene in exon 7, c.7973 C>G in the patient. The treatment included the administration of vitamin K and increased high-vitamin K food.

Conclusions: The heterozygous mutation of *GGCX* gene can cause recurrent bleeding symptoms and requires treatment with vitamin K until patient receives adequate amounts, namely more than the maintenance level, of vitamin K from food intake.

Keywords: Bleeding disorder; *GGCX* gene; Infant

Abbreviations: APCD: Acquired Prothrombin Complex Deficiency; APTT: Activated Partial Thromboplastin Time; Gla: Gamma-Carboxyglutamic Acid; GGCX: γ -Glutamyl Carboxylase; Glu: Glutamic acid; PCR: Polymerase Chain Reaction; IVKDI: Idiopathic Vitamin K Deficiency in Infancy; PIVKA: Proteins Induced by Vitamin K Absence;

PT: Partial Thromboplastin Time; VKCFD: Vitamin K-Dependent Coagulation Factors; VKOR: Vitamin K Epoxide Reductase

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Introduction

Congenital combined deficiency of the vitamin K-dependent coagulation factors (VKCFD) is a rare autosomal recessive bleeding disorder. The clinical presentations range from no bleeding to intracranial hemorrhage [1]. VKCFD can be diagnosed

after excluding acquired causes such as vitamin K deficiency, liver disease, intestinal malabsorption, or the ingestion of warfarin or rodenticide [2]. The etiologies of VKCFD are the mutations of γ -glutamyl carboxylase (GGCX), named VKCFD1, and the vitamin K epoxide reductase (VKOR) complex, named VKCFD2.

GGCX is a vitamin K-dependent enzyme which changes peptide-bound glutamic acid (Glu) into gamma-carboxyglutamic acid (Gla), a process which requires carbon dioxide, oxygen, and vitamin K hydroquinone (KH_2) [3]. The proteins containing Glu are factors II, VII, IX, X, protein C, protein S, protein Z, matrix Gla protein, osteocalcin, and periostin [1]. The function of vitamin K epoxidase is the conversion of vitamin K hydroquinone (KH_2) to vitamin K epoxide (KO).

A crucial investigation for vitamin K-dependent bleeding disorder is proteins induced by vitamin K absence (PIVKA-II), which is an inactive under- γ -carboxylated form of vitamin K-dependent clotting factor. PIVKAI level can be used to diagnose subclinical vitamin K deficiency. PIVKAI increases as the carboxylation process decreases [4].

The *GGCX* gene is located on chromosome 2 and has 15 exons. The protein contains 758 amino acids. Recently, there have been reports of mutations in the *GGCX* gene, most of which were compound heterozygous mutations [1,2]. Here, we report an infant with recurrent epistaxis and ecchymosis and found to have a heterozygous mutation in the *GGCX* gene.

Methods

After informed consent, blood specimens were collected in sodium citrate tube and EDTA tube. The blood in sodium citrate was spun at 3,000 rpm for 10 minutes to collect plasma for PIVKA II. The levels of PIVKA II, using enzyme immunoassay (Diagnostica Stago), were measured in the patient at the time of bleeding symptoms and in parents. DNA from EDTA tube was isolated by salting-out extraction method. Polymerase chain reaction (PCR) of all 15 exons of *GGCX* and 3 exons of *VKORC1* gene was amplified by using primers and conditions according to Oldenburge et al and Li T et al [5,6]. The PCR also included flanking intronic sequences and promoter regions. After that, the bidirectional sequencing was performed (ABI 370, Applied Biosystem, Foster City CA). The restriction fragment length polymorphism (RFLP) using DpnII enzyme was designed to identify the c.7973C>G in 56 normal subjects to confirm the mutation. The mutated allele showed 233 base pair band, while 133 and 100 base pair bands were found in the wild type.

Case report

A 1.2-year-old Thai girl was referred to our hospital for the diagnosis and management of a bleeding problem. Her history of bleeding began at the age of 7 months, with epistaxis and multiple ecchymosis. The initial screening at a local hospital revealed an activated partial thromboplastin time (APTT) of 231 sec (N 22-33) and a partial thromboplastin time (PT) of 160 sec (N 10.0-13.5). Her liver function test was normal. She had no underlying diseases or allergies, no surgical history, and no signs of being abused. Furthermore, there was no family history of bleeding disorders, except for her mother's cousin who had a history of bleeding at the age of 40. The patient experienced a normal term labor on the way to the hospital, received intravenous vitamin K after birth, and had a normal post-natal history. She was breast-fed and ate 2 daily meals of solid food containing rice, chicken, fish, and green vegetables. Her estimated vitamin K intake from

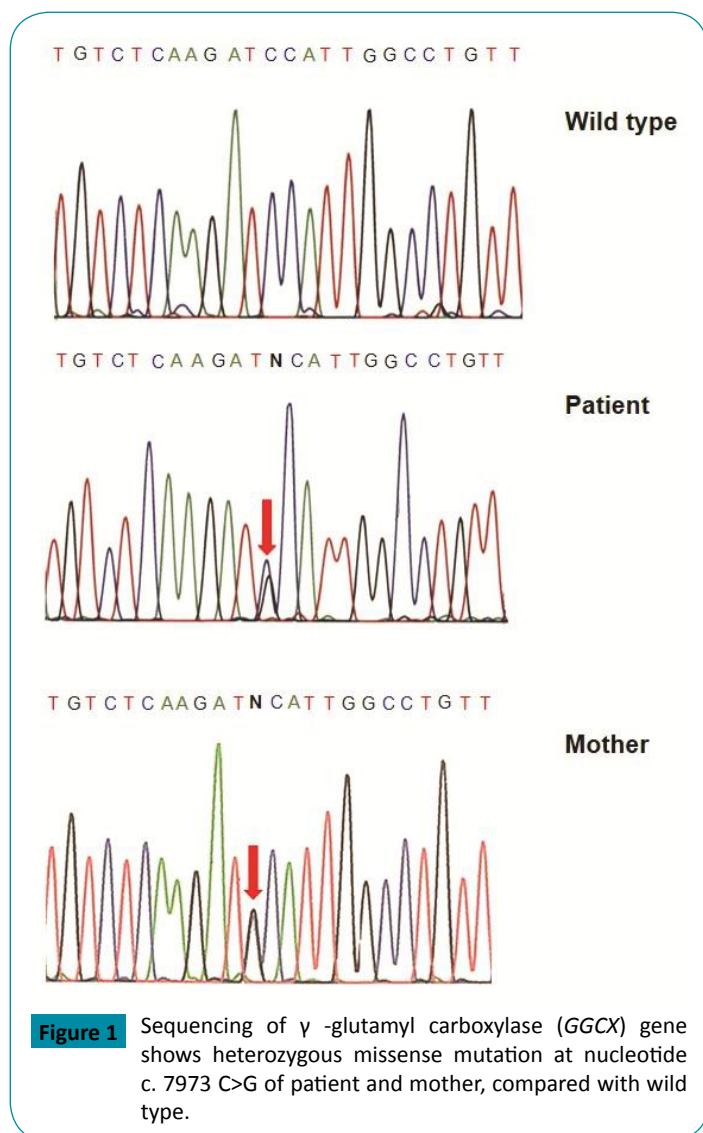
solid food was 45 mcg/day (4.5 mcg/kg/day, maintenance 1-5 mcg/kg). In addition, her mother maintained a balanced diet including fruit and vegetables, with an estimated vitamin K intake of 275 mcg/day (maintenance 90 mcg/day).

According to the bleeding symptoms and prolonged APTT and PT, she was suspected to have Idiopathic vitamin K deficiency in infancy (IVKDI) or acquired prothrombin complex deficiency (APCD). As a result, 2 mg of intravenous vitamin K was administered. The patient's coagulogram became normal and bleeding symptoms disappeared. Two months later, there was a recurrence of multiple ecchymosis and epistaxis, which was reversed with 5 mg of intravenous vitamin K. Eventually, she was referred for further investigation and management. Physical examination revealed epistaxis and ecchymosis on her trunk and extremities. No hepatosplenomegaly or lymphadenopathy was found. Investigations at Ramathibodi Hospital found the patient's APTT and PT were prolonged but those of the parents were normal (Table 1). Complete blood counts and liver enzymes tests were normal. The levels of PIVKA II were elevated in the patient, slightly elevated in the mother, and normal in the father. Genetic studies of *VKORC* and *GGCX* demonstrated a heterozygous mutation in the γ -glutamyl carboxylase gene in exon 7, c.7973 C>G in the patient and in the mother. The father was normal for the *GGCX* and *VKOR* gene mutation (Table 1 and Figure 1).

Due to a history of recurrent bleeding in the patient, she had been treated with regular intramuscular vitamin K of 5 mg weekly until the age of 2. The follow-up coagulogram before vitamin K became normal. At the same time, the patient was encouraged to consume more food containing high vitamin K, and then vitamin K was tapered off to every 2 weeks until the age of 3, when it was withdrawn without consequences. The estimated vitamin K intake was 128 mcg/day (8.5 mcg/kg). Six months after vitamin K withdrawal, APTT and PT were normal.

Discussion

This patient presented with epistaxis and ecchymosis at the age of 7 months. Laboratory findings showed prolonged APTT and PT, and a high level of PIVKAI. Due to the patient's age at presentation and the laboratory results, IVKDI or APCD was suspected even though the patient had received vitamin K at birth, as the incidence of APCD reported by the Thailand National Survey was 4.2-7.8 per 100,000 births [7]; higher than that of VKCFD [8]. The recurrence of symptoms after vitamin K administration was uncommon for IVKDI or APCD [9]. In addition, the immaturity of the liver is unlikely because vitamin K dependent factors reach the levels closed to adult levels at 6 months [10]. Therefore, a mutation in either the *GGCX* or the *VKORC* gene was suspected. In this patient, a genetic study of both genes was performed. The heterozygote of the *GGCX* gene was identified as a missense mutation in exon 7, c.7973 C>G, resulting in a change in amino acid from serine to cysteine at 277 position. In order to confirm that p.S277C is a mutation, the DNA of 56 healthy controls, aged 8.9 \pm 0.7 years and with a female to male ratio of 1:1, was performed by RFLP technique and no c.7973 C>G was detected. In addition, this mutation was not identified in the exome sequencing database in 156 Thai population.



The carboxylase polypeptide consists of a hydrophobic amino-terminal half, which has several transmembrane domains and is located on the cytoplasmic side, and a hydrophilic carboxy-terminal half, which is located on the luminal side of the endoplasmic reticulum [11]. The mutation in this patient was located in the hydrophobic region at the amino-terminal of the enzyme. Mutations in hydrophobic region have been shown to impair carboxylase activity [12]. To our knowledge, this mutation has never been reported. The heterozygote in this patient is proposed to cause partial defect in the enzyme activity. As a result of this mutation, patients may require a higher amount of vitamin

K in order to have adequate amounts of carboxylated coagulation proteins. After vitamin K treatment, bleeding symptoms in this patient were disappeared. Contrarily, in reported patients with compound heterozygote, vitamin K could only partially correct factor activities and may not prevent all bleeding [8]. Before obtaining the genetic findings, the treatment plan was to give the patient regular vitamin K injections. However, after receiving the genetic findings, effort was made to decrease the frequency of vitamin K injections to zero while the patient received more than the required amount of vitamin K from food intake. The laboratory results of the mother revealed normal coagulogram while carrying a similar mutation to the patient. The PIVKAI1 of the mother revealed a higher than normal level, indicating some defect in carboxylation. The patient did not have a non-bleeding phenotype such as midfacial hypoplasia, premature osteoporosis, cochlear hearing loss, or heart valve defects, which can be found in patients with mutations of the *GGCX* gene in both alleles [13-17].

The limitation of the study is that the method could not detect the large mutation or duplication. However, to our knowledge, the large mutation and duplication have never been reported. In addition, if a patient has compound heterozygote, the symptoms of bleeding would be more severe than what noted in this studied patient. The normal PIVKAI1 level in father as compared to mother may imply the possibility of wild type in father.

Conclusion

Although most reports of VKCFD have been of compound heterozygous mutations in the *GGCX* gene, the heterozygous mutation may cause bleeding symptoms and require treatment with vitamin K until patients receive adequate amounts, namely more than the maintenance level, of vitamin K from food intake.

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Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict of bias.

Table 1 Symptoms and laboratory results of patient and parents at the time of presentation.

	Patient	Father	Mother
Symptom	Recurrent epistaxis, ecchymosis	None	None
Coagulogram*			
• APTT (N 22-28 sec)	231	29	22.8
• PT (N 10-14 sec)	160	10.7	10.8
• INR	15.99	0.9	0.91
• TT (N 9-12 sec)	11.3		
PIVKA II (N<2 ng/ml)	223	1.6	2.79
VKORC1 gene	Normal	Normal	Normal
GGCX gene -Nucleotide (Amino acid)	Heterozygous c.7973C>G** (p.S277C)	Normal	Heterozygous c.7973C>G** (p.S277C)

S = Serine, C = Cysteine, GGCX = Gamma-Glutamyl Carboxylase, PIVKAII = Protein Induced by Vitamin K Absence II, VKORC1 = Vitamin K Epoxide Reductase Complex 1

*coagulogram is a group of laboratory testing's to determine the clot formation

**Nucleotide positions are specified according to Wu SM et al [14]

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