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Congenital factor V deficiency in Taiwan: identification of a novel variant p.Tyr1813* and two variants specific to East Asians

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Congenital coagulation factor V deficiency (FVD) is a rare, autosomal recessive bleeding disorder. We characterized the clinical presentations, laboratory features, and genetic alterations of Taiwanese patients with FVD. From 1983 to 2010, five women, one man, and one boy diagnosed with FVD were enrolled in this study. The factor V coagulant activity was determined using a one-stage prothrombin time-based test. The factor V antigen level was measured in an ELISA. Sanger sequencing was performed for genetic analyses of *F5*, the gene responsible for the disease. One novel and de novo *F5* genetic variant, p.Tyr1813*, was identified. Based on the presence of a premature termination codon with a resultant truncated factor V-protein lacking an intact light chain fragment, the variant is pathogenic. In addition, we identified seven variants previously found to cause FVD. Among them, p.Gly420Cys and p.Asp96His were repeatedly detected in five and four patients, respectively. Both variants are found to be specific to the East Asian populations. Various FVD-associated bleeding manifestations were observed, predominantly mucocutaneous bleeding and hypermenorrhea. All patients exhibited very low factor V coagulant activity (<1–2.5 IU/dl, reference range: 60–133 IU/dl). The factor V antigen level was less than 2% in six patients (reference range: 75–157%). The novel *F5* genetic variant p.Tyr1813* and two distinct, East Asians-specific, recurrent variants p.Gly420Cys and p.Asp96His were identified among seven index patients with FVD in Taiwan. Our clinical and laboratory findings support the reported features of FVD.

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Introduction

In the coagulation system, factor V is structurally and functionally homologous to factor VIII. Both proteins are essential biological cofactors that undergo proteolysis process for activation [1]. Factor V is a large, multidomain (A1–A2–B–A3–C1–C2) glycoprotein. Its gene, *F5*, mapped to the chromosome 1q23 locus, is composed of 25 exons and 24 introns and is 74.6 kb in length [2]. The activated form of factor V, FVa, is transformed by thrombin or activated factor X (FXa) [3]. FVa consists of a heavy chain (A1–A2) and light chain (A3–C1–C2) noncovalently linked via a calcium ion [4]. By serving as a cofactor, FVa associates with FXa in the prothrombinase complex, which converts prothrombin to thrombin to contribute to clot formation [5]. In addition to its procoagulant role, factor V exerts anticoagulant activity through the activated protein C (APC)-mediated inactivation of coagulation factor VIII [5]. Most circulating factor V is synthesized by hepatocytes in the liver. However, a small portion of factor V is contained in alpha granules within platelets (platelet factor V) and megakaryocytes [6].

Congenital factor V deficiency (FVD, OMIM *227400*), also known as parahemophilia, was first described by Owren in 1943 [7]. FVD is a rare, autosomal recessive bleeding disorder [8,9], with an estimated prevalence in the general population of 1:1000 000. Individuals with FVD harboring either homozygous or compound heterozygous causative variants in *F5* commonly have low or undetectable plasma factor V levels along with bleeding diathesis [10]. Manifestations of bleeding associated with FVD include mucosal bleeding, epistaxis, oral cavity bleeding, hemarthrosis, posttraumatic or postoperative bleeding, menorrhagia (in affected women), and rarely, life-threatening bleeding (such as central nervous system bleeding) [7–11]. Some specific *F5* genetic variants can predispose the affected individuals to venous thromboembolism; for example, factor V Leiden induces resistance to the inactivation mediated by APC. Nevertheless, bleeding-related phenotypes are associated with most recognized *F5* alterations [5]. Moreover, residual factor V activity and the relevant bleeding severity in FVD show a poor correlation [12].

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According to the Report on the Annual Global Survey 2020 published by the World Federation of Hemophilia [13], 2554 FVD cases have been identified worldwide. However, only a few cases were reported in Taiwan [14–18]. In this study, we characterized the clinical and laboratory features of seven unrelated index patients with FVD in Taiwan. We performed genetic analyses of patients with FVD and their family members.

Methods

Patient records from 1983 to 2010 were collected from Changhua Christian Hospital and National Taiwan University Hospital, which are two referral medical centers in central and northern Taiwan, respectively. Seven unrelated index patients with FVD were identified and included in the study. Their family members were recruited when available. Clinical characteristics, laboratory data, and genetic alterations were assessed. The relevant bleeding manifestation and severity were evaluated using the International Society on Thrombosis and Haemostasis bleeding assessment tool (ISTH-BAT) [19]. All enrolled participants and their family members provided informed consent. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board (IRB), CCH IRB No. 210130.

Blood samples were collected from the patients and their family members. Factor V coagulant activity (FV:C) was determined in a one-stage prothrombin time-based test. The factor V antigen (FV:Ag) level was measured using an ELISA (Zymutest Factor V, Hyphen Biomed, Neuville-sur-Oise, France), according to the manufacturer's protocol.

For *F5* mutational analysis, genomic DNA was purified from the separated buffy coat using the Quick-DNA Universal Kit (Zymo Research, Irvine, California, USA) according to the manufacturer's instructions. The primers were designed to span the intron/exon boundaries of *F5*. All 25 coding exons and the flanking intronic sequences of *F5* were amplified using PCR with the primers described by Jenny *et al.* [20]. The amplified DNA was sequenced by Sanger sequencing. The genetic findings were described following the guidelines of the Human Genome Variation Society [21], with reference to the Human Gene Mutation Database (HGMD) [22].

Results

Study participant characteristics

One 7-year-old boy, one 44-year-old man, and five women (aged 27–45) were enrolled. Their clinical features are shown in Table 1. The associated laboratory data, including prothrombin time, activated partial thromboplastin time (aPTT), template bleeding time, FV:C, FV:Ag, and genetic alterations are shown in Table 2.

Identification of a novel and de novo variant associated with factor V deficiency in patient II

One novel *F5* variant, c.5438_5439 delAC p.Tyr1813*, characterized by a genetic 2-bp frameshift deletion in exon 17, was identified in patient II. In addition, two distinct *F5* genetic alterations, that is, p.Gly420Cys and p.Asp2222Gly, were identified in this patient.

The clinical manifestations and relevant laboratory data of the proband (patient II) are summarized herein. The female patient was 33 years old when she was diagnosed with FVD. She reported prolonged bleeding after tooth extraction during childhood, recurrent ovary hemorrhage episodes which twice required laparotomy for bloody ascites evacuation at the age of 23 years, and frequent heavy menstrual bleeding during menstruation cycles, with resultant severe anemia that required blood transfusions since adolescence. The coagulation assay revealed prothrombin time of 31 s (reference range, 10–14 s),

Table 1 Clinical manifestations and bleeding scores[a] of seven index patients with congenital factor V deficiency

Patient	I	II	III	IV	V	VI	VII
Age[b]/Sex	44/M	33/F	43/F	7/M	27/F	34/F	45/F
ISTH/SCC bleeding score							
Symptoms (up to the time of diagnosis)							
Epistaxis	0	1	2	0	2	0	0
Cutaneous	2	1	0	0	2	0	0
Bleeding from minor wounds	1	0	0	0	1	0	1
Oral cavity	0	1	0	0	0	0	0
GI bleeding	0	0	0	0	0	0	0
Hematuria	0	4	0	0	0	0	1
Tooth extraction	2	2	0	0	4	2	3
Surgery	3	0	0	0	0	0	4
Menorrhagia		3	2		3	0	3
Postpartum hemorrhage		3	0		0	0	1
Muscle hematomas	0	0	0	0	0	0	0
Hemarthrosis	0	0	0	0	0	0	0
CNS bleeding	0	0	0	0	0	0	0
Other bleedings	0	0	0	0	0	0	0
Total	8	15	4	0	12	2	13

CNS, central nervous system; F, female; GI, gastrointestinal; ISTH/SSC, International Society on Thrombosis and Haemostasis/Scientific and Standardization Committee; M, male. [a] Bleeding scores were determined using the ISTH/SCC bleeding assessment tool. [b] Age when diagnosed.

Table 2 **Genetic defects and laboratory data of seven patients with congenital factor V deficiency**

Index patient	Age/ Sex	Codon	Exon	Nucleotide substitution	Amino acid substitution	Comment	PT (s)	aPTT (s)	BT (min)	FV:C (IU/dl)	FV:Ag (%)	Platelet counts ($\times 10^3/\mu l$)
I	44/M					Compound heterozygous	45	165	65	<1	<2	242
		420	8	c.1258G>T	p.Gly420Cys	From mother						
		558	11	c.1673A>C	p.Tyr558Ser	From father						
II	33/F					Compound heterozygous	31	70	16	<1	<2	135
		420	8	c.1258G>T	p.Gly420Cys	From mother						
		2222	25	c.6665A>G	p.Asp2222Gly	From father						
		1813[a]	17	c.5438_5439delAC	p.Tyr1813*	De novo and to daughter						
III	43/F					Compound heterozygous	31	70	NA	1.4	NA	300
		96	3	c.286G>C	p.Asp96His	To daughter						
		420	8	c.1258G>T	p.Gly420Cys	To son						
IV	7/M					Homozygous	29	47	7	2.5	<2	324
		96	3	c.286G>C	p.Asp96His	From parents						
V	27/F					Compound heterozygous	37	97	7	<1	<2	355
		420	8	c.1258G>T	p.Gly420Cys	From mother						
		2059	22	c.6175C>T	p.Gln6175*	From father						
VI	34/F					Compound heterozygous	23	67	6.5	2.2	<2	406
		96	3	c.286G>C	p.Asp96His	From father						
		175	4	c.524A>G	p.His175Arg	From mother						
		2102	23	c.6304C>T	p.Arg2102Cys	From mother						
VII	45/F					Compound heterozygous	26	47	4.0	2.5	<2	256
		96	3	c.286G>C	p.Asp96His	To two daughters						
		420	8	c.1258G>T	p.Gly420Cys	To son						

Normal range: PT 10–14 s, aPTT 27–40 s, BT 2–9 min, FV:C 60–133 IU/dl, FV:Ag 75–157%, platelet counts 157–377 $\times 10^3/\mu l$. aPTT, activated partial thromboplastin time; BT, bleeding time; FV, factor V; FV:Ag, FV antigen; FV:C, FV coagulant activity; NA, not available; PT, prothrombin time. [a]Novel mutation.

aPTT of 70 s (27–40 s), and template bleeding time of 16 min (2–9 min). The FV:C and FV:Ag levels were substantially low at less than 1 IU/dl (60–133 IU/dl) and less than 2% (75–157%). The results for all other coagulation factors were normal. The prothrombin time and aPTT of the patient's parents and the patient's daughter were normal (data not shown).

Further genetic investigation of the family members revealed that the genetic variant p.Tyr1813* was de novo (Fig. 1), as neither parent harbored this mutation (father, heterozygous, p.Asp2222Gly; mother, heterozygous, p.Gly420Cys). The patient's daughter inherited this variant along with p.Asp2222Gly.

Identification of two frequent genetic mutations associated with factor V deficiency

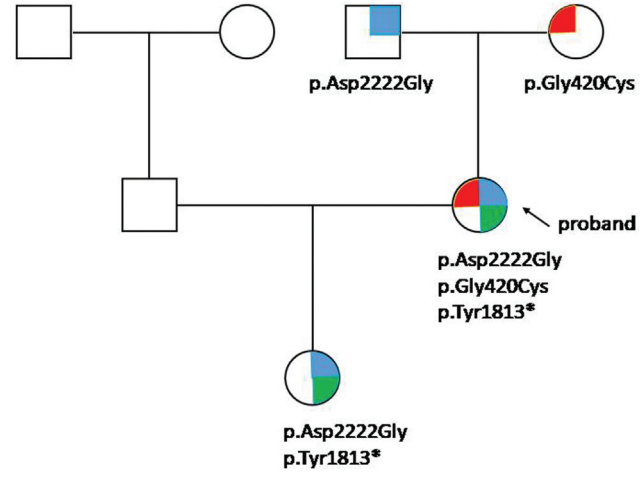
Two genetic variants were frequently detected in our cohort: c.1258 G>T p.Gly420Cys in exon 8 was detected in five patients (I, II, III, V, and VII), and c.286 G>C p.Asp96His in exon 3 in four patients (III, IV, VI, and VII).

Other genetic, clinical, and laboratory findings associated with factor V deficiency

Eight distinct genetic alterations in *F5* were identified in our cohort (Table 2). One patient (patient IV) was homozygous, whereas the remaining patients showed as compound heterozygous genotype.

Most patients suffered from mucocutaneous bleeding (Table 1), whereas their ISTH-BAT scores were in a wide range (0–15). The most common symptoms (until the time of diagnosis) included menorrhagia (observed in four of five females, 80%) and bleeding after dental extraction (71%), followed by epistaxis (43%), cutaneous bleeding (43%), and prolonged bleeding from minor wounds (43%). Hematuria (29%), postoperative bleeding (29%), and oral cavity bleeding (14%) were also noted. In

Fig. 1



Pedigree of patient II with congenital factor V deficiency. The patient (patient II, proband) expressed a factor V deficiency phenotype, as a result of compound heterozygosity with the involvement of three distinct disease-associated genetic variants. They included p.Tyr1813* (green, the novel variant), p.Asp2222Gly (blue) (both in the paternal allele), and p.Gly420Cys (red, in the maternal allele). Inheritance of the double-mutant, paternal allele by the patient's daughter is also shown.

addition, postpartum hemorrhage was observed in two affected female patients (40%). Paradoxically, one affected individual reported no remarkable abnormal bleeding events (asymptomatic, patient IV). This patient was born to parents of a consanguineous marriage. No thrombotic episodes were observed in any of our patients.

All patients exhibited prolonged prothrombin time and aPTT (Table 2). The FV:C level was between less than 1 and 2.5 IU/dl (reference range, 60–133 IU/dl) in all index patients. The FV:Ag level, which was measured in six patients, was less than 2% (75–157%). Bleeding manifestation and factor V activity showed no correlation.

Discussion

One novel *F5* genetic variant, c.5438_5439 delAC p.Tyr1813*, was identified in patient II and the patient's daughter. This variant introduces a premature termination codon at residue 1813 in the A3 domain of factor V, resulting in a truncated factor V protein without an intact light chain fragment. According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines [23], this variant is considered as pathogenic.

In addition to p.Tyr1813* (Fig. 1), the patient's daughter inherited another genetic alteration, p.Asp2222Gly, a single nucleotide polymorphism that can induce a reduction in the factor V level [24,25]. The factor V level in the patient's daughter was not directly assessed. Nevertheless, both prothrombin time and aPTT in the patient's daughter were found to be within the reference limits. These findings disagree with the typical coagulation abnormalities observed in FVD-affected individuals, indirectly demonstrating that the patient's daughter did not have FVD. Considering the importance of p.Asp2222Gly in reducing the factor V level and pathogenic implication of p.Tyr1813*, these two variants carried by the patient's daughter were likely only at the same allele rather than at different alleles, as the latter positioning may form the compound heterozygous genotype associated with FVD. Hence, the novel variant, p.Tyr1813*, is at the p.Asp2222Gly mutant allele that originated from the patient's father, rather than at the other allele from the patient's mother. This genetic positioning suggests that p.Tyr1813* is of paternal origin.

In addition, de novo variant, defined as variants that appear in the patient but not in the patient's parents, occurs either during gamete formation or postzygotically. The mutation rate is correlated with the number of cell divisions; in general, the number of cell divisions during spermatogenesis is extremely high [26]. So the most likely scenario is that the de novo variant p.Tyr1813* developed through mutation in a single sperm cell [27] that already possessed p.Asp2222Gly (Fig. 1).

Significantly, two variants were frequently found in our cohort, p.Asp96His (4/7 patients) and p.Gly420Cys (5/7 patients). According to the HGMD, 134 pathogenic variants with a definite FVD phenotype have been identified and registered, including missense mutations (accounting for approximately half of the variants), nonsense mutations, small deletions, splicing mutations, and small insertions [22]. Because of the large size of *F5* and lack of mutational hotspots, most FVD-causing mutations are considered as unique and segregated to a specific family or region. To our knowledge, p.Asp96His has been reported only in Taiwan [15,16,18], China [28,29], and Korea [30], whereas p.Gly420Cys has been identified in Taiwan [14], China [31,32], Japan [33], and not in Western regions. Hence, these two genetic variants may be specific to East-Asian populations. Review of the available information among several genomic databases, including dbSNP (https://www.ncbi.nlm.nih.gov/snp/), GnomAD (https://gnomad.broadinstitute.org/), Ensembl (https://asia.ensembl.org/index.html), VarSome (http://varsome.com/), and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), validated the proposed regional distinct genetic predisposition. Nevertheless, further studies are needed to confirm the possible 'founder effects' associated with these two variants.

Seven of the genetic variants associated with FVD identified in our study have been previously described in the literature, including p.Asp2222Gly [18,24,25], p.Asp96His [15,16,18], p.Gly420Cys [14,31–33], p.His175Arg [15], p.Tyr558Ser [34], p.Gln2059* [35], and p.Arg2102Cys [15,36]. We previously reported the functional and molecular characteristics of the variants p.Asp96His [16] and p.His175Arg [15].

According to several published large series [9–11,37–39], the most frequent symptoms of FVD are mucosal bleeding, such as epistaxis, menorrhagia, and oral cavity bleeding, followed by postsurgical bleeding. Our findings mostly corroborate the previously reported bleeding characteristics associated with FVD. There were no cases of hemarthrosis, hematoma formation, or life-threatening bleeding episodes in our cohort, although these manifestations are occasionally reported as associated with FVD [9–11].

All patients showed a very low FV:C. The associated FV:Ag level was less than 2% among the six patients evaluated. These findings indicate that the six patients have type I (quantitative) deficiency. However, varying FVD-associated manifestations and severities were observed, ranging from no remarkable abnormal bleeding to severe postpartum hemorrhage. Our results highlight the unpredictable correlation between clinical presentation and residual factor V activity, a recognized feature of FVD [12]. Several mechanisms have been proposed to explain the discrepancies observed between factor V activity and bleeding manifestations related to FVD [6], including the sufficient thrombin generation capacity of residual factor

V activity and the potential contribution from platelet factor Vs, which account for 20% of the total circulating factor Vs. Nevertheless, we did not evaluate the platelet factor V, although one patient displayed a prolonged bleeding time (patient II). In addition, the FVD-related clinical presentations may have been complicated or modified by the presence of various thrombophilic and/or procoagulant factors in different individuals. These predictions support the asymptomatic phenotype of patient IV, although the specific investigation was not performed in this case.

In our experience, the initial abnormal coagulation findings of FVD are often suggested by prolonged prothrombin time and aPTT, observed during screening for bleeding diathesis or when evaluating abnormal bleeding episodes. Subsequent detection of low factor V activity suggests the diagnosis of FVD. However, FVD should be differentiated from the rare bleeding disease known as combined factor V and factor VIII deficiency, as both diseases exhibit similar clinical manifestations, autosomal recessive inheritance, and routine coagulation laboratory abnormalities [40]. Nevertheless, the level of factor VIII is normal in FVD. Conclusive diagnosis based on these features should be made cautiously by determining the activities of relevant coagulation factors.

The current study had several limitations. First, functional experiments of our newly identified variant are needed to validate its molecular mechanism and significance. Second, some relevant data, such as platelet factor V levels in the patients and the factor V activity and antigen levels in the family members, were not available when this article was written. Furthermore, highly mutational heterogeneity associated with FVD was recently detected using modern sequencing technologies [39,41]. However, we used the conventional sequencing method for genetic analysis.

Conclusion

We investigated *F5* genetic alterations and determined the relevant clinical presentations and laboratory findings in seven patients with inherited FVD in Taiwan, thereby contributing to the understanding of the inherited rare bleeding disorder. We identified a novel and de novo, pathogenic variant, p.Tyr1813*, which is associated with early termination factor V-protein production. In addition, two variants, p.Asp96His and p.Gly420Cys, were recurrently found in our cohort. Both variants appeared to be specific to East-Asian populations after the database research. Furthermore, we characterized the FVD-associated clinical manifestations and laboratory information, which mainly corroborate the reported features of FVD.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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