

CASE REPORT

Combined factor V and factor VIII deficiency in a Thai patient: a case report of genotype and phenotype characteristics

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Summary. A Thai woman, with no family history of bleeding disorders, presented with excessive bleeding after minor trauma and tooth extraction. The screening coagulogram revealed prolonged activated partial thromboplastin time and prothrombin time. The specific-factor assay confirmed the diagnosis of combined factor V and factor VIII deficiency (F5F8D). Her plasma levels of factor V and factor VIII were 10% and 12.5% respectively. The medications and blood product treatment to prevent bleeding from invasive procedure included 1-deamino-8-D-arginine vasopressin, cryoprecipitate, factor

VIII concentrate, fresh frozen plasma and antifibrinolytic agent. Gene analysis of the proband identified two *LMAN1* gene mutations; one of which is 823-1 G → C, a novel splice acceptor site mutation that is inherited from her father, the other is 1366 C → T, a nonsense mutation that is inherited from her mother. Thus, the compound heterozygote of these two mutations in *LMAN1* cause combined F5F8D.

Keywords: bleeding, combined factor V and factor VIII deficiency, ER-Golgi intermediate compartment protein, *LMAN1*

Introduction

Combined factor V and factor VIII deficiency (F5F8D) is a rare autosomal recessive bleeding disorder first described by Oeri *et al.* [1]. To date more than 100 recognized cases have been reported, usually in the context of consanguineous marriage [2–6]. The estimated prevalence of the disease is about 1:100 000.

The presentation is mild to moderate bleeding symptoms such as easy bruising, menorrhagia, epistaxis, gum bleeding, muscle bleeding and bleeding after surgery or tooth extraction. The reported plasma level of both factors ranges between 5%

and 30% and the successful treatments in order to prevent bleeding from surgery include administration of 1-deamino-8-D-arginine vasopressin (DDAVP) and plasma transfusion [7,8].

Previous mechanisms proposed for this condition included deficiency of a protein C inhibitor [9] and coinheritance of both factor VIII and factor V deficiency [10]. In 1997, Nichols *et al.* [11] used homozygosity mapping to identify a 2.5 cM locus on chromosome 18 that was tightly linked to the gene for F5F8D. Shortly after that, the recombinant interval at chromosome 18q21 was matched to the gene *LMAN1* (previously identified as ERGIC-53), a protein of the endoplasmic reticulum (ER)-Golgi intermediate compartment with previously unknown function [12–14]. At present, *LMAN1* mutations are identified in 74% of the patients [15,16]. Until 2003, Zhang *et al.* [17] described mutations in a second gene, called multiple combined factor deficiency 2 (*MCFD2*) on chromosome 2, which forms the complex to the ERGIC through a direct, calcium-

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dependent interaction with *LMAN1*. The mutation found on *MCFD2* can explain this disease in almost all of the remaining patients.

We report the clinical presentations and the identification of *LMAN1* mutations in a non-consanguineous, Thai ethnic background patient who was diagnosed with F5F8D.

Methods

After obtaining the informed consent, blood samples were collected by standard double syringe technique from an antecubital vein into evacuated tubes containing EDTA and sodium citrate for complete blood count and coagulation tests, respectively. The samples were centrifuged at 1,600 g for 15 min within 2 h after collection. Then, the plasma was frozen at -70°C until further study. Coagulation tests included activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT). Factor VIII and factor V clotting activities (factor VIII:C and factor V:C) were performed using the one stage method, based on the APTT and PT, respectively, with human lyophilized factor deficient plasma obtained from Instrumentation Laboratory, USA.

The DNA fragments containing coding exons and exon-intron junctions of *MCFD2* and *LMAN1* genes from the proband and family members were amplified by polymerase chain reaction (PCR) and sequenced as described [16,17].

Results

A 25-year-old Thai woman was born after an uneventful pregnancy with no family history of bleeding disorders or consanguineous marriage. She was found with superficial bruising a few times during her toddler period and a traumatic bleeding episode in her left eye, which subsequently caused her vision loss of that eye at the age of 2. She also had a history of prolonged bleeding after tooth extraction at the age of 10 and was referred to our centre when she was 11 years old for the investigation of the aetiology of bleeding and a heart murmur problem.

Clinical examination showed a few bruises on her forearm, an atrophic left eye, dental caries and mild cardiomegaly with systolic ejection murmur grade 4/6 and early diastolic murmur grade 2/6 at the left upper sternal border.

At that time, her laboratory findings revealed a normal complete blood count, normal liver profile and normal blood chemistry. The coagulogram showed a prolonged APTT 68 s [normal range (N) 32–38 s] and PT of 18.5 s (N 11.5–14.5 s) but

normal TT of 10 s (N 9–12 s). Coagulation factors showed fibrinogen 292 mg dL^{-1} , prothrombin 120%, factor VII clotting activity 63%, factor IX clotting activity 90%, factor X clotting activity 62%, factor V clotting activity 10%, factor VIII clotting activity 12.5%, factor VIII antigen 8% and von Willebrand factor antigen 80%. The bleeding time was 6 min and platelet aggregation induced by ADP, adrenalin, collagen and ristocetin was normal. Thus, the diagnosis of F5F8D was made.

The results of the chest X-ray, electrocardiogram and 2D-echocardiography all supported the diagnosis of subaortic ventricular septal defect and aortic valve insufficiency.

During the hospitalized period, a clinical trial of $0.3\text{ }\mu\text{g kg}^{-1}$ of DDAVP via intravenous route was conducted. The factor VIII:C peak response increased from 10% to 70% at 30 min, 28% at 12 h and 18% at 24 h; however, factor V:C was not changed. As fresh frozen plasma (FFP) had been used in order to increase factor V:C, the dose of 20 mL kg^{-1} was able to increase factor V:C from 10% to 25%.

For tooth extraction, oral tranexamic acid was given. The haemostasis was achieved by giving 20 mL kg^{-1} of FFP and $0.3\text{ }\mu\text{g kg}^{-1}$ of DDAVP for raising factor V:C and factor VIII:C, respectively, prior to procedure. The celluloid splint was applied as a local haemostatic measure immediately after tooth extraction. Subsequently, FFP, at 10 mL kg^{-1} , and DDAVP were given every 12 h for three times. Before removal of the splint, 20 mL kg^{-1} of FFP and DDAVP were given. No excessive bleeding occurred.

For open heart surgical correction, factor VIII concentrate had been used at $50\text{ }\mu\text{g kg}^{-1}$ in order to raise the level to 100%. The level was maintained with continuous infusion of factor VIII concentrate. During surgery and the postoperative period, the rate of infusion was adjusted by maintaining the plasma level at about 80–100% on days 1–3. The FFP at 10 mL kg^{-1} , was infused within 1 h before operation and 10 mL kg^{-1} during operation to maintain the haemostatic level of factor V. After surgery FFP, at 10 mL kg^{-1} , was given every 8 h on days 1–3. Subsequently both factor VIII concentrate and FFP infusions were gradually decreased after day 4 postoperatively. On days 4–8, the factor VIII levels were maintained at 40–50% and FFP, at 10 mL kg^{-1} , was given every 12 h until the sutured material stitches were removed.

To identify the mutation causing F5F8D in this patient, DNA was amplified by PCR using gene analysis of the proband. The smaller *MCFD2* gene was first sequenced. No mutations were identified.

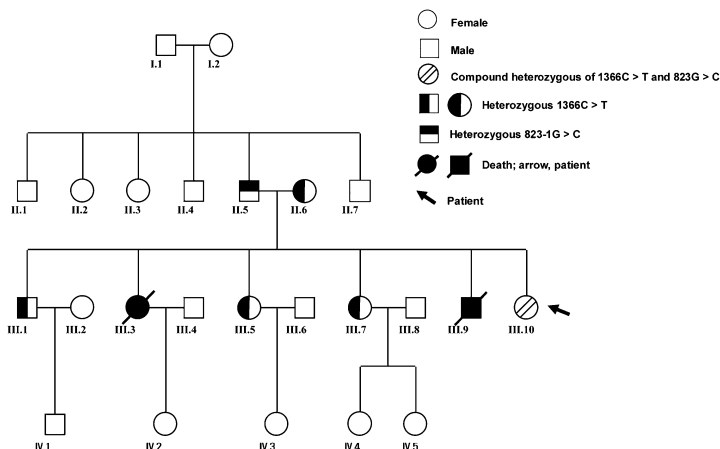


Fig. 1. Family pedigree of the patient.

We then sequenced the *LMAN1* gene and identified two mutations. One is 823-1G \rightarrow C, a splice site mutation at the exon 8 splice acceptor site and the other is 1366C \rightarrow T, a nonsense mutation in exon 11 that causes a change from a codon for arginine (CGA) to a stop codon (TGA).

Family pedigree is shown in Fig. 1. Her family members, numbers II.2, II.5, II.6, III.1, III.5, III.7, IV.1 and IV.4, have no history of bleeding disorders. The coagulation screening revealed normal APTT, PT and TT. Their factor V and factor VIII levels were in the normal range. The gene analyses for family members are as follows: numbers II.6, III.1, III.5 and III.7 show heterozygous for the 1366C \rightarrow T muta-

tion while number II.5 showing heterozygous for the 823-1G \rightarrow C mutation. Family numbers III.3 and III.9 succumbed to stomach cancer at age 38 and sudden death because of an unidentified cause at age 26 respectively. The details are shown in Table 1 and Fig. 1.

Discussion

Congenital F5F8D is a rare bleeding disorder, usually found in families with a history of consanguineous marriage [2–6]. The affected families have been reported from many places such as the Middle East, North America, Europe and the Far East. The highest

Table 1. History and laboratory findings in a Thai woman with F5F8D and her family.

No.	Sex	Age (year)	Bleeding history	Platelet ($\times 10^3 \mu\text{L}^{-1}$)	Coagulogram (s)			FV:C (%)	FVIII:C (%)	Mutation in <i>LMAN1</i> gene
					APTT (N 32–38)	PT (N 11.5–14.5)	TT (N 9–12)			
II.2	F	52	No	321	30	12	13	89.5	105	Not done
II.5	M	61	No	312	32.5	12	11.6	93.5	96	Heterozygous 823-1G \rightarrow C mutation
II.6	F	56	No	222	35	13	12.5	89.5	93	Heterozygous 1366C \rightarrow T mutation
III.1	M	41	No	254	36	12	11	86.5	89	Heterozygous 1366C \rightarrow T mutation
III.5	F	34	No	369	34.5	12	11.5	68	91	Heterozygous 1366C \rightarrow T mutation
III.7	F	31	No	281	37.8	12	11.4	53.5	62	Heterozygous 1366C \rightarrow T mutation
III.10 (patient)	F	23	Yes	252	68	18.5	10	10	12.5	Compound heterozygote for the 823-1G \rightarrow C mutation and 1366C \rightarrow T mutation
IV.1	M	3	No	267	41	13.6	15	80	67	Not done
IV.4	F	5	No	348	37	12	11.5	72	86	Not done

N, normal range; APTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time; FV:C, factor V clotting activity; FVIII:C, factor VIII clotting activity.

frequency of this disorder is found in Jews of Sephardim and Middle Eastern origin living in Israel. To our knowledge, this paper describes the only reported case of combined F5F8D in Thailand. The presentation of this disorder is usually a mild to moderate bleeding tendency, which may require treatment when patients are undergoing a surgical procedure. Our patient has had a history of easy bruising and bleeding after trauma and tooth extraction. However, no history of menorrhagia, epistaxis or gastrointestinal bleeding was found. The diagnosis for this disorder can be made by screening coagulogram and factor assay. The range of factor V and factor VIII levels are between 5% and 30%. For our patient, her factors V and VIII levels are at 10% and 12.5% respectively.

Treatment for the bleeding episodes consists of DDAVP, which can increase the factor VIII level up to three times [18]. The FFP at a dose of 20 mL kg⁻¹ can increase factor V level to 30% [19]. An antifibrinolytic agent is also useful to prevent clot lysis especially at the mucosal surface such as tooth extraction or tonsillectomy [7,8]. Our patient showed a good response to initial DDAVP administration, which resulted in a sevenfold increase in factor VIII:C. For factor V:C, the haemostatic level was raised to 25–30% by giving a loading dose of FFP 20 mL kg⁻¹.

We found that the patient had compound heterozygous mutations in the *LMAN1* gene which includes two mutations. One is 823-1G → C, a mutation that destroys the essential acceptor consensus (AG) of exon 8 and is expected to result in a null allele. This is the first reported splice acceptor site mutation in *LMAN1*. The most likely consequence of this mutation is the skipping in the exon 8 and frameshift in exon 9. A less likely scenario is the use of a cryptic splice site which results in the disruption of the open reading frame of *LMAN1* mRNA. The other mutation is 1366C → T, a nonsense mutation in exon 11 that causes a change from a CGA to a TGA (R456X). The same mutation had been reported previously [15]. Her father is heterozygote for the 823-1G → C mutation and her mother is heterozygote for the 1366C → T mutation. Therefore the proband is a compound heterozygote that has inherited two different null mutations from her parents. Most of the previously described patients have homozygous mutations because of consanguineous marriage [15,16]. This report describes a native Thai family with no consanguinity. Previous studies have demonstrated that *LMAN1* is required for efficient secretion of factor VIII and factor V and that this is mediated by

oligosaccharide structures within their respective B domains [11–17]. In the absence of *LMAN1*, *MCFD2* is also not retained with the ER or intermediate compartment. This patient has two null mutations which predicts a loss in the function of *LMAN1* and could explain her clinical presentation and laboratory findings. Family members who have the heterozygous mutations do not have any history of bleeding tendency and all coagulation test results are normal suggesting that only low level of *LMAN1* expression is required for efficient factor V and factor VIII expression and is consistent with previous reports [15,16].

References

- Oeri J, Matter M, Isenschmid H, Hauser F, Koller F. Congenital factor V deficiency (parahemophilia) with true hemophilia in two brothers. *Bibl Paediatr* 1954; 58: 575–88.
- Seligsohn U, Ramot B. Combined factor-V and factor-VIII deficiency: report of four cases. *Br J Haematol* 1969; 16: 475–86.
- Seligsohn U, Zivelin A, Zwang E. Combined factor V and factor VIII deficiency among non-Ashkenazi Jews. *N Engl J Med* 1982; 307: 1191–5.
- Sweeney J, Wenz B. Combined factor V and factor VIII deficiency. *N Engl J Med* 1983; 308: 656–7.
- Ozsoylu S. Combined congenital deficiency of factor V and factor VIII. *Acta Haematol* 1983; 70: 207–8.
- Peyvandi F, Tuddenham EG, Akhtari AM, Lak M, Mannucci PM. Bleeding symptoms in 27 Iranian patients with the combined deficiency of factor V and factor VIII. *Br J Haematol* 1998; 100: 773–6.
- Chuansumrit A, Mahaphan W, Pintadit P, Chaichareon P, Hathirat P, Ayuthaya PI. Combined factor V and factor VIII deficiency with congenital heart disease: response to plasma and DDAVP infusion. *Southeast Asian J Trop Med Public Health* 1994; 25: 217–20.
- Takai Y, Hayashi H, Ishimaru F *et al.* [DDAVP administration in a case of congenital combined factor V and factor VIII deficiency]. *Rinsho Ketsueki* 1989; 30: 2035–40.
- Marlar RA, Griffin JH. Deficiency of protein C inhibitor in combined factor V/VIII deficiency disease. *J Clin Invest* 1980; 66: 1186–9.
- Giddings JC, Seligsohn U, Bloom AL. Immunological studies in combined factor V and factor VIII deficiency. *Br J Haematol* 1977; 37: 257–64.
- Nichols WC, Seligsohn U, Zivelin A *et al.* Linkage of combined factors V and VIII deficiency to chromosome 18q by homozygosity mapping. *J Clin Invest* 1997; 99: 596–601.
- Ginsburg D, Nichols WC, Zivelin A, Kaufman RJ, Seligsohn U. Combined factors V and VIII deficiency – the solution. *Haemophilia* 1998; 4: 677–82.

- 13 Nichols WC, Seligsohn U, Zivelin A *et al.* Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII. *Cell* 1998; **93**: 61–70.
- 14 Neerman-Arbez M, Antonarakis SE, Blouin JL *et al.* The locus for combined factor V–factor VIII deficiency (F5F8D) maps to 18q21, between D18S849 and D18S1103. *Am J Hum Genet* 1997; **61**: 143–50.
- 15 Neerman-Arbez M, Johnson KM, Morris MA *et al.* Molecular analysis of the ERGIC-53 gene in 35 families with combined factor V–factor VIII deficiency. *Blood* 1999; **93**: 2253–60.
- 16 Nichols WC, Terry VH, Wheatley MA *et al.* ERGIC-53 gene structure and mutation analysis in 19 combined factors V and VIII deficiency families. *Blood* 1999; **93**: 2261–6.
- 17 Zhang B, Cunningham MA, Nichols WC *et al.* Bleeding due to disruption of a cargo-specific ER-to-Golgi transport complex. *Nat Genet* 2003; **34**: 220–5.
- 18 Hill FGH, Gidding JC, William CE, Pragnell D. Combined deficiency of factor V and VIII. Study of a family and response to cryoprecipitate and DDAVP infusions including protein C inhibitor measurement (Abstract 0646). *Thromb Haemost* 1982; **50**: 210.
- 19 Gongsakdi C. Dental care in patients with bleeding tendency using celluloid splint. *Southeast Asian J Trop Med Public Health* 1979; **10**: 298–300.