Vitamin K-Dependent Coagulation Factors Deficiency, Diagnosis, and Management

10

Maryam Sadat Hosseini and Mariasanta Napolitano

10.1 Introduction

Vitamin K-dependent coagulation factors deficiency (VKCFD) is a rare bleeding disorder and literature is only restricted to a few case reports and small case series [1], even if the progress of genotyping techniques has recently allowed to improve the knowledge of the disease. VKCFD is an autosomal recessive disorder, which arises from defects in either γ-glutamyl carboxylase (*GGCX*) or subunit 1 of vitamin K epoxide reductase complex (*VKORC1*) genes. If there is a mutation in the former enzyme it will be considered as type I disorder, and if the responsible mutation involves the latter, it will be considered as type II [2]. These genes are encoding proteins that are involved in the γ-carboxylation of the glutamate (Glu) residues of several vitamin K-dependent (VKD) proteins including a number of coagulation factors (FII, FVII, FIX, and FX), and also some non-hemostatic proteins entangled in mineralization or cell signaling. VKCFD is usually symptomatic since infancy with life-threatening bleeding events [3]. VKCFD can be diagnosed by prolongation of PT, APTT with normal TT, and parallel reduction of VKD coagulation factors, usually around 1–30%. However, the definite diagnosis is based on molecular analysis of GGCX and VKORC1 genes. Management of the disorder is mainly through administration of vitamin K1 or four-factor prothrombin complex concentrate (PCC) [4].

M. S. Hosseini
Tehran, Iran

M. Napolitano (✉)
PROMISE Department, University of Palermo, Palermo, Italy

Hematology Unit-Reference Regional Center for Thrombosis and Hemostasis, Università di Palermo, Palermo, Italy
e-mail: mariasanta.napolitano@unipa.it

A. Dorgalaleh (ed.), *Congenital Bleeding Disorders*,
https://doi.org/10.1007/978-3-031-43156-2_10

10.2 Structure and Function of GGCX and VKOR

GGCX is an integral trans-membrane protein, which is located on the ER membrane. It consists of 758 amino acids with a disulfide bond between cysteines 99 and 450 [3]. There is limited information on the structure of GGCX. However, it seems that GGCX contains 5 trans-membrane domains. The amino terminal of this enzyme is located in cytoplasm and the carboxyl terminal is exposed to ER lumen [5]. Based on the studies on the structure and mechanism of action, different binding and catalytic sites are considered for GGCX. Accordingly, GGCX contains a propeptid binding site, a glutamate binding site, a vitamin K binding site, a carboxylation active site, an epoxidation active site, and probably, a CO_2 binding site; however, the information on the exact location of these functional regions is limited [6].

The VKOR protein is also an integral protein in the ER membrane with 163 amino acids [3]. There are two topology models which consider three or four trans-membrane domains for the protein. According to the different studies, three trans-membrane model is more reasonable. In this model, the amino terminal of VKOR is located in the ER lumen and the carboxy terminal is located in the cytoplasm [5]. For a long period, it was assumed that the VKOR was a multi-enzyme complex, a theory, which is now questioned [5].

10.2.1 Vitamin K Cycle

VKD carboxylation is a post-translational modification, which is critical for proper function of VKD proteins. The most important VKD proteins are coagulation factors (F) FII, FVII, FIX, and FX, natural anticoagulants protein C, protein S, and protein Z, and non-hemostatic proteins including osteocalcin (also known as bone Gla protein, BGP), matrix Gla protein (MGP), growth arrest-specific protein 6 (Gas6), and Gla rich protein (GRP) [7].

In VKD carboxylation, specific glutamate (Glu) residues are modified to gamma-carboxyglutamate (Gla). Each VKD factor contains 10–12 Gla residues in the amino terminus which is called Gla domain. The responsible enzyme for this conversion is GGCX which requires reduced vitamin K (KH_2), CO_2, and O_2 as cofactors. When each Glu is modified, one KH_2 molecule is oxidized to vitamin K 2, 3 epoxide (KO). For restoration of KH_2, this KO molecule needs to be converted back to the reduced form (Fig. 10.1). This conversion occurs in a two-step reaction, first KO is reduced to vitamin K using VKOR and then vitamin K is reduced to KH_2 using vitamin K reductase [5, 8].

Recognition of protein substrates by GGCX is mediated via binding to the N-terminal propeptide of the substrate which plays a pivotal role in the carboxylation of clotting factors and determines the carboxylation efficiency. The propeptide of coagulation factors exerts various affinities towards GGCX. Although naturally occurring mutations involving the propeptide of coagulation factors do not have a

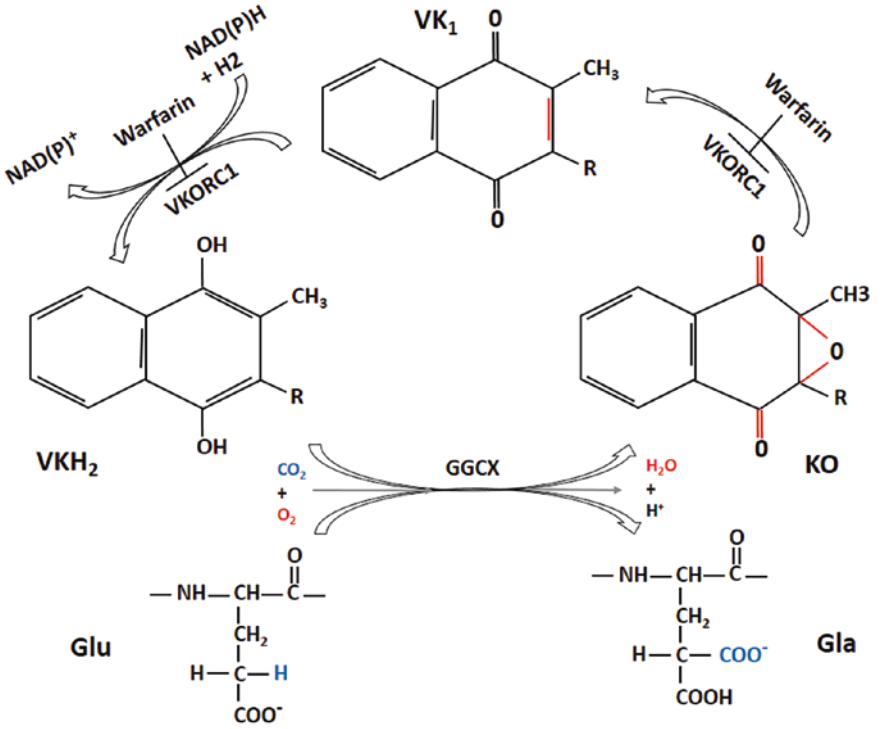

Fig. 10.1 Carboxylation of glutamate (Glu) to carboxyglutamate (Gla) residues by GGCX is necessary for activation of vitamin K-dependent coagulation factors. VKH_2 is the cofactor of this conversion. In this process, VKH_2 is oxidized into KO and then reduced to vitamin K quinone and VKH_2, respectively by VKORC1. Warfarin can block the VKORC1. Another reductase, NQO1 can also convert VK to VKH_2. Warfarin can inhibit VKORC1 but not NQO1 [9]. *GGCX* γ-glutamyl carboxylase; *VKH_2* vitamin K hydroquinone; *KO* vitamin K epoxide; *VKORC1* vitamin K epoxide reductase complex subunit 1; *NQO1* NADPH quinone oxidoreductase; *VK* vitamin K

considerable impact on the carboxylation and the normal levels of active VKD clotting factors, it has been recently suggested that such mutations may predispose to warfarin hypersensitivity during anticoagulant treatment [10].

Gamma-carboxylation of the Glu residues is necessary for binding of calcium ions which then allows binding of VKD factors to phospholipid membranes such as surface of activated platelets or damaged endothelium. This phenomenon leads to concentration of clotting factors at the site of vascular injury [5].

Non-hemostatic VKD proteins also possess several biological functions for which carboxylation is necessary, such as vascular calcification, bone metabolism, and signal transduction. Therefore, disrupted carboxylation of VKD proteins may also lead to comorbid phenotypes in VKCFD patients including skeletal, dermatological or cardiac abnormalities [11].

10.3 Vitamin K-Dependent Coagulation Factors Deficiency

VKCFD (OMIM #277450 and #607473) was first described in 1966 by McMillan and Robert in a 4-month-old girl [12]. She presented with several bruises and bleeding events, a prolonged PT and APTT, and undetectable levels of FII, FVII, FIX, and FX by clotting assays. However, neither liver disease nor malabsorption had been detected. Low level of coagulation factors showed a partial recovery following administration of high doses of vitamin K [12]. The patient was further investigated at age of 15 years and the clotting factors were reevaluated by immunologic assays [13]. However, the molecular mechanism remained unclear.

Now, VKCFD is known as an autosomal recessive bleeding disorder that arises from defects in either *GGCX* or *VKORC1* genes. Plasma level of VKD coagulation factors in VKCFD may be around 1–30% [14].

10.4 Clinical Manifestations

VKCFD usually manifests in infancy, although it may also remain latent for a short time. Severity of clinical manifestations depends on the level of reduced coagulation factors [15]. However, the clinical picture is not closely correlated to the activity of VKD coagulation factors [4]. Severe bleeding such as intra cranial hemorrhage (ICH) or umbilical cord bleeding has been described in affected neonates [16–18]. Mucocutaneous, soft tissue bleeding and post traumatic hemorrhages are also reported in these patients (Table 10.1) [17, 19]. Less commonly, VKCFD may present with hemorrhagic events in adulthood or even may be found incidentally [4].

Some affected individuals may also suffer from mental retardation, skin, cardiac and skeletal abnormalities which are attributed to the impaired γ-carboxylation of other VKD proteins [16, 20]. Skeletal abnormalities including nasal hypoplasia, distal digital hypoplasia, and epiphyseal stippling are similar to those seen in warfarin embryopathy [21]. Pseudoxanthoma elasticum-like (PXE-like) disorders have also been reported in patients affected by VKCFD with GGCX mutations [22]. Recently, the effect of GGCX variants on their ability to γ-carboxylate non-hemostatic VKD proteins in the presence of different concentrations of vitamin K

Table 10.1 Clinical manifestations of vitamin K-dependent coagulation factors deficiency

Clinical manifestations	Incidence
Intracranial hemorrhage	34%
Ecchymoses/easy bruising	21%
Skeletal abnormalities/growth or developmental retardation	21%
Umbilical cord bleeding	17%
Post-trauma/post-operative	17%
Epistaxis	17%
Gingival/oral cavity	12%
Hemarthrosis	4%

has been explored; variants with a markedly reduced ability to γ-carboxylate the upper zone of the growth plate and cartilage matrix-associated protein (UCMA/GRP) have been identified in patients with a PXE-like phenotype [23].

Some natural anticoagulants including protein C, protein S, and protein Z also require Glu residues to be modified into γ-carboxyglutamate (Gla) residues and therefore there are also low levels of protein C and protein S in the deficiencies of GGCX or VKOR. The fact that no cases with thrombosis have been reported in the literature so far, may suggest the dominant effect of these two enzymes in procoagulant activities [1, 24].

10.5 Molecular Basis

The gene encoding for GGCX with 13 kb length is located on chromosome 2p11.2 and comprises 15 exons. The responsible gene for VKORC1 protein that is called *VKORC1* is located on chromosome 16p11.2. It is a small gene with 5126 bp length and includes 3 exons [15]. Defect in GGCX is known as type I VKCFD. Another enzyme that plays an important role in this cycle is VKORC1. VKORC1 catalyzes reconversion of vitamin K epoxide (KO), which is produced during the last reaction, to KH2. Defect in VKORC1 is known as type II VKCFD [4, 7]. It seems that a missense mutation that leads to the substitution of tryptophan to arginine at amino acid number 98 is the only reported mutation involving *VKORC1* (Table 10.2).

To date, at least 34 mutations have been reported in the *GGCX* gene, which are associated with VKCFD and the majority of them are point mutations (Table 10.2) [1, 15]. The mutations can be observed in homozygous or compound heterozygous. Jin et al. showed that 1657delA and IVS13-6G>A are the underlying mutations of the first case of VKCFD which was reported by McMillan and Robert [25].

Considering various VKD proteins as substrates of GGCX, different mutations of GGCX have been linked to distinct bleeding and non-bleeding phenotypes. A number of these mutations lead to PXE-like disorder combined with reduced activity of VKD coagulation factors with or without abnormal bleeding tendency. The variable clinical picture of patients harboring GGCX mutations, regarding both hemostatic and non-hemostatic features, may be explained by the recent findings of Hao et al. and Gosh et al. The study of the first group revealed that GGCX mutations have differential impacts on the carboxylation efficiency of structurally/functionally discrete VKD proteins, including FIX, BGP, and MGP in a cell-based study. Furthermore, the effect of administrating vitamin K as a cofactor of carboxylation was affected differentially, a finding which also explains why vitamin K administration improves bleeding but not non-bleeding disorders/manifestations [26]. Another in vitro study by Gosh et al. was indicative of the differential impacts of GGCX mutations on carboxylation of VKD coagulation factors. According to this study, different GGCX mutations also have variable impacts on the responsiveness to vitamin K. Such findings can be useful in predicting the risk of bleeding and also the effectiveness of vitamin K therapy based on the molecular defect [27].

Table 10.2 Vitamin K-dependent coagulation factors deficiency causing mutations in the *GGCX* and *VKORC1* genes

	Type	Nomenclature	Gene region
Mutations in *GGCX* gene (type I disorder)	Undefined[a]	IVS1del14bp	Intron 1
	Splicing	IVS1-1G>A	Intron 1
	Missense	Asp31Asn	Exon 2
	Splicing	IVS2+1G>T	Intron 2
	Splicing	IVS2-1G>T[b]	Intron 2
	Missense	Pro80Leu	Exon 3
	Missense	Arg83Pro	Exon 3
	Missense	Arg83Trp	Exon 3
	Missense	Cys139Trp	Exon 4
	Missense	Cys139Tyr	Exon 4
	Missense	Asp153Gly	Exon 4
	Missense	Trp157Arg	Exon 4
	Missense	Met174Arg	Exon 4
	Missense	Asp183Val	Exon 5
	Missense	Arg204Cys	Exon 5
	Missense	Val255Met	
	Missense	Ser284Pro	
	Missense	Phe299Ser	
	Missense	Ser300Phe	
	Nonsense	Trp315Ter	
	Nonsense	Gln374X	Exon 8
	Missense	Gly386Val	
	Missense	Leu394Arg	Exon 9
	Missense	His404Pro	Exon 9
	Missense	Arg476Cys	Exon 10
	Missense	Arg476His	Exon 10
	Missense	Arg485Pro	Exon 11
	Missense	Trp493Cys	Exon 11
	Missense	Trp501Ser	Exon 11
	Missense	Arg513Lys	Exon 11
	Missense	Ile532Thr	Exon 11
	Missense	Gly537Ala	Exon 11
	Missense	Gly558Arg	Exon 11
	Splicing	IVS11+3A>G	Intron 11
	Frameshift	1657delA	Exon 12
	Missense	Thr591Lys	Exon 13
	Splicing	IVS13-6G>A	Intron 13
	Nonsense	Arg704X	Exon 15
	Missense	Ser741Leu	Exon 15
Mutations in *VKORC1* gene (type II disorder)	Missense	Arg98Trp	Exon 3

GGCX γ-glutamyl carboxylase; *VKORC1* vitamin K epoxide reductase complex subunit 1

[a]It seems that the deleted region in intron 1 is probably associated with cis-acting elements and thereby is involved in gene regulation [28]

[b]This splice site mutation in intron 2 results in a loss of exon 3 (Gly72-Leu124del)

Most recently, Rishavy et al. reported the significance of GGCX processivity and complete carboxylation for appropriate function of VKD proteins. The assay was based on comparing the carboxylase activity of wild type and mutant (V255M) GGCX enzymes in the presence of a VKD protein (FIX or MGP) and a challenge protein as an interfering agent for VKD protein carboxylation. In the presence of wild type enzyme, the VKD protein became fully carboxylated before the initiation of Challenge protein carboxylation, while both VKD protein and Challenge protein became carboxylated simultaneously but not completely in the presence of mutant carboxylase, indicating that GGCX V255M mutant has lost its processivity in carboxylation which subsequently results in poor clotting activity of FIX. the latter was obtained by an in vitro-study using FIX-HEK293 cells, a finding that explains the impaired hemostasis in patients with mutant GGCX [28].

10.6 Diagnosis

There is a weak relationship between severity of clinical manifestations and laboratory results of VKCFD [4]. VKCFD can be diagnosed by prolongation of PT, APTT with normal TT, and parallel reduction of FII, FVII, FIX, and FX activity [4, 14]. Although both PT and APTT are prolonged in VKCFD, PT test is slightly more affected. Factor activities are usually around 0.2–0.6 IU/mL and less commonly are <0.1 IU/mL at baseline [4]. Presence of inhibitors can be excluded by mixing study.

Differentiation of VKCFD and acquired vitamin K deficiency must be intentioned with normal fasting serum KH_2 (reduced vitamin K) concentration in VKCFD. Acquired vitamin K deficiency may arise from intestinal malabsorption of vitamin K in the inflammatory bowel disease or celiac disease, liver cirrhosis, and exposure to coumarin anticoagulants [4]. The differential diagnosis of the disorder in neonates mainly includes vitamin K deficiency. In healthy newborns the levels of VKD coagulation factors gradually increase up to the age of 6 months. Therefore, the diagnosis in neonates has to be confirmed by repeating the test at 6 months of age [4, 29].

In the subject of type I and II of the disorder, it should be considered that oxidized vitamin K (KO) is typically undetectable in VKCFD type I, even after vitamin K supplementation, but in VKCFD type II, an elevation of KO level can be observed following vitamin K supplementation [2, 4]. In addition, VKCFD must be distinguished from congenital factor deficiency including FII deficiency, isolated FVII and FIX deficiencies, and FX deficiency, as well as combined FVII and FX deficiency. In this setting, inhibitor against FIX (acquired hemophilia B) and FVII must be assayed [2]. For definite diagnosis, molecular analysis for mutations of *VKORC* or *GGCX* is necessary. Recently, a cell-based system for the detection of GGCX activity has been developed, it may thus be adopted for the diagnosis of VKCFD1 caused by GGCX variants [30].

10.7 Management

Management of the disorder is mainly through administration of vitamin K1 (phytomenadion), but in severely affected patients and in major surgeries, four-factor prothrombin complex concentrate (PCC) (containing prothrombin, FVII, FIX, and FX) is also required (Table 10.3) [31]. Most of the cases (not all) may show a partial restoration in the level of deficient factors with high doses of vitamin K [15].

As VKCFD may lead to life-threatening hemorrhagic events, prophylaxis at diagnosis is highly recommended. Dose of treatment for long-term prophylaxis is 5–20 mg/day of oral vitamin K1 and when response is poor, 5–20 mg/week of parenteral vitamin K1 is recommended [4, 31]. In the subject of mild bleeding or minor surgery, 15–20 mL/kg tranexamic acid or 1 g four times daily alone is recommended [4]. In connection with severe bleeding or major surgery, 20–30 IU/kg of four-factor PCC combined with 5–20 mg of vitamin K1 is recommended. In the lack of four-factor PCC, 15–25 mL/kg of virus inactivated FFP can be administered [4, 31].

In normal pregnancy, the levels of FVII and FX usually increase, while FII and FIX levels do not alter. The level of FVII in some pregnant women may elevate even up to tenfold [32]. However, in some pregnant women affected by VKCFD, the physiologic increase in the deficient factors may be inadequate for an uneventful delivery. Pregnant women in whom each of the VKD factors has an activity below 20 IU/mL in the third trimester must be managed carefully at the time of delivery. In this condition, one dose of four-factor PCC 20–30 IU/kg before delivery or before cesarean section is recommended, treatment with PCC should be continued for at least 72 h [4].

It currently seems that prenatal diagnosis of VKCFD is not preferred, taking into account the potential bleeding risk of the procedure but also because major hemorrhagic events in the affected newborn can be prevented by the administration of vitamin K in the third trimester of pregnancy in mothers which are prone to have a

Table 10.3 Different therapeutic choices for management of patients with vitamin K-dependent coagulation factors deficiency (VKCFD) in different conditions

Condition	Recommended dosages
Long-term prophylaxis	Oral vitamin K1 (5–20 mg/day) *If response is poor:* Parenteral vitamin K1 (5–20 mg/week)
Mild bleeding events/minor surgeries	Tranexamic acid (15–20 mL/kg or 1 g four times daily)
Severe bleeding events/major surgeries	Four-factor PCC (20–30 IU/kg) + Vitamin K1 (5–20 mg) *Four-factor PCC can be substituted by virus inactivated FFP (15–25 mL/kg)*
Pregnancy (each of the vitamin K-dependent factors activity <20 IU/mL in the third trimester)	Four-factor PCC (20–30 IU/kg), one dose at the time of delivery or before cesarean section and continued for at least 3 days

PCC prothrombin complex concentrate

child with VKCFD. Antenatal management and timing of Vitamin K administration for prophylaxis of intrauterine bleeding need, however, to be better defined also in order to prevent bone and skin manifestations of the disease [2, 33].

References

1. Brenner B, Kuperman AA, Watzka M, Oldenburg J, editors. Vitamin K-dependent coagulation factors deficiency. Seminars in thrombosis and hemostasis, vol. 35; 2009. p. 439.
2. Napolitano M, Mariani G, Lapecorella M. Hereditary combined deficiency of the vitamin K-dependent clotting factors. Orphanet J Rare Dis. 2010;5(1):21.
3. Stafford D. The vitamin K cycle. J Thromb Haemost. 2005;3(8):1873–8.
4. Mumford AD, Ackroyd S, Alikhan R, Bowles L, Chowdary P, Grainger J, et al. Guideline for the diagnosis and management of the rare coagulation disorders. Br J Haematol. 2014;167(3):304–26.
5. Tie JK, Stafford DW. Structural and functional insights into enzymes of the vitamin K cycle. J Thromb Haemost. 2016;14(2):236–47.
6. Hosseini MS, Shams M, Dorgalaleh A, Mansouritorghabeh H. Multiple coagulation factor deficiency. Congenital bleeding disorders. Springer; 2018. p. 219–38.
7. Oldenburg J, Marinova M, Müller-Reible C, Watzka M. The vitamin K cycle. Vitamins Hormones. 2008;78:35–62.
8. Oldenburg J, Bevans CG, Müller CR, Watzka M. Vitamin K epoxide reductase complex subunit 1 (VKORC1): the key protein of the vitamin K cycle. Antioxid Redox Signal. 2006;8(3–4):347–53.
9. Gallieni M, Fusaro M. Vitamin K and cardiovascular calcification in CKD: is patient supplementation on the horizon? Kidney Int. 2014;86(2):232.
10. Hao Z, Jin D-Y, Stafford DW, Tie J-K. Vitamin K-dependent carboxylation of coagulation factors: insights from a cell-based functional study. Haematologica. 2020;105(8):2164.
11. Watzka M, Geisen C, Scheer M, Wieland R, Wiegering V, Dörner T, et al. Bleeding and non-bleeding phenotypes in patients with GGCX gene mutations. Thromb Res. 2014;134(4):856–65.
12. McMillan CW, Roberts HR. Congenital combined deficiency of coagulation factors II, VII, IX and X: report of a case. N Engl J Med. 1966;274(23):1313–5.
13. Chung K-S, Bezeaud A, Goldsmith JC, McMillan CW, Menache D, Roberts HR. Congenital deficiency of blood clotting factors II, VII, IX, and X. Blood. 1979;53(4):776–87.
14. Castaman G, Linari S. Diagnosis and treatment of von Willebrand disease and rare bleeding disorders. J Clin Med. 2017;6(4):45.
15. Zhang B, Ginsburg D. Familial multiple coagulation factor deficiencies: new biologic insight from rare genetic bleeding disorders. J Thromb Haemost. 2004;2(9):1564–72.
16. Oldenburg J, Von Brederlow B, Fregin A, Rost S, Wolz W, Eberl W, et al. Congenital deficiency of vitamin K-dependent coagulation factors in two families presents as a genetic defect of the vitamin K-epoxide-reductase-complex. Throm Haemost. 2000;84(6):937–41.
17. Brenner B, Sánchez-Vega B, Wu S-M, Lanir N, Stafford DW, Solera J. A missense mutation in γ-glutamyl carboxylase gene causes combined deficiency of all vitamin K-dependent blood coagulation factors. Blood. 1998;92(12):4554–9.
18. Spronk HM, Farah RA, Buchanan GR, Vermeer C, Soute BA. Novel mutation in the γ-glutamyl carboxylase gene resulting in congenital combined deficiency of all vitamin K–dependent blood coagulation factors. Blood. 2000;96(10):3650–2.
19. Lunghi B, Redaelli R, Caimi T, Corno A, Bernardi F, Marchetti G. Novel phenotype and γ-glutamyl carboxylase mutations in combined deficiency of vitamin K-dependent coagulation factors. Haemophilia. 2011;17(5):822–4.
20. Hauschka PV, Lian JB, Cole D, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiol Rev. 1989;69(3):990–1047.

21. Pauli R, Lian J, Mosher D, Suttie J. Association of congenital deficiency of multiple vitamin K-dependent coagulation factors and the phenotype of the warfarin embryopathy: clues to the mechanism of teratogenicity of coumarin derivatives. Am J Hum Genet. 1987;41(4):566.
22. Vanakker OM, Martin L, Gheduzzi D, Leroy BP, Loeys BL, Guerci VI, et al. Pseudoxanthoma elasticum-like phenotype with cutis laxa and multiple coagulation factor deficiency represents a separate genetic entity. J Investig Dermatol. 2007;127(3):581–7.
23. Ghosh S, Kraus K, Biswas A, Müller J, Forin F, Singer H, et al. GGCX variants leading to biallelic deficiency to γ-carboxylate GRP cause skin laxity in VKCFD1 patients. Hum Mutat. 2022;43(1):42–55. https://doi.org/10.1002/humu.24300.
24. Palla R, Peyvandi F, Shapiro AD. Rare bleeding disorders: diagnosis and treatment. Blood. 2015;125:2052.
25. Jin D-Y, Ingram BO, Stafford DW, Tie J-K. Molecular basis of the first reported clinical case of congenital combined deficiency of coagulation factors. Blood. 2017;130(7):948–51.
26. Hao Z, Jin D-Y, Chen X, Schurgers LJ, Stafford DW, Tie J-K. γ-Glutamyl carboxylase mutations differentially affect the biological function of vitamin K–dependent proteins. Blood. 2021;137(4):533–43.
27. Ghosh S, Kraus K, Biswas A, Müller J, Buhl AL, Forin F, et al. GGCX mutations show different responses to vitamin K thereby determining the severity of the hemorrhagic phenotype in VKCFD1 patients. J Thromb Haemost. 2021;19(6):1412–24.
28. Rishavy MA, Hallgren K, Wilson LA, Hiznay JM, Runge KW, Berkner KL. GGCX mutants that impair hemostasis reveal the importance of processivity and full carboxylation to VKD protein function. Blood. 2022;140:1710.
29. Thomas A, Stirling D. Four factor deficiency. Blood Coagul Fibrinolysis. 2003;14:S55–S7.
30. Gao W, Liu H, Su G, Xu Y, Wang Y, Cui L, et al [Development of a cell-based diagnostic system for vitamin K-dependent coagulation factor deficiency 1]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2020;37(8):811–4. https://doi.org/10.3760/cma.j.issn.1003-9406.2020.08.002.
31. Peyvandi F, Menegatti M. Treatment of rare factor deficiencies in 2016. ASH Educ Program Book. 2016;2016(1):663–9.
32. Prisco D, Ciuti G, Falciani M, editors. Hemostatic changes in normal pregnancy. Hematology Meeting Reports (formerly Haematologica Reports); 2009.
33. Guzzardo GM, Ghosh S, Pezeshkpoor B, Pavlova A, Czogalla-Nitsche K, et al. Novel gamma-Glutamyl carboxylase mutation causing hemorrhagic and non-hemorrhagic VKCFD1 phenotypes. Blood. 2022;140(Supplement 1):8480–2. https://doi.org/10.1182/blood-2022-169105.