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Familial Deficiency of Vitamin K–Dependent Clotting Factors

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SUMMARY

Combined deficiency of vitamin K–dependent clotting factors II, VII, IX, and X (and proteins C, S, and Z) is usually an acquired clinical problem, often resulting from liver disease, malabsorption, or warfarin overdose. A rare inherited form of defective γ-carboxylation resulting in early onset of bleeding was first described by McMillan and Roberts in 1966 and subsequently has been termed *vitamin K–dependent clotting factor deficiency* (VKCFD). Biochemical and molecular studies identify 2 variants of this autosomal recessive disorder: VKCFD1, which is associated with point mutations in the γ-glutamylcarboxylase gene (GGCX), and VKCFD2, which results from point mutations in the vitamin K epoxide reductase gene (VKOR). Bleeding ranges in severity from mild to severe. Therapy includes high oral doses of vitamin K for prophylaxis, usually resulting in partial correction of factor deficiency, and episodic use of plasma infusions. Recent molecular studies have the potential to further our understanding of vitamin K metabolism, γ-carboxylation, and the functional role this posttranslational modification has for other proteins. The results may also provide potential targets for molecular therapeutics and pharmacogenetics.

Keywords

γ-carboxylation; vitamin K; reductase; polymorphisms; hemorrhage

INTRODUCTION

Vitamin K–dependent clotting factor deficiency (VKCFD) is a rare autosomal recessive bleeding disorder that often presents with severe hemorrhage during infancy. The first case of VKCFD was reported in 1966 and described a 3-month-old girl with multiple bruises and hemorrhages [1]. She had no evidence of malabsorption, liver disease, or warfarin poisoning. She was found to have a prothrombin time of 95 seconds and a partial

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LINKS TO ORGANIZATIONS
The International Registry of Rare Bleeding Disorders (RBDs): http://www.rbdd.org http://www.rbdd.eu
FDA news release dated September 17, 2007: The Nanosphere Verigene Warfarin Metabolism Nucleic Acid Test; FDA's Center for Drug Evaluation and Research Web site: www.fda.gov/cder
CDC Universal Data Collection/Rare Bleeding Disorders Working Group
Medical Co-Chair: www.ihtc.org
Centers for Disease Control UDC Working Group:
http://www.cdc.gov/ncbddd/hbd/surveillance.htm
FDA news release dated September 17, 2007: The Nanosphere Verigene Warfarin Metabolism Nucleic Acid Test; FDA's Center for Drug Evaluation and Research Web site: www.fda.gov/cder

thromboplastin time of 305 seconds. These times corrected on mixing 1:1 with normal plasma, indicating factor deficiency rather than inhibition of coagulation. Her plasma showed less than 3% activity of factors II, VII, IX, and X.

The proband was further studied at the age of 15 years and found to have immunologically recognizable coagulation factors II, VII, IX, and X that lacked γ-carboxyglutamic acid residues [2]. Both parents of the children were found to have reduced levels of γ-carboxylated proteins in their urine, suggesting heterozygous defects in the vitamin K metabolic pathway (H.R. Roberts, personal communication). The roband's obstetric care at the age of 34 years was also reported [3]; management continued to involve high doses of oral vitamin K and plasma infusions for surgical procedures and significant hemorrhages. Additional VKCFD cases and pedigrees were reported over the years; bleeding has ranged from mild to severe [4–10].

Subsequent characterization of anticoagulant proteins C, S, and Z in VKCFD cases showed defective γ-carboxylation of these proteins as well, and a modest propensity to thrombotic events has been suggested by a few cases [8]. Skeletal defects have also been reported in some cases, likely due to defective γ-carboxylation of certain bone matrix proteins [7,9,10] or from vitamin K interactions with other target genes in osteoblasts [11].

The isolation and characterization of the human genes encoding the γ-glutamyl carboxylase (GGCX) in 1991 by forward genetics [12,13] and the vitamin K epoxide reductase (VKOR) in 2004 by reverse genetics and expression cloning [14–16] greatly advanced our understanding of VKCFD, as well as the metabolism of vitamin K and the biological roles of γ-carboxylation. Subsequent pedigree studies have looked at missense mutations in each gene, resulting in the subtype designations VKCFD1 and VKCFD2 [17,18]. Common single nucleotide polymorphisms (SNPs) in these genes (particularly VKOR) and CYP2C9 (cytochrome p450 2C9) have been examined in large populations of various ethnic backgrounds in relation to warfarin dosing [reviewed in 19]. Recent work has also focused on drug development and potential molecular therapeutics using the knowledge gained by VKCFD, making this rare coagulation disorder relevant in new ways.

METHODS

All current publications in PubMed and other scientific databases were searched. Approximately 300 articles pertaining to the subject were reviewed in detail. Original studies published in the past 3 years were chosen for additional analysis. Polymorphisms in GGCX and VKOR were reviewed in standard genome databases and registries. Selected investigators working in this area were also contacted. Several recent reviews and perspectives are also recommended [20–24].

INCIDENCE, RACIAL AND ETHNIC PREDILECTION

VKCFD1 and VKCFD2 are extremely rare autosomal recessive disorders with fewer than 30 cases reported. Carrier incidence, racial distribution, and ethnic predilections are not known. Cases and pedigrees have been reported in Africa, Asia, Europe, and North America. As mentioned previously (19), SNP frequencies in the GGCX and VKOR genes in several large population-based studies have been reported, but obviously these rarely result in the clinical syndrome VKCFD.

PATHOPHYSIOLOGY

Glutamate residues in coagulation factors II, VII, IX, and X (and the anticoagulant factors proteins C, S, and Z) are carboxylated by GGCX into γ-carboxyglutamate residues. Nine to

13 of these residues are found in the amino-terminal region of the circulating form of each of these proteins, constituting the γ-carboxyglutamic acid-rich Gla domain. Full activity of these factors (but not immunologic recognition) is provided by this posttranslational modification of the proteins in the endoplasmic reticulum. Once carboxylated, the proteins have a calcium-dependent conformation that allows binding to phospholipids and/or endothelial cells.

Vitamin K in reduced form is required as a cofactor by GGCX during the catalytic reaction:

Glutamic acid ----------> γ-carboxyglutamic acid

GGCX

Cofactor = Vitamin K reduced

Once vitamin K is oxidized to the epoxide form in this reaction, the reduced form of vitamin K must be regenerated by the vitamin K epoxide reductase (VKOR):

Vitamin K epoxide----------> Vitamin K reduced

VKOR

The primary target for warfarin's action is VKOR, resulting in lower levels of the reduced form of vitamin K. This in turn leads to failure of carboxylation and therefore to decreased function of coagulation factors in patients treated with coumarin derivatives. Detailed review of the vitamin K cycle and carboxylation can be found in several recent reviews [20–22].

GENETICS AND MOLECULAR BASIS

These rare autosomal disorders arise from point mutations in either the GGCX or VKOR genes. Several compound heterozygous cases and pedigrees have been described [5,7,15]. Carriers are asymptomatic. As Ginsberg notes [23], the rarity of VKCFD and the fact that only missense mutations have been identified suggest embryonic lethality with complete deficiency of either enzyme in humans; this is supported by knockout mice results [23].

CLINICAL MANIFESTATIONS

The first case of VKCFD was described in an infant with multiple bruises and hemorrhages, and subsequent case reports are often similar. VKCFD may present with intracranial hemorrhage in the neonate or early in life, similar to hemorrhagic disease of the newborn resulting from acquired vitamin K deficiency [8,14]. A few patients have dysmorphic features vaguely resembling warfarin embryopathy, but this is a heterogeneous group. Some of the described patients have also had developmental abnormalities and/or skeletal defects [7,9,10], and there appears to be a high incidence of fetal wastage [7,9–11,25], although the small numbers preclude a definitive statement. Milder cases with later onset of diagnosis have also been reported [5,6]. The most detailed clinical descriptions, however, are found in the original case report [1], with published followup reports many years later [2,3].

VKCFD patients have markedly prolonged prothrombin time and activated partial thromboplastin time results that correct with plasma mix. Factors II, VII, IX, and X activity levels show variably reduced values (though usually quite low) that partially improve with vitamin K treatment of the patient. Proteins increased in vitamin K's absence (PIVKA-II; undercarboxylated prothrombin) are increased, even following therapeutic correction with plasma infusion. Although the PIVKA-II immunoassay is more sensitive than vitamin K–dependent factor activity levels, its limited specificity does not allow differentiation of VKCFD from some forms of liver disease or other disorders resulting in vitamin K

deficiency. Proteins C, S, and Z activities are also reduced; however, the propensity to thrombotic events seems to be much less common [8]. This is primarily a bleeding diathesis, not a thrombophilic disorder.

DIAGNOSIS

Diagnosis of vitamin K deficiency rests on the persistence of bleeding manifestations and reduced levels of vitamin K–dependent coagulation and anticoagulation factors. Warfarin ingestion, malabsorption, and liver disease must be ruled out [2]. Reduced vitamin K levels due to antibiotic therapy can also lead to bleeding in VKCFD patients, and additional treatment and monitoring may be indicated during infection.

Genotyping for VKORC (5 kb) and GGCX (13 kb) is possible in several research laboratories [12–16] and should be strongly considered rather than allowing the patient to have multiple and/or severe bleeding episodes while not taking vitamin K. Point mutations have also greatly contributed to our understanding of both GGCX and VKOR structure and function [26–28].

MANAGEMENT

Administration of large doses of oral vitamin k (for example, 15 mg daily in adults) may partially correct the low factor assay results in severely affected patients to about 15–20% but may not prevent significant bleeding [2–4]. These partially corrected factor activities approach the levels seen in milder cases of VKCFD [5,6]. In fact, massive parenteral doses of vitamin k do not always correct factor II, VII, IX and X activities, and there is clear biochemical evidence that the molecules are not fully carboxylated by such treatment [2,7]. Continued daily treatment with high dose oral vitamin k is, however, successful in preventing some bleeding complications [2–8] and is generally recommended for these patients.

Plasma infusions for surgical procedures and overt hemorrhage are indicated, and VKCFD patients often require multiple doses. Alternatively, so-called 4-factor prothrombin complex concentrates (PCCs), which contain factors II, VII, IX, and X and proteins C and S in variable amounts [29] could be considered. Although these concentrates have rarely been used in VKCFD in the published literature [7], they have been used effectively in reversing warfarin anticoagulation and offer a therapeutic option that includes pathogen-inactivation steps and lower risk of volume overload with repeated dosing when compared with plasma.

PROGNOSIS

Prenatal diagnosis is possible if both alleles are sequenced and familial SNPs are defined. Prognosis of VKCFD can be guarded based on the propensity for perinatal intracranial hemorrhage, which can result in permanent neurological damage and developmental disabilities [8]. Other cases, however, have been milder and have favorable outcomes over time. Early intervention with appropriate treatment and prevention modalities would likely improve the prognosis, as with any coagulation disorder. As noted previously, factor levels partially improve with vitamin K therapy, and regular vitamin K dosing may maintain hemostasis, though not preventing all complications.

CONCLUSIONS

Future areas of potential clinical relevance resulting from this work include prediction of warfarin dose using SNPs in VKOR and other genes, design of new small molecule

inhibitors of VKOR and GGCX, and further definition of defective carboxylation of nonhematologic proteins.

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