Instructions to Contributors

Dear Contributor:

Enclosed in this document please find the page proofs, copyright transfer agreement (CTA), and offprint order form for your article in *Seminars in Thrombosis and Hemostasis*. Please print this document and complete and return the CTA and offprint order form, along with corrected proofs, within 72 hours.

1) Please read proofs carefully for **typographical** and **factual** errors only; mark corrections in the margins of the proofs in blue or black pen, or use **Adobe Acrobat tools** to mark the changes in the PDF file directly. Please be sure to write as clearly as possible so no errors are introduced into your article. **Answer (on the proofs) all author queries marked in the margins of the proofs.** Check references for accuracy. **Please check on the 1st page of your article that your titles and affiliations are correct.** Avoid elective changes, because these are costly and time consuming and will be made at the publisher's discretion.

2) Please pay particular attention to the proper placement of figures, tables, and legends. Please provide copies of any formal letters of permission that you have obtained.

3) Please return the corrected proofs, signed copyright transfer agreement, and your offprint order form.

4) After PDF approval, the author will not be able to request changes to the published article.

5) As a contributor to this journal, you will receive a complimentary PDF file of the article after publication.

- If you wish to order offprints, **please circle the quantity required** (left column) **and the number of pages in your article.** If you wish to order copies of the journal, please enter the number of copies on the indicated line.

- If you do not want to order offprints or journals, simply put a slash through the form, **but please return the form.**

Please return all materials within 72 hours. E-mail is the easiest way to ensure your corrections are received in a timely manner. Please return the corrected proofs to:

Mansi Agarwal, Project Manager
Thieme Medical and Scientific Publishers
Fax: +91-120-4556649
E-mail: Mansi.Agarwal@thieme.com

Please do not return your materials to the editor or the typesetter.

Please note: Due to a tight schedule, if the publisher does not receive the return of your article proofs within 7 days of the date the e-mail was sent to you, the publisher reserves the right to proceed with publication without author changes. Such proofs will be proofread by the editor and the publisher.

Thank you for your contribution to this journal.

Permission to Publish and Copyright Transfer Agreement

Manuscript Information:

Journal:	Seminars in Thrombosis & Hemostasis
Manuscript Title:	Clinical, Laboratory, and Molecular Characteristics of Inherited Vitamin K–Dependent Coagulation Factors Deficiency
Manuscript Number:	03283
Authors:	Salvatore Perrone, MD Simona Raso, MD, PhD Mariasanta Napolitano, MD, PhD
Corresponding author's contact data:	Mariasanta Napolitano, MD, PhD, Haematology Unit, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University Hospital "P. Giaccone," University of Palermo, Via Del Vespro, 129, Palermo 90127, Italy
Corresponding author's e-mail address:	mariasanta.napolitano@unipa.it
Contact at the publishers:	Mansi Agarwal
E-mail address at the publishers:	Mansi.Agarwal@thieme.com

Dear Author,

Please

- *read this form carefully,*
- *check all manuscript information,*
- *sign this form with your digital signature and*
- *reply directly to the e-mail with which you received this document and leave the subject unchanged.*

Thank you very much in advance.

Assignment of Rights

We – the Thieme Publishing Group – do not accept for publication in a journal any manuscript that has previously been published elsewhere.

Your consent to the following assignments of rights, also on behalf of the other authors (if several authors contribute to the manuscript), and the signing of this Copyright Transfer Agreement is a necessary requirement for the publication of your manuscript.

Upon acceptance of your manuscript by us you assign to us (on behalf of all authors), without geographical or language restriction and for the duration of the legal copyright term, the rights to use your article, for all print runs/updates, including the rights to:

- reproduce and distribute copies of the article in printed form (e. g., in a periodical or journal, medical textbook or other target group oriented book, paperback book, special edition for secondary markets or special customers, brochures, advertising supplements, edited volumes, etc.) including the publication of the article under an Open Access license;

- reproduce and distribute the article in electronic media formats (e. g., CD-Rom, DVD, USB memory stick, databases, cloud-based service, ebook and other forms of electronic publishing) and also make available to the public (e. g., internet, intranet or other wired or wireless data networks), in particular by displaying on stationary or mobile visual display units, monitors, smart phones, tablets or other devices by download (e. g., e-pub, PDF, App) or retrieval in any other form;

- publish ourselves or to authorize the publication of excerpts in other works or articles, in audio-visual accompanying materials or interactive products or services, and including the transfer of rights of use to third parties (e. g., under the terms of licensing agreements);

Please use the latest Adobe Reader version independently of your system software (Windows, Linux, Mac OS X).
To download Adobe Reader: click here.

© Thieme Publishing Group – January 2019

Thieme

Permission to Publish and Copyright Transfer Agreement

– translate, transfer and process into other languages or versions (e. g., podcast, audiobook or other image and sound carriers), broadcast by means of television, cable or satellites, radio or other audio-visual media, to rent out and lend, store in an electronic archive and to use in any other type of format that may become known in the future and – where applicable – for all other rights protected by organizations assessing and/or collecting fees for copyright use. Furthermore you assign to us all statutory royalty claims under relevant law insofar we mandate a reproduction rights organization to administer such rights for publishers and authors; we accept the assignments.

The aforesaid rights to use are assigned to us exclusively. Your rights to use the article as author in accordance with the provisions in the following section “Authors' rights and Green Open Access” shall remain unaffected.

Without prejudice to this assignment of rights, the moral rights in connection with the article shall remain with you and the other authors, whereas we are entitled to all rights with regard to the editorial process and publishing service in connection with the reproduction and distribution of the article in our journal and making it available to the public.

Any adaptions, if appropriate for the exercise of the rights of use granted to us, shall be processed by us.
Please forward any inquiries that are addressed to you regarding the above-mentioned rights of use for our attention and response.

Authors’ rights and Green Open Access

The rights of use are assigned to us exclusively – subject to your rights in accordance with our Open Access Guidelines. Our Open Access Guidelines state that immediately after the publication of the article by us, you and the other authors are entitled to make the published version of the article available to the public on your homepage for your own scientific and other non-commercial purposes. Twelve months after publication by us, you and the other authors are entitled to make the accepted manuscript version available to the public on other non-commercial websites, provided that you make full reference to the published version (“Green Open Access”). For further details please click the button “Authors’ rights and Green Open Access.”

For more Information on our Open Access Program please visit **http://open.thieme.com**.

If you wish to publish your article Gold Open Access, please contact **openthieme@thieme.de**.

Duties of care

Product liability laws set high standards for your duty of care as the author of a scientific manuscript. This is especially the case when you give therapeutic information and/or specify doses. Therefore please check this information carefully in the typeset page-proofs of your article. Your task will be much easier if you have the information counterchecked – depending on the sensitivity of the information within the article – by specialist colleagues. Only you, as the author, have the specialist knowledge to be able to assess the accuracy of the information. For further information on how to indicate corrections, please click the button “Correction markup symbols”.

Author’s Declaration

I have taken note of the information on the duties of care under product liability law; I agree to the assignments of rights in accordance with the foregoing sections “Assignment of Rights” and “Authors’ rights and Green Open Access” also on behalf of the other authors (if several authors have contributed to the article).

I declare that no third party rights will be infringed through the publication. Any material contained in the manuscript (including illustrations, tables, or other material) from third-party sources will be identified as such through citation, indicating the source. If required, I have obtained the copyright permission from the publishers concerned.

The above-mentioned assignments of rights also relate to the illustrations in your manuscript. We do not accept any illustrations for which it has not been granted all rights of use in accordance with this contract.

Should one of the foregoing regulations be or become invalid in whole or in part this shall not affect the validity of the other provisions. Any invalid provision shall be replaced by a regulation that comes as close as possible to the purpose of the invalid provision in economic terms, insofar as legally permissible.

This article is ready to print after the execution of the corrections indicated by me.

21/10/2024	MARIASANTANAPOLITANO	Firmato digitalmente da MARIASANTANAPOLITANO Nome distinto: cn=MARIASANTANAPOLITANO gn=MARIASANTANAPOLITANO c=IT Italy I=IT Italy Motivo: Sono l'autore del documento Luogo: Data: 2024-10-21 09:53+02:00
Date	Digital Signature	

OPEN ACCESS

Authors’ rights and Green Open Access

Instructions for Authors

Digital signature

© Thieme Publishing Group – January 2019

Thieme

Open Access Publishing – Author Information

Open Access (OA) means free reading and use of scientific literature and related content via the Internet.

1. OA Publishing at Thieme

There are two main ways to publish OA articles: “gold” and “green.”

Gold OA means that immediately upon publication your article will be made free to read via the Internet with additional usage rights. In return for this instant and global dissemination and availability of your article, an Article Publication Charge (APC) is paid either by yourself as the author or by your institution or funding organization.

Green OA means that after 12 months you may self-archive the accepted manuscript. You may not use the final published version, but you may deposit your publication as the accepted manuscript in institutional online repositories.

Thieme offers authors the choice by providing both gold and green OA options, with the preference for gold as it provides immediate access to the edited, published version of your article with well-defined reuse rights under a sustainable financial model.

2. General Information — Thieme Open

The Thieme Publishing Group is one of the market-leaders for scientific information and services in medicine and chemistry. Since the end of 2013, we have offered authors an OA publication service called Thieme Open, **http://open.thieme.com**.

This OA service includes rigorous peer-review, professional in-house editorial services, fast publication times, and worldwide access via Thieme’s electronic journals, **https://www.thieme.de/en/thieme-connect/home-3939.htm**.

It also includes reference linking and long-term archiving, distribution, including the Digital Object Identifier (DOI), as well as entry into PubMed Central and indexing in OA-relevant search engines.

Our services for the Thieme OA authors:

1. Publish under a gold OA model in our suite of gold OA journals or choose from one of our more than 150 classic journals that also offer you an OA option. Transfer your copyright and declare no conflict of interest electronically by signing the Copyright Transfer Agreement and Conflict of Interest forms.
2. Be certain that your OA manuscript will enjoy the same high-quality standards of peer-review and superior production services as you enjoy in all our journals.
3. Post the final version of your article immediately onto any repository if published under the gold OA option.
4. Deposit the final published version of your article immediately as a PDF on your personal homepage.
5. Protect your rights as an author when you publish your article under a “CC - BY - NC - ND license.” Information at: **https://creativecommons.org**
6. Be compliant with requirements, mandates, and policies of funders such as NIH, OSTP, Horizon 2020, DFG, and RCUK. For details see: **http://www.sherpa.ac.uk/romeo/search.php**
7. Be assured that your work will be archived long-term at archiving services such as CLOCKSS or Portico.
8. Be assured that your paper is discovered on major search engines and indexing services such as PubMed Central, Directory of Open Access Journals (DOAJ), and Scopus.

© Thieme Publishing Group – January 2019

Thieme

3. Why is Open Access important for you?

OA is an additional publication service among the services and products that are provided by the Thieme Publishing Group.

The most important reasons to publish OA are the immediate, global access, and sharing via the citable version of an article. This means a high visibility leading to a potentially higher usage, more citations, and a higher impact of your article by extended search ability and discoverability. Your article quickly gains wide scientific impact. These play an increasing role if you, your project, and the publication of your results and data are publicly funded. Please find a glossary of bibliometrics at: **http://www.bibliothek.kit.edu/cms/english/bibliometrics-glossary.php**

Maintaining a high-level quality standard undergoing a rigorous peer-review process by international and leading specialists, Thieme OA articles are published quickly and may include multi-media enhancements. Furthermore, your OA publication meets equivalent funder mandates, requirements, and policies.

The advantages of publishing OA in summary:

4. What does this mean for you?

What you should be considering before the transfer of your copy- and usage rights:

Your position as the copyright holder

As copyright holder you own exclusive rights on usage and re-usage of your work by third parties. The type and level of protection of copyrights and licenses vary between countries.

© Thieme Publishing Group – January 2019

Open Access Publishing – Author Information

The Rights Transfer — your rights as an author

When you decide to publish with Thieme, we kindly ask you to assign your copyright to us as your publisher.

OA licenses permit the copyright owner and holder to grant and impose conditions on the reuse of the content. By using a Creative Commons License you agree to a partial disclaimer of your rights as a copyright holder. You renounce irrevocably parts of your copyright and agree to the dissemination of your content to third parties free of charge.

Please find below further information about the CC-licenses and their acceptance by authors:

On the CC website: **https://wiki.creativecommons.org/Considerations_for_licensors_and_licensees**

About the acceptance by authors: **Scholarlykitchen-acceptance authors-licenses**

CC-BY-NC-ND license license enjoys the highest acceptance among authors, the CC-BY license the lowest.

5. CC-BY-NC-ND

To abide by and fully comply with our responsibility as publisher of your scientific work and record, Thieme publishes your OA paper under the most recent CC-BY-NC-ND license:

CC-BY-NC-ND (Attribution-Noncommercial-No Derivatives)

Users may **share**, copy, and redistribute the material in any medium or format under the following **conditions:**

Attribution — user must give appropriate credit, provide a link to the license, and indicate if changes were made. User may do so in any reasonable manner, but not in any way that suggests the licensor endorses the user or her/his use.

Noncommercial — user may not use the material for commercial purposes.

No Derivatives — if user remixes, transforms, or builds upon the material, the user may not distribute the modified material.

No additional restrictions — user may not apply legal terms or technological measures that legally restrict others from doing anything the license permits.

Thieme's default license is CC-BY-NC-ND. However, in case of a funder requiring it, you as the author may select another license and we will arrange your publication accordingly.

Please find the relevant information on the submission page of the journal of your choice, as well as on our Copyright Transfer Agreements.

Should you have any questions or would like more information, please visit our Open Access page:
http://open.thieme.com/de/web/19/faq

Or contact us: the editorial team of your journal

or

thiemeopen@thieme.de

© Thieme Publishing Group – January 2019

Instructions for Author Corrections

Dear Author,

Many thanks for choosing to publish your manuscript with us! The reviewing of the typeset article by yourself is one of the last steps before publication, and we now ask for your approval to proceed with publication.

The manuscript has been prepared for publication by our copy-editors and typeset into the journal layout. Suggested changes, new formulations, deletions or restructuring of the text, if they are significant, along with questions to you are marked with a square (■). Please consider carefully whether the changes made are suitable and are correct and respond to each question within the pdf proof. Please also check that the images have been reproduced correctly when incorporated into the layout (please note that the resolution will be higher in the print version).

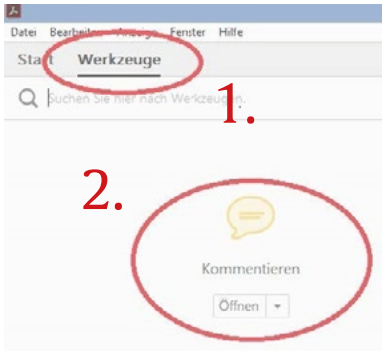
During the editorial process your article has been reviewed, and (if applicable) dosages outlined in the text were scrutinized. However, only you, as the author, have the expertise to assess the accuracy and validity of the information within the text. The responsibility for the accuracy rests solely with you.

We ask that you return corrections in the following way:

To prevent misunderstandings and errors due to readabilty of handwriting, please make any necessary changes in the PDF document using the following edit functions in Adobe Acrobat Reader DC (freeware) or Adobe Acrobat Pro DC and Adobe Acrobat Pro (full version):

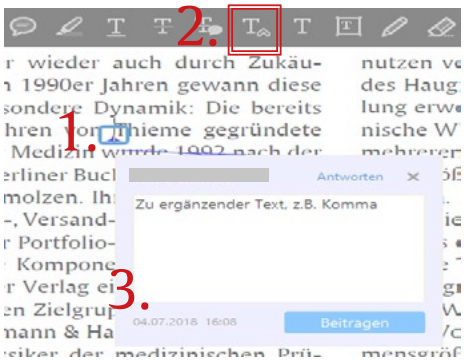
Activating the comment function

Acrobat Pro:
1. Tools
2. Comment



Function: **Inserting** text at the cursor location

1. Move cursor to the desired location
2. Click on button „Insert text at cursor“
3. Enter the desired text in the pop-up note box

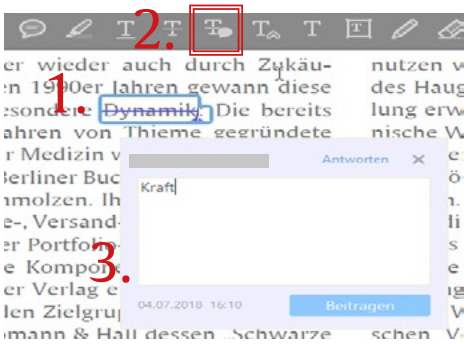


© Thieme Publishing Group – January 2019

Thieme

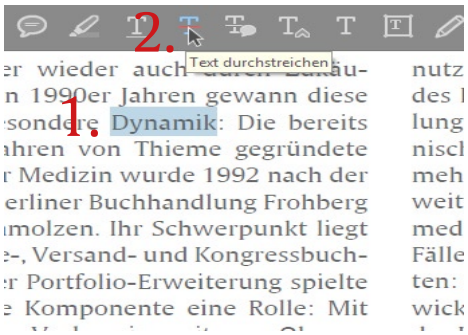
Function: **Replacing** text

1. Highlight text to be replaced
2. Click on button „Add note to replace text“
3. Enter new text in the pop-up note box



Function: **Crossing out (Deleting)** text

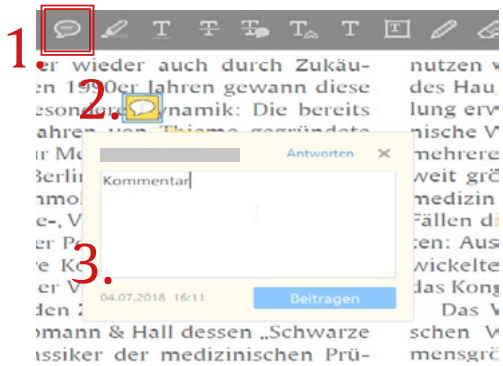
1. Highlight text to be deleted
2. Click on button „Strikethrough text“



Function: **Adding a sticky note / comment**

1. Click on button „Add sticky note“
2. Click on where you want to place the note in the text
3. Enter comments in the pop-up note box

Please never use this function for text corrections!



Please do not use the Note, Text comment and “Edit Text and Images” function as they will cause technical problems and may cause serious delay in the publication process.

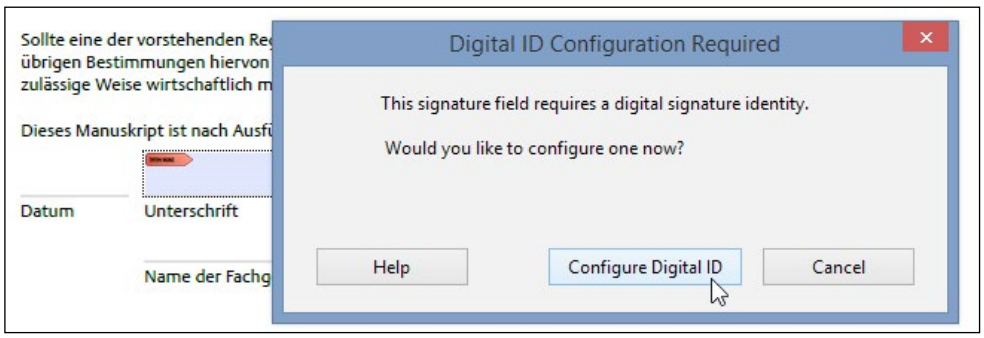
You should return the PDF with the marked corrections by e-mail to your contact at the publishers.

© Thieme Publishing Group – January 2019

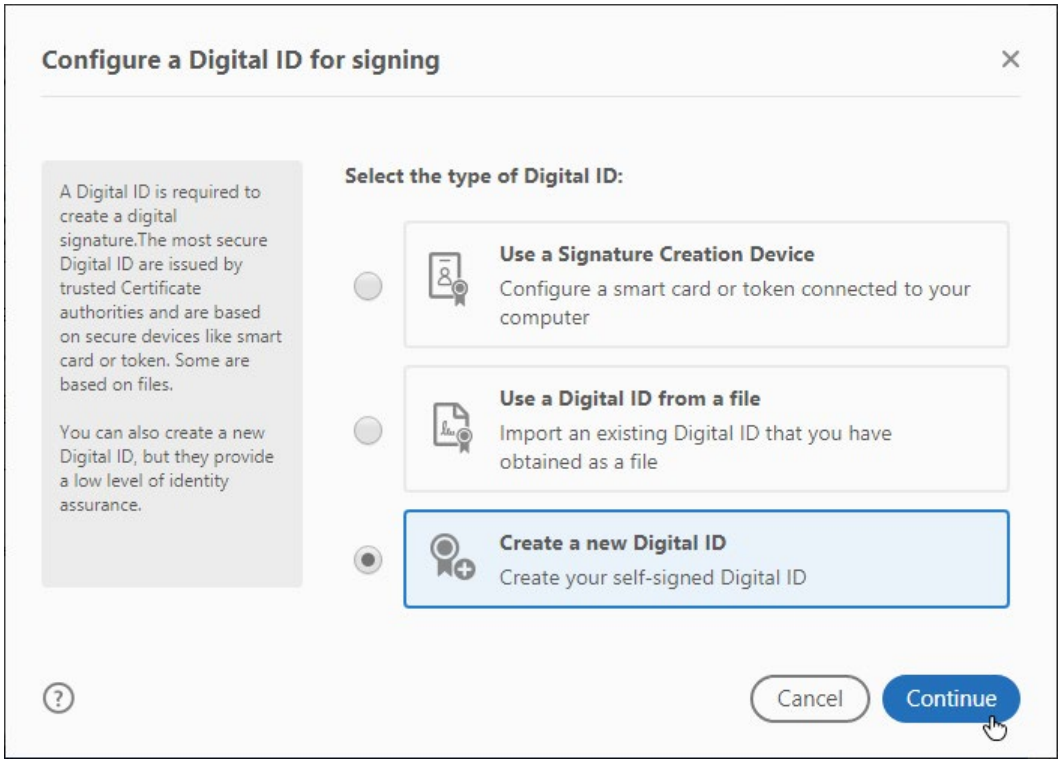
How to Set Up a Digital Signature in Adobe Reader or Adobe Acrobat

Please note: depending on the version of the Adobe program, the depiction of the dialog boxes and the wording can vary. The following screenshots were taken from Adobe Acrobat Reader DC.

To begin the digital ID setup process simply click in the appropriate signature field. Select “Configure Digital ID”.

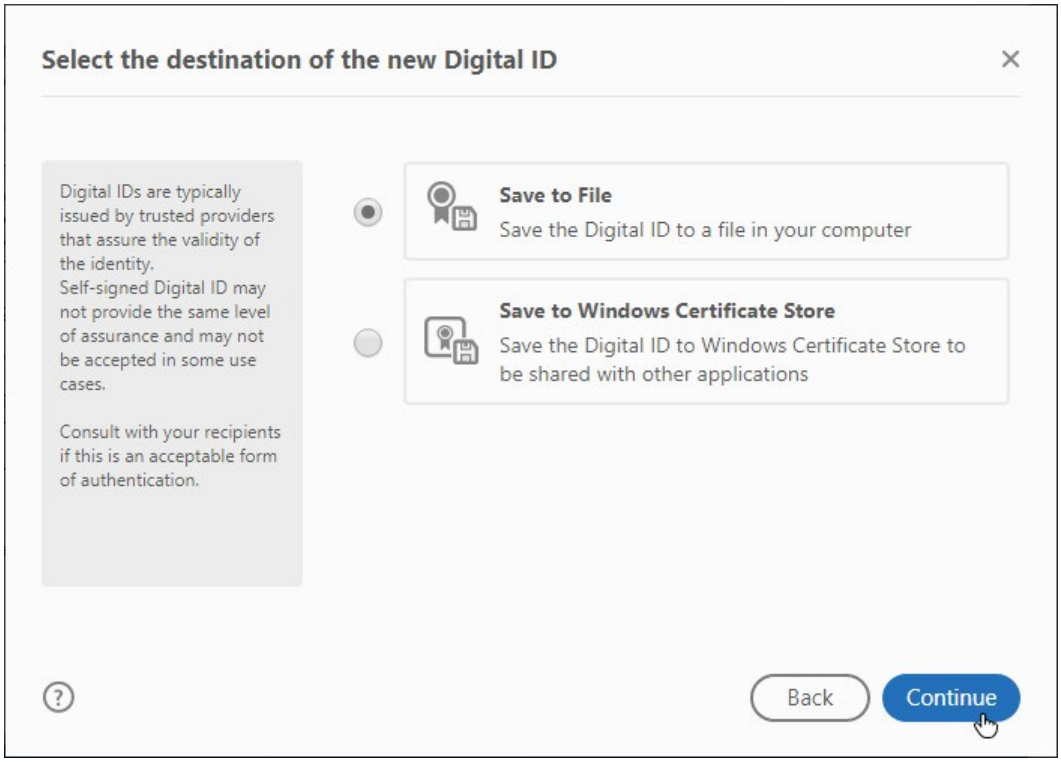


In the following dialog box, please select “Create a new Digital ID” and click Continue.

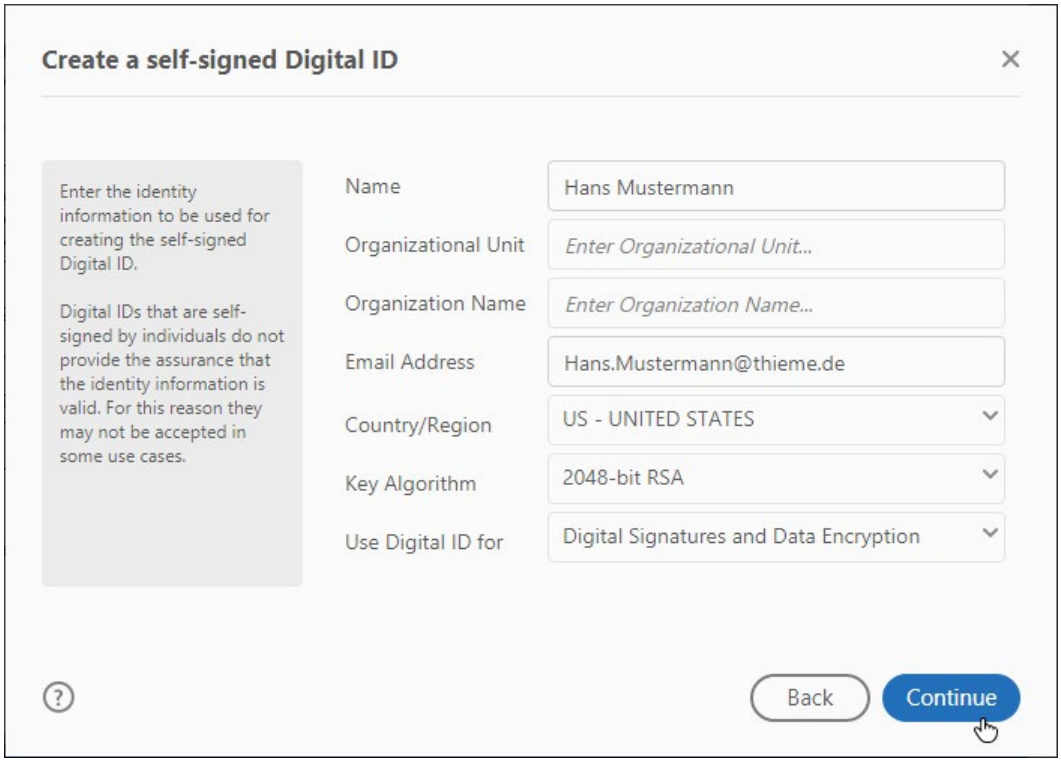


© Thieme Publishing Group – January 2019

Specify where you are going to store the digital ID – select “Save to File” and click Continue.

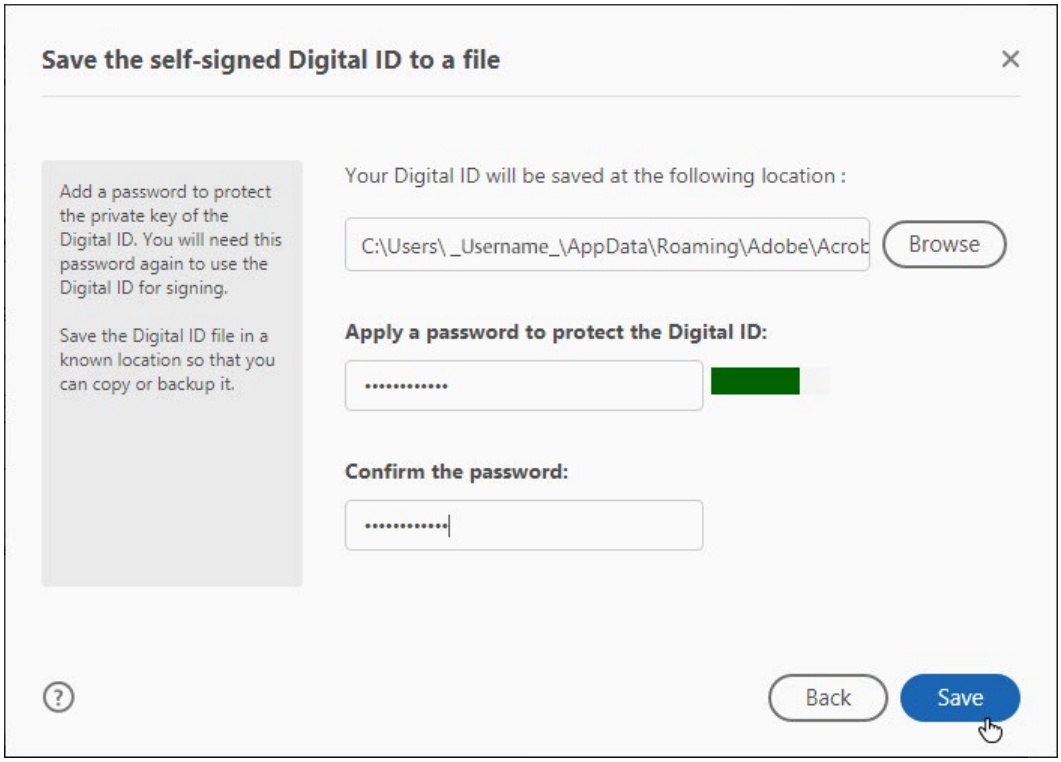


Type in your personal information (name, organization unit, organization name and email address, country) in all fields and click Continue.



© Thieme Publishing Group – January 2019

Enter a file location for your new Digital ID file – either use the default location or enter a different location if you prefer. Then create and enter a password for the ID into both password fields and click Save.



© Thieme Publishing Group – January 2019

Order Form for Offprints and additional copies of *Seminars in Thrombosis and Hemostasis*

Please circle the cost of the quantity/page count you require (orders must be in increments of 100)

	Pages in Article / Cost				
Quantity	**1 to 4**	**5 to 8**	**9 to 12**	**13 to 16**	**17 to 20**
100	$298	$497	$746	$968	$1,158
200	$397	$646	$970	$1,258	$1,495
300	$496	$798	$1,198	$1,568	$1,869
400	$549	$886	$1,330	$1,735	$2,075
500	$598	$966	$1,450	$1,886	$2,262
1000	$1,076	$1,739	$2,610	$3,385	$3,995

Volume/Issue #: _____ Page Range (of your article): _____

Article Title: _____

MC/Visa/AmEx No: _____ Exp. Date: _____

Signature: _____

Name: _____

Address: _____

City/State/Zip/Country: _____

Corresponding author will receive a complimentary PDF of the article after publication.

Number of **additional** copies of the journal, at the discounted rate of $25.00 each: _____

Notes

1. The above costs are valid only for orders received before publication of the issue. **Reprints ordered after printing will be substantially more expensive.**

2. **A shipping charge will be added to the above costs.**

3. Reprints are printed on the same coated paper as the journal and saddle-stitched.

4. For larger quantities or late orders, please contact reprints department:

Phone: +1(212) 584-4662
Fax: +1(212) 947-1112
E-mail: reprints@thieme.com

Clinical, Laboratory, and Molecular Characteristics of Inherited Vitamin K–Dependent Coagulation Factors Deficiency

Salvatore Perrone[Q1], MD[1,*] Simona Raso, MD, PhD[2,*] Mariasanta Napolitano, MD, PhD[3]

[1] Department of Hematology, S. M. Goretti Hospital, Polo Universitario Pontino, Latina, Italy
[2] Department of Hematology and Rare Diseases, V Cervello Hospital, Azienda Ospedaliera Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy
[3] Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo, Italy

Address for correspondence Mariasanta Napolitano, M
[Q2]Haematology Unit, Department of Health Promotion
Child Care, Internal Medicine and Medical Specialties (P
University Hospital "P. Giaccone," University of Palermo
Vespro, 129, Palermo 90127, Italy
(e-mail: mariasanta.napolitano@unipa.it).

Q1 ,Yes, I confirm that these informati on are correct

Q1

Q2

Semin Thromb Hemost 2024;00:1–10.

Q2: Please mo

Abstract

Vitamin K–dependent coagulation factors deficiency (VKCFD) is a rare autoso recessive genetic disease characterized by impaired levels of multiple coagulation factors (II, VII, IX, and X) and natural anticoagulants (proteins C and S). VKCFD is part of familial multiple coagulation factor deficiencies, reporting overall 50 affected families thus far. Disease manifestations are quite heterogeneous, bleeding symptoms may vary, and even, although generally mild, some patients may succumb to fatal outcomes. VKCFD diagnosis may be delayed because the disease phenotype simulates the most frequently acquired deficiencies of vitamin K. First-line coagulation assays, prothrombin time/international normalized ratio (PT/INR) and activated partial thromboplastin time (aPTT), are both prolonged; mixing test typically normalizes the clotting times; and vitamin K–dependent coagulation factors will be variably decreased. Molecularly, VKCFD is associated with mutations in *γ-glutamyl-carboxylase* (*GGCX*) or *vitamin K epoxide reductase* complex subunit 1 (*VKORC1*) genes. Vitamin K is involved not only in the biosynthesis of coagulation proteins but also in bone metabolism and cell proliferation. Therapeutic options are based on vitamin K supplementation, coagulation factors (prothrombin complex), and fresh frozen plasma, in case of severe bleeding episodes. Two case studies here illustrate the diagnostic challenges of VKCFD: case 1 depicts a woman with a history of bleeding episodes, diagnosed, only in her third decade of life with inherited homozygous *GGCX* gene mutation. Case 2 shows a man with an acquired vitamin K deficiency caused by Crohn's disease. Better understanding of *GGCX* and *VKORC1* mutations aids in prognosis and treatment planning, with emerging insights suggesting potential limitations in the effectiveness of vitamin K supplementation in certain mutations.

Keywords
- VKCFD
- γ-Glutamyl carboxylase
- VKORC1
- rare bleeding disorders
- familial multiple factor deficiencies
- vitamin K antagonists

* *These authors contributed equally to this article.*

Issue Theme Precision Medicine in Rare Bleeding Disorders; Guest Editors: Maha Othman, MD, PhD, Akbar Dorgalaleh, PhD

© 2024. Thieme. All rights reserved.
Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

DOI https://doi.org/10.1055/s-0044-1792031.
ISSN 0094-6176.

Vitamin K–dependent coagulation factors deficiency (VKCFD) is a very rare autosomal recessive genetic disease characterized by multiple decrease in the levels of several coagulation factors, involving the glutamination, vitamin K (VK) mediated, of coagulation factors II (FII), VII (FVII), IX (FIX), and X (FX), as well as natural anticoagulants protein C (PC), protein S (PS), and protein Z (PZ). Two separate genes are involved in the pathogenesis of this disease: γ-glutamyl-carboxylase (*GGCX*, OMIM 277450) and vitamin K epoxide reductase complex subunit 1 (*VKORC1*, OMIM 607473).[1–3] Patients harboring defects in *GGCX* have VKCFD type 1, while those with *VKORC1* defects have VKCFD type 2. For classification purposes, VKCFD is part of the familial multiple coagulation factor deficiencies (FMCFDs), a group of rare inherited disorders characterized by a simultaneous decrease in the levels of two or more coagulation factors arising from a genetic defect or defects.[4] The prevalence of VKCFD is unknown, but less than 50 affected families and 272 individuals with publicly available variants have been reported worldwide in the literature to date.[5,6] Indeed, all available information are mainly based on multiple case reports and small clinical series,[2,3,7–37] the first identified in 1966.[32] Clinical manifestations of VKCFD are variable from one individual to another; they typically involve not only the coagulation system but also connective tissue's health and cell signaling. Indeed, VK is involved in the carboxylation of several proteins in different tissues and organs: K2-dependent proteins activate a protective mechanism preventing the development of vascular calcification involved in the occurrence of cardiovascular disease[38] and patients treated with VK inhibitors develop calcifications of vessels and cardiac valves.[39] It is thus hypothesized that lectin and adiponectin are involved in an intricate network for glucose metabolism and osteocalcin promotes proliferation of pancreatic β cells; so, the implications of these proteins in diabetes are under active research.[40,41] VK is also implicated in inhibiting the proliferation, growth, and differentiation of cancer cells through various mechanisms, including induction of c-myc and c-fos genes, regulation of B cell lymphoma-2 (Bcl-2) and p21 genes, and angiogenesis inhibition[42]; therefore, its involvement in cancer has been suggested.[43] With reference to the hemostatic profile of VKCFD, bleeding symptoms are generally mild. In the most severe forms of the disease, symptoms appear at birth, while in milder forms their onset may be later in life. Bleeding manifestations at birth must be differentiated from VK deficiency of the newborn,[44] while symptoms with late onset in adulthood are more frequently related to acquired forms secondary to disorders of the bowel,[45] liver disease,[46] dietary VK deficiency,[47,48] or drugs[49,50] like VK antagonists.[51] Subjects affected by VKCFD may show other nonbleeding abnormalities due to defective carboxylation in extracellular connective tissue proteins such as osteocalcin and Gla proteins, including midfacial hypoplasia, premature osteoporosis, cochlear hearing loss, heart valve defects, pulmonary stenosis, or pseudoxanthoma elasticum-like phenotype.[52]

The primary aim of this narrative review is to analyze, starting from two paradigmatic cases, the scientific data on the clinical, laboratory, and molecular aspects characterizing VKCFD. We highlight current and future challenges in the diagnostic workup and treatment choices of VKCFD.

Case 1

A 34-year-old Caucasian[Q3] woman was referred to our hemophilia center, for prolonged clotting times incidentally found before an elective colonoscopy: the activated partial thromboplastin time (aPTT) was mildly prolonged (37 seconds) and the prothrombin time/international normalized ratio (PT/INR) was remarkably increased (INR = 3.63); fibrinogen, D-dimer, and platelet counts were normal. She had a previous history of repeated nose and gum bleedings, excessive menstrual blood loss, severe postpartum hemorrhage requiring blood transfusions, and hemoperitoneum cyst rupture. She had also received a diagnosis of precocious "osteoporosis." The family history was negative for hemorrhagic diathesis. Despite a personal history characterized by several, even severe, bleeding symptoms with a bleeding score of 6, calculated by International Society on Thrombosis and Haemostasis Bleeding Assessment Tool (ISTH-BAT),[53] she was never investigated for hemorrhagic disorders. A revision of her past exams showed the presence of abnormal standard coagulation assay results over time, although with variable values, and an anecdotal positive lupus anticoagulant (LA) assay.

At first step, PT/INR and aPTT assays were repeated in the hospital clinical laboratory (HCL), confirming the abnormal results. The second laboratory step was taken, according to clinical suspicious and past bleeding history, by performing a mixing study, and, following its correction, VK-dependent factors with the following results: FVII:C = 10% (activity), FIX:C = 21%, FX:C = 8%, FII:C = 13%, PC = 34% (normal range: 70–140%), and PS = 21% (normal range: 74–146%).

Protein C was measured on an ACL 9000 Coagulometer (Instrumentation Laboratories, Lexington, MD) using IL Test ProClot (Instrumentation Laboratory) reagent. Protein S was measured on an ACL 3000 Coagulometer by the method of IL-Test Protein S (Instrumentation Laboratory).

In the presence of a laboratory diagnosis of VKCFD, the genetic defect was then investigated by Sanger sequencing, and a pathogenetic homozygous mutation in the γ-glutamyl-carboxylase (GGCX) gene was found. To prepare the patient for an invasive procedure, an intravenous (IV) single dose of vitamin K1 (10 mg) was administered 1 hour before the colonoscopy; this allowed a fast, efficient, and sustained normalization of the both coagulation times (PT and aPTT).[54] The patient did not develop any procedure-related complication and she was started on prophylactic treatment with twice weekly oral VK with the purpose of reducing her excessive menstrual blood losses.

Case 2

A 45-year-old Caucasian[Q4] man presented to the emergency room of our hospital, due to a recent onset of extensive bruising and widespread subcutaneous hematomas; he had no family or personal history of previous bleeding episodes,

 © 2024. Thieme. All rights reserved.

even after a hernia surgery procedure. He was on oral medication (ACE inhibitors) for high blood pressure, and he did not take any additional drug recently. Moreover, the patient complained of diarrhea and rare episodes of rectal bleeding in the last 2 months. The first-line coagulation assays showed a nondetectable PT/INR and a considerably prolonged aPTT (57 seconds) while fibrinogen, D-dimer levels, and platelet count were normal. He underwent second-level coagulation assays in HCL, including a mixing test, LA assay, platelet function tests, levels of coagulation factors, and fibrinolytic proteins (plasminogen, α2-antiplasmin). Second-level coagulation assays revealed decreased VK-dependent factors levels: FII:C = 17%, FVII:C = 2.9%, FIX: C = 11%, FX: C = 8.5%, PC = 58%, PS = 64%; AT = 94%, and plasminogen 116% (normal range: 80–130%), α2-antiplasmin = 108% (normal range: 98–122%).

Platelet function testing (PFA100) was normal and LA was negative. Based on laboratory findings and patient history of recent onset of bleeding symptoms, an acquired deficiency of VK factors was suspected. To define the etiology of acquired VK deficiency, further laboratory assays were performed including liver function tests and fecal calprotectin. Results of these investigations showed an increase in C-reactive protein (22 mg/dL), gamma-globulin plasma concentration (3.45 g/dL), and a high level of fecal calprotectin (504 µg/g). Endoscopic examinations of the gastroenteric tract (gastroscopy and colonoscopy with biopsy) led to the diagnosis of Crohn's disease causing VK deficiency due to malabsorption.[55]

During the acute phase of Crohn's disease, intravenous VK1 was regularly administered at the dosage of 10 mg to control rectal bleeding. Parenteral VK treatment induced a fast, efficient, and sustained normalization of coagulation times and controlled clinical symptoms; after the acute phase, VK treatment was discontinued.

The Hemostatic and Nonhemostatic Role of Vitamin K

VK is a lipo-soluble vitamin involved in the process of carboxylation of several proteins including coagulation factors; among VK-dependent nonhemostatic proteins, there are matrix Gla protein (MGP), osteocalcin (BGLAP), proline-rich Gla proteins (PRGPs) 1 and 2, upper zone of the growth plate and cartilage matrix-associated protein (UCMA/GRP), transmembrane Gla proteins (TMGs) 3 and 4, and growth arrest-specific 6 (GAS6m atrix-Gla protein). These proteins have several functions: BGLAP, MGP, and GRP regulate the calcification processes of bone and soft tissues. GAS6 is involved in cell signaling and platelet aggregation.[30,34,36]

VK1 (phylloquinone) is present in fruits and vegetables (in particular green leafy vegetables, herbs, vegetable oils), and represents the main source of VK in diet (90%). VK2 (menaquinones) is found in animal-based and fermented foods or produced by intestinal bacteria, but its total amount is quite limited.[56] In Europe, the recommended VK daily allowance is 75 µg (Commission Directive 2008/100/EC).[57] Additionally, VK2 has been suggested to be suitable for a specific dietary recommendation intake.[58] Vitamin K2 has a higher bioactivity than vitamin K1 for several molecular processes, including γ-glutamylcarboxylation activity, inhibitory effect on bone resorption, antioxidant, and anticancer effects.[43,59]

The process of carboxylation consists in the posttranslational modification of some particular glutamate (Glu) amino acids that receive a carboxylic group to form a carboxyl glutamate (Gla), which generates a calcium-binding module that is essential for the activity of the protein.[60]

Glu is targeted for carboxylation because they contain an exosite binding domain (EBD) that can be recognized by the enzyme. The GGCX, which is an integral membrane protein localized into the endoplasmic reticulum, allows interactions with the hydrophobic VK and generates Gla residues by the oxygenation of reduced VK, which in turn becomes converted to VK epoxide during this reaction (►**Fig. 1**).[61] In particular, GGCX catalyzes a reaction between oxygen and reduced VK to generate a VK superbase that extracts a hydrogen from Glu, thus generating a Glu carbanion that then reacts with CO_2 to form Gla.[62] The VK epoxide produced by GGCX must be reduced by VKORC to regenerate VK hydroquinone, which is required for continual carboxylase activity.[63] VKORC is also targeted by warfarin; therefore, when VK hydroquinone levels are decreased, carboxylase activity is disrupted and, in turn, levels of active VK-dependent proteins including those involved in hemostasis are decreased; this explains the clinical activity of VK antagonist drugs.[64] Hereditary mutations in *VKORC1* cause warfarin resistance and multiple coagulation factor deficiency type 2.[65] Indeed, in clinical practice, VK is used to reverse VK antagonist overdose or rodenticide poisonings,[66] and it is also administered in newborns to prevent the classic hemorrhagic disease of the newborn.[67]

Molecular Basis of VKCFD

GGCX is a well preserved and not polymorphic gene; indeed, only two variants (rs699664 and rs6173310) of the 400 known variants have an allelic frequency >0.0001.[65] Mice lacking the homologous GGCX protein show a complete lethality secondary to hemorrhages, suggesting the critical role of GGCX and the lack of alternative pathways.[68] Only 40 gene mutations occurring in *GGCX* have been detected by genetic screenings of patients with VK-related disorders (►**Fig. 2**). De Vilder et al revised available cases and tried to correlate the genomic and phenotypic spectrum of mutations in *GGCX* gene. Beyond coagulation, other systems can be involved: bones, cardiac, skin, and ocular systems. In particular, VKCFD1 can be associated with skeletal (midfacial hypoplasia, reduced bone mass, chondrodysplasia punctata) or cardiac abnormalities (patent ductus arteriosus Botalli, septal closure defects) in some patients.[69,70] In the systematic review from De Vilder, 47 patients with mutations of *GGCX* were identified; of these, 33 patients had VKCFD1, 25 of which had a deficiency of all VK-dependent coagulation factors. Reduced FX activity was observed in all patients, FII impairment in 30 patients, FVII deficiency in 31 patients, and FIX was abnormally low in 24 patients. Of note, FIX deficiency was present only in those patients in whom all the other coagulation factors were also deficient. Of the 33 patients with VKCFD1, 21 patients were symptomatic (spontaneous mucosal bleeding, intra-articular bleeding, abnormal bleeding after injuries, vaccination, and surgery). Ten patients were severely affected with

 © 2024. Thieme. All rights reserved.

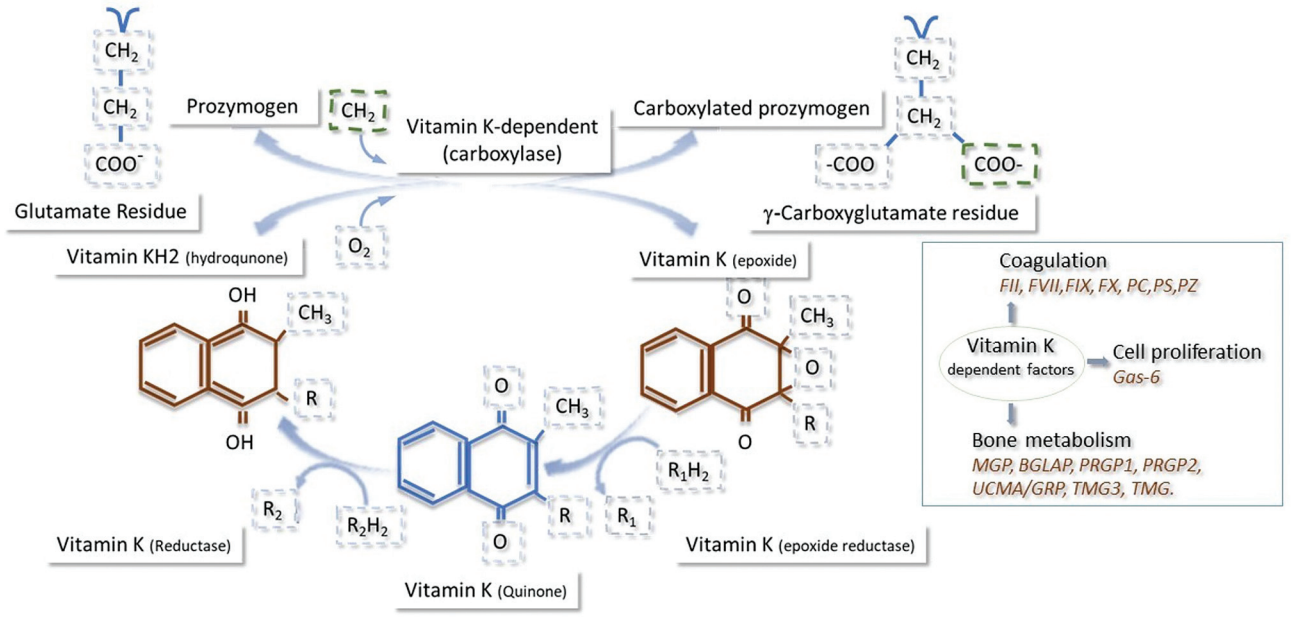


Fig. 1 Vitamin K metabolism and function in coagulation and beyond. Coagulation: factor II (FII), factor VII (FVII), factor IX (FIX), factor X (FX), protein C (PC), protein S (PS), protein Z (PZ). Beyond coagulation: matrix Gla protein (MGP), osteocalcin (BGLAP), proline-rich Gla proteins (PRGPs) 1 and 2, upper zone of the growth plate and cartilage matrix-associated protein (UCMA/GRP), transmembrane Gla proteins (TMGs) 3 and 4, and growth arrest-specific 6 (GAS6).

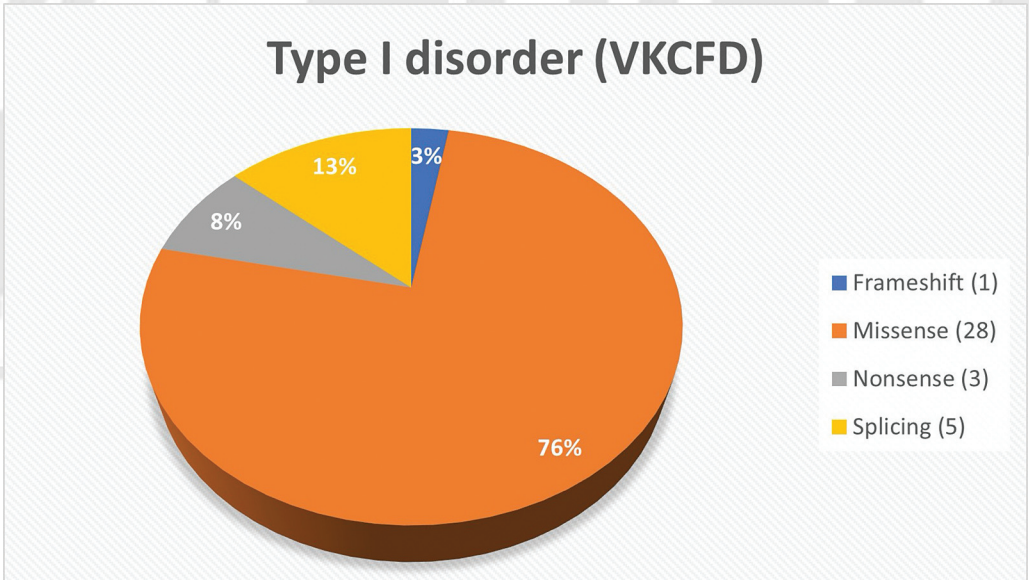


Fig. 2 This diagram shows the relative frequency of reported cases of mutations involving GGCX. To date, at least 34 mutations have been reported in the GGCX gene, which are associated with VKCFD and the majority of them are point mutations. The mutations can be observed in homozygous or compound heterozygous.

symptoms onset before the age of 1 year: all had a combined deficiency of FII, FVII, FIX, and FX; none of the severely affected patients had cutis laxa or other pseudoxanthoma elasticum (PXE)-like[71] skin or eye manifestations. Eleven patients developed a bleeding, four of them had skin symptoms and three had eye manifestations.[69]

In two unrelated patients with VKCFD type 2, the same homozygous point mutation was identified by Rost et al in the third exon of the *VKORC1* gene (located on chromosome 16p11.2): a 292C-T transition resulting in an arg98-to-trp (R98W) substitution.[65] The families of the two subjects were of Lebanese and German origin.[11] Their haplotypes in the region of homozygosity encompassing the mutated gene were different. Furthermore, cytosine-292 is part of a CpG dinucleotide, which is a known mutation hotspot. Rost et al suggested that gene mutation in these families probably arose independently.[65] Subsequently, Marchetti et al reported a patient with moderate to severe bleeding tendency and found to be homozygous for the unique *VKORC1* mutation (Arg98Trp) so far detected in VKCFD2. This R98W mutation causes mislocalization from endoplasmic reticule and degradation of the VKORC protein, and thus reduces most of its activity.[54] This patient also carried a homozygous

 © 2024. Thieme. All rights reserved.

FVII gene polymorphism (613878.0013) associated with further reduction in factor VII levels.[72]

Clinical Manifestations of VKCFD

Even if a reduced activity of natural anticoagulants might be potentially associated with thrombotic risk, there are no reports of venous or arterial thrombosis in patients affected by VKCFD, commonly presenting, on the contrary, with bleeding symptoms and a quite variegate clinical phenotype.[73]

Most patients seek medical attention for bleeding episodes ranging from mild bruises,[23] or epistaxis[24] to severe intracranial bleeding.[34] The vast majority of available reports are from pediatric patients and focused on prenatal diagnosis, familial screening, and genetic counseling[11,36,74]; however, cases of prolonged bleeding after surgical procedures are also described.[33]

This last clinical scenario was described in case 1 with major bleeding episodes occurring mainly postpartum and after the rupture of ovarian cysts. However, the patient also experienced spontaneous, mild hemorrhagic symptoms.

The case underlines how VKCFD is defined by a wide spectrum of symptoms. Usually, the cluster of symptoms involves skin and mucosae with frequent easy bruising. Symptoms occur either spontaneously or in a surgical or trauma setting. Mucocutaneous bleeding, such as gastrointestinal bleeding, may also appear in conjunction with antibiotic therapy, as a consequence of the reduced VK2 production by gut bacteria.[1,42]

VKCFD can sometimes cause fatal intracranial bleeding in the first weeks of life, thus resembling the acquired hemorrhagic disease of the newborns.[10,32,34] Umbilical cord bleeding has also been described,[32,73] while hemarthrosis seems quite rare.[73]

Clinical burden depends on the residual coagulation factor activity, which is also based on the availability of VK.[16] Moreover, antibiotic and anticonvulsant therapy administration must be carefully evaluated, as these drugs can contribute to worsen the bleeding pattern.[73]

VKCFD-affected patients may also suffer from non–hemostasis-related symptoms, including mental retardation, osteoporosis, increased fracture rates, and risk of cardiovascular diseases, due to the defective γ-carboxylation of several other VKD proteins.

In case 1, the pattern of VKCFD symptoms included "precious osteoporosis" in the absence of any additional risk factor.

Skeletal abnormalities of subjects affected by VKCFD include nasal hypoplasia, distal digital hypoplasia, and epiphyseal stippling; they all resemble those observed in warfarin embryopathy.[8] Pseudoxanthoma elasticum-like (PXE-like) disorders have also been reported in patients carrying *GGCX* gene mutations[71] leading to skin hyperlaxity and folds characterized by severe fragmentation and calcification of elastic fibers. Cardiac abnormalities including calcified peripheral arteries, subclinical atherosclerosis, and congenital atrial septal defects are also detected in PXE-like disease. An increased rate of fetal loss has been hypothesized by some authors as a result of bone metabolism during embryogenesis; however, the exact incidence of abortion is unknown due to the rarity of the disease.[2,8]

Laboratory and Differential Diagnosis

Case 1 highlights that VKCFD is a very rare hemorrhagic disorder for which the clinical suspicion is crucial; the diagnostic delay observed may be due to several reasons. First, it is possible that the hemorrhagic phenotype of the woman was considered mild and mainly due to concomitant risk factors; another potential reason for this delay may be due to the variable prolonged values of first-line coagulation assay results (PT/INR and aPTT), probably attributed to the detection of LA.

While the diagnosis of a single-coagulation factor deficiency is often straightforward, the diagnosis of VKCFD can be challenging due to a wide heterogeneity of presentations and often indistinct clinical and laboratory features.

Facing a clinical suspicion of coagulation disorder, a fundamental step is to collect an accurate personal bleeding history and to systematically evaluate the severity of bleeding symptoms by adopting BATs.[53]

The patient from case 1 had an abnormal BAT score highlighting the need for further investigations. Familial history may be negative because the defect is autosomal recessive even if carriers of genetic missense mutations in *GGCX* or *VKORC*[75] may be symptomatic. The diagnostic workup requires the exclusion of other, more frequent, conditions mimicking VKCFD. Initial laboratory finding is characterized by prolonged PT/INR and aPTT. The differential diagnosis of a prolonged coagulation time assay includes artifacts such as a high hematocrit, deficiency of one or more clotting factors, the presence of inhibitors, therapeutic anticoagulation, or sample contamination with anticoagulant medications. The most common cause of a prolonged aPTT reported in the acute care setting is LA, while factor deficiencies are rare.[76]

Liver failure, disseminated intravascular coagulation, or defects in the common coagulation pathway are warranted in the differential diagnosis.[77]

In VFCKD, the extent to which the PT and aPTT are prolonged may be influenced by the degree of individual coagulation factor reduction.[75,78] Usually, PT/INR is slightly more prolonged than aPTT because FVII has the shortest half-life. VK-dependent coagulation factors activity can be variably decreased, usually ranging between 20 and 60% and less commonly below 10%.[79]

Abnormal screening coagulation tests are followed by mixing studies (50:50) to rule out an inhibitor's interference like in acquired hemophilia or in the very rare presence of inhibitors against FVII (►**Fig. 3**). When mixing studies correctly, as in case of VKCFD, specific factor assays are performed to identify the deficiency.[80]

Other rare inherited bleeding disorders may mimic the clinical presentation of VFCKD and they should be kept in mind in differential diagnosis. These include, in order of frequency, single coagulation defects of FIX (hemophilia B); FVII deficiency; the rarest combined FII and FX deficiencies; as well as other combined defects like combined FVII and FX deficiency or type 2N von Willebrand disease or a FV disorder with overexpression of FV short.[78,81]

 © 2024. Thieme. All rights reserved.

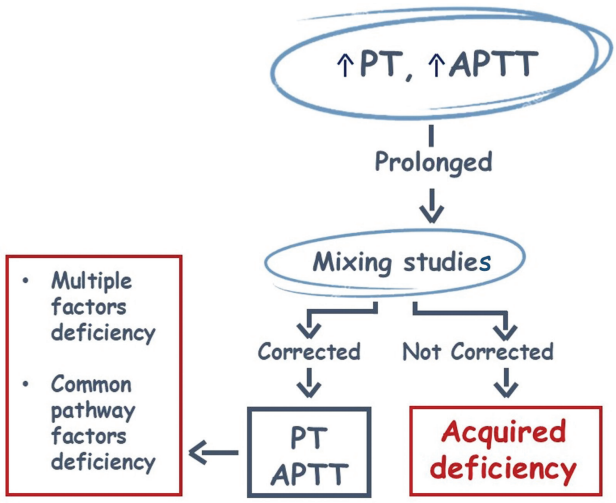


Fig. 3 Laboratory approach to the differential diagnosis of VKCFD. Prolongation of PT and aPTT requires a mixing study with normal plasma, a correction of both assays after mixing implies that multiple factor deficiencies are present while persistently prolonged PT and aPTT suggest inhibitors or acquired conditions like high titer lupus anticoagulant or severe disseminated intravascular coagulation.

Coagulation assays are extensively adopted in clinical practice as indicators of vitamin K deficiency; however, this approach lacks sensitivity and specificity because it mirrors only markedly decreased VK-dependent coagulation factors. Usually, an approximate 50% decrease in prothrombin levels (FII) is required for prolonging PT values.[82] The measurement of phylloquinone (K1) concentrations in serum is a marker of vitamin K status and it reflects abundance of the vitamin. Concentrations of K1 below 0.15 µg/L are suggestive of deficiency, but they do not necessarily mirror its use by target tissues.[83] Moreover, the disadvantages of this approach include exclusion of the other vitamin K homologues, interference by dietary lipid content, and a not-easy-to-access to the assay.[84]

Oxidized vitamin K (KO) is typically undetectable in VKCFD type I, even after vitamin K supplementation, but in VKCFD type II, an increase in KO serum levels can be observed following VK supplementation. In increased protein induced by VK absence/antagonism (PIVKA-II), also called Des-gamma-carboxy prothrombin (DCP), circulating levels can be detected in case of vitamin K deficiency. PIVKA-II or DCP levels can thus be adopted as an indirect indicator of hepatic vitamin K status, and their abnormal levels precede any subsequent change in PT.[85] PIVKA-II can be measured by automated immunoassay, manual ELISA, or liquid chromatography-tandem mass spectrometry (LC-MS/MS) and show minimal variability for age or dietary lipid content.[85,86] In patients with hemorrhagic phenotype, VKCFD diagnosis is based on disproportional bleeding symptoms in comparison to the reduced levels of the affected clotting factors. A clinical distinction is, however, not always easy and requires a precise laboratory assessment as well as genotype confirmation. As shown in case 2, it is essential to differentiate between acquired VK deficiencies and VKCFD. The acquired forms can be attributable to (1) intestinal malabsorption of VK (inflammatory bowel diseases or celiac disease); (2) severe liver dysfunction (e.g., liver cirrhosis); (3) drug interactions caused by voluntary or accidental ingestion of warfarins and superwarfarins); (4) hemorrhagic disease of the newborn.[79] Case 2 showed an atypical early acquired form of VK deficiency due to Crohn's disease onset; VK deficiency in fact usually occurs late during the clinical course of Crohn's disease and is associated with abnormal bone metabolism.[55] Indeed, deficit of dietary VK can be rarely observed,[87] especially in newborns whose parents refuse prophylactic administration of VK at birth.[88] Interference in metabolism of VK and antibiotics has also been reported.[89] In addition, surreptitious use of VK antagonist and suicidal use of rodenticides[90] must be accurately evaluated; the diagnostic work-up should include a thorough psychosocial and psychiatric assessment, to rule out attempted suicide and factitious purpura.[66] Unfortunately, toxicology testing with liquid chromatography–mass spectrometry (LC-MS/MS) may be unavailable in clinical laboratories of community hospitals.[91] Re-testing after administration of oral or parenteral doses of VK can show transient shortening of PT/INR and aPTT, with consequent increased expression of VK-dependent coagulation factors. Typically, in VKCFD, coagulation parameters improve after VK administration, but after a few hours or days this beneficial effect is lost.[4]

When VKCFD is highly suspected, a research laboratory can perform genotyping of *GGCX* and *VKORC1* genes to confirm the diagnosis.[92]

The genotyping of the two involved genes *VKORC* (spanning 5 kb and containing 3 exons on chromosome 16)[93] and *GGCX* (spanning 13 kb and containing 15 exons on chromosome 2)[94] can be performed in selected laboratories, to confirm the homozygous presence of mutations. Gene analysis is mandatory to exactly define a complex coagulation defect that must be distinguished from many acquired conditions as well as from other single or multiple congenital coagulation defects. In selected cases with severe bleeding, the study of the pedigree of the family, encompassing genetic and psychological counseling, is advised. On a molecular basis, we can distinguish two forms of the disease: VKCFD type 1 (VKCFD1) presenting mutations in *GGCX* and VKCFD type 2 (VKCFD2) presenting mutations in *VKORC* gene.[95,96] To date, prenatal diagnosis of VKCFD is not suggested, considering the potential hemorrhagic risk of the procedure and the prevention of major hemorrhagic events in the affected newborn by the routine replacement therapy with VK1 in the third trimester of pregnancy in mothers which are at risk of VKCFD. However, the antenatal management and timing of replacement therapy to prevent intrauterine bleeding and bone or skin manifestations of the disease should be implemented.[30]

Therapeutic Considerations

The first explored and approved therapeutic option adopted since the discovery of VKCFD consists in the supplementation with exogenous phylloquinone (VK1), since clotting times improve after its administration (►**Table 1**). However, the exact mechanisms by which VK bypasses the gene defect are

 © 2024. Thieme. All rights reserved.

Table 1 Treatment of vitamin K–dependent coagulation factors deficiency

Treatment	Dosage	Schedule	Route of administration	Indication
Vitamin K1[79]	5–20 mg	According to PT/INR	IV or oral	Minor bleeding
Tranexamic acid[79,97]	15–20 mL/kg or 1 g	Up to every six hours	IV or oral	Minor bleeding
FFP[98]	15–20 mL/kg	According to clinical situation	IV	Severe bleeding
3 or 4 PCCs[99]	20–30 units/kg	According to clinical situation	IV	Severe bleeding and major surgery
rFVIIa[33,103]	10–20 μg/Kg	According to clinical situation	IV	Severe bleeding and major surgery

Abbreviations: FFP, fresh frozen plasma; IV, intravenous; PCCs, prothrombin complex concentrates; rFVIIa, recombinant activated FVII.
Note: Data on recommended trough levels for each treatment are not available.

not fully understood, and it is not clear how and if the nonbleeding manifestations of the disease can be affected by prolonged exposure to VK (i.e., by impairing the vascular calcification process).[79]

Oral administration of VK1 may partially correct low coagulation factor levels of approximately 15 to 20%, but it may not be able to prevent major bleeding.[1,79] In cases where oral treatment is not well tolerated or ineffective, intravenous infusion of VK1 can be considered as a valid option, and the schedule of treatment could vary according to PT/INR values. However, a specific VK treatment schedule has not been yet approved for VKCFD. In milder forms or in minor surgery setting, tranexamic acid can be administered as single agent at the dosage of 15 to 20 mL/kg or 1 g every 6 hours.[79,97] During major surgical procedures and severe hemorrhage, fresh frozen plasma (FFP) infusion is indicated; usually, multiple infusions are needed at the recommended dose of 15 to 20 mL/kg of body weight.[98]

As an alternative to FFP, three or four prothrombin complex concentrates (PCCs) should be considered,[78,79,97] although data on use in VKCFD are extremely limited.[98] PCCs have been effectively adopted in warfarin reversal; thus, available schedules derive from this indication, with an initial dose of 500 units (U) given intravenously for a PT/INR below 5.[99]

With reference to the potential risk of transmission of blood-borne pathogens, even if theoretically possible, it is extremely low[100] due to viral inactivating procedures, while a low but appreciable thrombotic risk should be taken into account, especially in the surgical setting.[101] Treatment options of VKCFD also include recombinant activated FVII (rFVIIa), usually indicated for acquired hemophilia, congenital FVII deficiency, or Glanzmann's disease. In clinical practice, rFVIIa has been successfully used off-label for hemorrhagic conditions including warfarin overdose or severe bleeding during warfarin treatment.[102]

In detail, rFVIIa was specifically administered in VKCFD during an emergency minor surgical procedure.[33] A single dose of 1.2 mg (rFVIIa $\sim$ 20 μg/kg of body weight) allowed a successful control of hemostasis and a prompt normalization of the PT/INR. According to the different peaks of action of rFVIIa (2 hours) and VK (24 hours), their combined administration can contribute to achieve a sustained normalization of coagulation parameters, thus representing a valid option in case of life-threatening bleeding or more complex major surgical procedures.[103] In consideration of potential life-threatening bleeding episodes, prophylaxis at diagnosis of VKCFD is strongly recommended. Usually, an oral administration of VK1 5 to 20 mg/day twice or three times per week avoids mucocutaneous bleeding. In case of poor response, VK should be administered intravenously at the dosage of 5 to 20 mg/week.[78] It is, however, known that response to replacement therapy with VK1 is widely unpredictable, depending both on the administration route and on individual sensitivity to VK.[61]

In vitro studies of carboxylation of differently mutated *GGCX* showed that some proteins have a very low or absent enzymatic activity that cannot be easily rescued with conventional doses of vitamin K.[61] It is thus suggested that VKCFD1 and VKCFD2 differently respond to VK. In the meta-analysis performed by De Vilder et al, 17 patients responded well to VK treatment, 5 patients showed no or only limited response, and 3 patients did not show any response. Nine patients had no new bleeding episodes during VK prophylaxis, two patients had recurrent bleeding while on VK treatment, and the clinical outcome was not reported for six patients. In three patients, VKCFD1 was an incidental finding. In six patients, the clinical effect of VK treatment was mentioned. For five of them, a good response to VK supplementation was reported without details on the clinical outcome.[69] An in vitro study suggested that not all mutations of *GGCX* gene can be corrected by VK administration, in particular mutations affecting structural or catalytically important sites as the glutamate binding site (GGCX:p.(L394R), GGCX:p.(H404P)), or the propeptide binding site (GGCX:p.(R485P), GGCX:p.(G558R), GGCX:p.(T591K)).[104] Probably these last mutations should be managed with PCCs.[102] Nevertheless, these predictive investigations are not available in clinical practice so that treatment is commonly guided by the lack of response to oral or parenteral administration of VK1.

Conclusion

Rare inherited coagulation disorders like VKCFD may challenge clinicians and laboratory specialists, in particular

 © 2024. Thieme. All rights reserved.

for the lack of awareness of this very rare disease. To avoid misdiagnosis, the combination of comprehensive screening of residual coagulation factor activities, molecular analysis, and family segregation analysis should be performed. An accurate characterization of the disease may contribute to reach important goals in its management. However, monitoring hemostasis might be an insufficient biomarker of treatment response due to the effects of the *GGCX* mutation on several other systems. Advances in the treatment of VKCFD may not only allow to reduce bleeding complications but also offer solutions to nonbleeding manifestations of the disease. VK1 supplementation may control cardiovascular calcification and osteoporosis with an impact on the morbidity of the disease.[84] VK2 shows a longer half-life in the circulation and may be longer available for the extrahepatic tissues.[105] Therefore, a combined treatment with VK1 and VK2 could be considered as a valid option for managing bleeding and nonbleeding symptoms, respectively. A multidisciplinary team, including hematologists, cardiologists, and orthopedic specialists could ensure a comprehensive care for individuals with VKCFD.

Conflict of Interest
None declared.

References

1 Napolitano M, Mariani G, Lapecorella M. Hereditary combined deficiency of the vitamin K-dependent clotting factors. Orphanet J Rare Dis 2010;5(21):21
2 Darghouth D, Hallgren KW, Shtofman RL, et al. Compound heterozygosity of novel missense mutations in the gamma-glutamyl-carboxylase gene causes hereditary combined vitamin K-dependent coagulation factor deficiency. Blood 2006;108(06): 1925–1931
3 Fregin A, Rost S, Wolz W, Krebsova A, Muller CR, Oldenburg J. Homozygosity mapping of a second gene locus for hereditary combined deficiency of vitamin K-dependent clotting factors to the centromeric region of chromosome 16. Blood 2002;100(09): 3229–3232
4 Zhang B, Ginsburg D. Familial multiple coagulation factor deficiencies: new biologic insight from rare genetic bleeding disorders. J Thromb Haemost 2004;2(09):1564–1572
5 Orphanet: Hereditary combined deficiency of vitamin K dependent clotting factors. Accessed October 29, 2023 at: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=98434
6 The GGCX gene homepage - Global Variome shared LOVD. Accessed February 12, 2024 at: https://databases.lovd.nl/shared/genes/GGCX
7 Pechlaner C, Vogel W, Erhart R, Pümpel E, Kunz F. A new case of combined deficiency of vitamin K dependent coagulation factors. Thromb Haemost 1992;68(05):617
8 Pauli RM, Lian JB, Mosher DF, Suttie JW. 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(04):566–583
9 Vicente V, Maia R, Alberca I, Tamagnini GPT, Lopez Borrasca A. Congenital deficiency of vitamin K-dependent coagulation factors and protein C. Thromb Haemost 1984;51(03):343–346
10 Bhattacharyya J, Dutta P, Mishra P, et al. Congenital vitamin K-dependent coagulation factor deficiency: a case report. Blood Coagul Fibrinolysis 2005;16(07):525–527
11 Oldenburg J, von Brederlow B, Fregin A, 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. Thromb Haemost 2000;84 (06):937–941
12 Spronk HM, Farah RA, Buchanan GR, Vermeer C, Soute BA. Novel mutation in the gamma-glutamyl carboxylase gene resulting in congenital combined deficiency of all vitamin K-dependent blood coagulation factors. Blood 2000;96(10):3650–3652
13 Brenner B, Sánchez-Vega B, Wu SM, Lanir N, Stafford DW, Solera J. A missense mutation in gamma-glutamyl carboxylase gene causes combined deficiency of all vitamin K-dependent blood coagulation factors. Blood 1998;92(12):4554–4559
14 Ghosh K, Shetty S, Mohanty D. Inherited deficiency of multiple vitamin K-dependent coagulation factors and coagulation inhibitors presenting as hemorrhagic diathesis, mental retardation, and growth retardation. Am J Hematol 1996;52(01):67
15 Leonar CO. Vitamin K responsive bleeding disorder: a genocopy of the warfarin embryopathy. Proceeding of the Greenwood Genetic Center 1988;7:165–166
16 Ekelund H, Lindeberg L, Wranne L. Combined deficiency of coagulation factors II, VII, IX, and X: a case of probable congenital origin. Pediatr Hematol Oncol 1986;3(02):187–193
17 Goldsmith GH Jr, Pence RE, Ratnoff OD, Adelstein DJ, Furie B. Studies on a family with combined functional deficiencies of vitamin K-dependent coagulation factors. J Clin Invest 1982;69 (06):1253–1260
18 Puetz J, Knutsen A, Bouhasin J. Congenital deficiency of vitamin K-dependent coagulation factors associated with central nervous system anomalies. Thromb Haemost 2004;91(04):819–821
19 Thomas A, Stirling D. Four factor deficiency. Blood Coagul Fibrinolysis 2003;14(Suppl 1):S55–S57
20 Mickleson KN, Whyte G. Severe deficiency of vitamin K dependent coagulation factors in an infant. N Z Med J 1979;90(645): 291–292
21 Johnson CA, Chung KS, McGrath KM, Bean PE, Roberts HR. Characterization of a variant prothrombin in a patient congenitally deficient in factors II, VII, IX and X. Br J Haematol 1980;44 (03):461–469
22 Fischer M, Zweymuller E. Kongenitaler Mangel der faktoren II, VII und X. Zeitschrift fuer kinderheikunde 1966;95:309–323
23 Arora R, Hartwig E, Kannikeswaran N. Toddler with unexplained multiple bruises. Pediatr Emerg Care 2014;30(09):646–648
24 Al Absi HS, Abdullah MF. Congenital combined deficiency of the vitamin K-dependent clotting factors (VKCFD): a novel gamma-glutamyl carboxylase (GGCX) mutation. J Pediatr Hematol Oncol 2019;41(04):e224–e226
25 Titapiwatanakun R, Rodriguez V, Middha S, Dukek BA, Pruthi RK. Novel splice site mutations in the gamma glutamyl carboxylase gene in a child with congenital combined deficiency of the vitamin K-dependent coagulation factors (VKCFD). Pediatr Blood Cancer 2009;53(01):92–95
26 Chung KS, Bezeaud A, Goldsmith JC, McMillan CW, Ménaché D, Roberts HR. Congenital deficiency of blood clotting factors II, VII, IX, and X. Blood 1979;53(04):776–787
27 Darghouth D, Hallgren KW, Issertial O, et al. Compound heterozygosity of a W493C substitution and R704/premature stop codon within the γ-glutamyl carboxylase in combined vitamin K-dependent coagulation factor deficiency in a French family. Blood 2009;114(22):1302–1302
28 Lunghi B, Redaelli R, Caimi TM, Corno AR, Bernardi F, Marchetti G. Novel phenotype and γ-glutamyl carboxylase mutations in combined deficiency of vitamin K-dependent coagulation factors. Haemophilia 2011;17(05):822–824
29 Jin DY, Ingram BO, Stafford DW, Tie JK. Molecular basis of the first reported clinical case of congenital combined deficiency of coagulation factors. Blood 2017;130(07):948–951

 © 2024. Thieme. All rights reserved.

30 Guzzardo GM, Ghosh S, Pezeshkpoor B, et al. Novel gamma-glutamyl carboxylase mutation causing hemorrhagic and non-hemorrhagic VKCFD1 phenotypes. Blood 2022;140 (Suppl 1):8480–8482
31 Rost S, Fregin A, Koch D, Compes M, Müller CR, Oldenburg J. Compound heterozygous mutations in the γ-glutamyl carboxylase gene cause combined deficiency of all vitamin K-dependent blood coagulation factors. Br J Haematol 2004;126(04): 546–549
32 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):1313–1315
33 Lapecorella M, Napolitano M, Bernardi F, et al. Effective hemostasis during minor surgery in a case of hereditary combined deficiency of vitamin K-dependent clotting factors. Clin Appl Thromb Hemost 2010;16(02):221–223
34 Boneh A, Bar-Ziv J. Hereditary deficiency of vitamin K-dependent coagulation factors with skeletal abnormalities. Am J Med Genet 1996;65(03):241–243
35 Brenner B, Tavori S, Zivelin A, et al. Hereditary deficiency of all vitamin K-dependent procoagulants and anticoagulants. Br J Haematol 1990;75(04):537–542
36 Mathonnet A, Cunat S, Allias F, et al. GGCX-related congenital combined vitamin K-dependent clotting factors deficiency-1: description of a fetus with chondrodysplasia punctata. Am J Med Genet A 2022;188(01):314–318
37 Preisler B, Pezeshkpoor B, Banchev A, et al. Familial multiple coagulation factor deficiencies (FMCFDs) in a large cohort of patients - a single-center experience in genetic diagnosis. J Clin Med 2021;10(02):347
38 Wen L, Chen J, Duan L, Li S. Vitamin K–dependent proteins involved in bone and cardiovascular health (Review). Mol Med Rep 2018;18(01):3–15
39 Kosciuszek ND, Kalta D, Singh M, Savinova OV. Vitamin K antagonists and cardiovascular calcification: a systematic review and meta-analysis. Front Cardiovasc Med 2022;9:938567
40 Li Y, Chen JP, Duan L, Li S. Effect of vitamin K2 on type 2 diabetes mellitus: a review. Diabetes Res Clin Pract 2018;136:39–51
41 Liu J, Qin L, Zheng J, et al. Research progress on the relationship between vitamins and diabetes: systematic review. Int J Mol Sci 2023;24(22):16371
42 Chen A, Li J, Shen N, Huang H, Hang Q, Vitamin K. Vitamin K: new insights related to senescence and cancer metastasis. Biochim

Q5 Q5. Yes, it is correct, sorry for the mistake, Please modify the reference as follows: Chen A, Li J, Shen N,

and the prophylaxis strategy in term healthy infants: a multicentre study. Eur J Clin Invest 2023. Doi: 10.1111/eci.14141
45 Vernia F, Burrelli Scotti G, Bertetti NS, et al. Low vitamin K and vitamin D dietary intake in patients with inflammatory bowel diseases. Nutrients 2023;15(07):1678
46 Sultana H, Komai M, Shirakawa H. The role of vitamin K in cholestatic liver disease. Nutrients 2021;13(08):2515
47 Wu Q, Wang L, Zhao R. Neglected vitamin K deficiency causing coagulation dysfunction in an older patient with pneumonia: a case report. BMC Geriatr 2022;22(01):628
48 Dounousi E, Liakopoulos V. Editorial for the special issue "vitamin K in chronic disease and human health". Nutrients 2022;14 (13):2595
49 Yu Z, Chen H. Piperacillin/tazobactam-induced coagulopathy in a patient through a vitamin K-dependent mechanism. Eur J Hosp Pharm 2021;28(04):237–238
50 Fotouhie A, Desai H, Parsa NA, King S. Gastrointestinal bleeding secondary to trimethoprim-sulfamethoxazole-induced vitamin K deficiency. BMJ Case Rep 2016;2016:bcr2016214437
51 Tuttolomondo A, Pignatelli P, Pola R. Editorial: Anticoagulation in cardiovascular diseases: evolving role, unmet needs, and grey areas. Front Cardiovasc Med 2023;10:1219033
52 Watzka M, Geisen C, Scheer M, et al. Bleeding and non-bleeding phenotypes in patients with GGCX gene mutations. Thromb Res 2014;134(04):856–865
53 Rydz N, James PD. The evolution and value of bleeding assessment tools. J Thromb Haemost 2012;10(11):2223–2229
54 Marchetti G, Caruso P, Lunghi B, et al. Vitamin K-induced modification of coagulation phenotype in VKORC1 homozygous deficiency. J Thromb Haemost 2008;6(05):797–803
55 Agnello L, Bellia C, Lo Coco L, et al. Vitamin K deficiency bleeding leading to the diagnosis of Crohn's disease. Ann Clin Lab Sci 2014;44(03):337–340
56 Simes DC, Viegas CSB, Araújo N, Marreiros C. Vitamin K as a diet supplement with impact in human health: current evidence in age-related diseases. Nutrients 2020;12(01):138
57 Official Journal of the European Union. Commission Directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. Accessed August 8, 2024 at: https://eur-lex.europa.eu/eli/dir/2008/100/oj/eng
58 Pinto E, Viegas C, Martins PV, Nascimento T, Schurgers L, Simes D. New Food Frequency Questionnaire to estimate vitamin K intake in a Mediterranean population. Nutrients 2023;15(13):3012
59 Buitenhuis HC, Soute BA, Vermeer C. Comparison of the vitamins K1, K2 and K3 as cofactors for the hepatic vitamin K-dependent carboxylase. Biochim Biophys Acta 1990;1034(02):170–175
60 Berkner KL. Vitamin K-dependent carboxylation. Vitam Horm 2008;78:131–156
61 Hao Z, Jin DY, Chen X, Schurgers LJ, Stafford DW, Tie JK. γ-Glutamyl carboxylase mutations differentially affect the biological function of vitamin K-dependent proteins. Blood 2021;137 (04):533–543
62 Rishavy MA, Berkner KL. Vitamin K oxygenation, glutamate carboxylation, and processivity: defining the three critical facets of catalysis by the vitamin K-dependent carboxylase. Adv Nutr 2012;3(02):135–148
63 Chu PH, Huang TY, Williams J, Stafford DW. Purified vitamin K epoxide reductase alone is sufficient for conversion of vitamin K epoxide to vitamin K and vitamin K to vitamin KH2. Proc Natl Acad Sci U S A 2006;103(51):19308–19
66 Schulman S, Furie B. How I treat poisoning with vitamin K antagonists. Blood 2015;125(03):438–442
67 Hand I, Noble L, Abrams SA. Vitamin K and the newborn infant. Pediatrics 2022;149(03):e2021056036
68 Zhu A, Sun H, Raymond RM Jr, et al. Fatal hemorrhage in mice lacking γ-glutamyl carboxylase. Blood 2007;109(12):5270–5275
69 De Vilder EYG, Debacker J, Vanakker OM. GGCX-associated phenotypes: an overview in search of genotype-phenotype correlations. Int J Mol Sci 2017;18(02):240
70 Tie JK, Carneiro JDA, Jin DY, Martinhago CD, Vermeer C, Stafford DW. Characterization of vitamin K-dependent carboxylase mutations that cause bleeding and nonbleeding disorders. Blood 2016;127(15):1847–1855
71 Vanakker OM, Martin L, Gheduzzi D, et al. Pseudoxanthoma elasticum-like phenotype with cutis laxa and multiple coagulation factor deficiency represents a separate genetic entity. J Invest Dermatol 2007;127(03):581–587
72 Czogalla KJ, Biswas A, Rost S, Watzka M, Oldenburg J. The Arg98Trp mutation in human VKORC1 causing VKCFD2 disrupts

 © 2024. Thieme. All rights reserved.

a di-arginine-based ER retention motif. Blood 2014;124(08): 1354–1362
73 Brenner B, Kuperman AA, Watzka M, Oldenburg J. Vitamin K-dependent coagulation factors deficiency. Semin Thromb Hemost 2009;35(04):439–446
74 Ayyash M, Chitlur M, Oldenburg J, Shaman M. Suspected vitamin K-dependent coagulation factor deficiency in pregnancy: a case report. Case Rep Womens Health 2022;34:e00416
75 Weston BW, Monahan PE. Familial deficiency of vitamin K-dependent clotting factors. Haemophilia 2008;14(06): 1209–1213
76 Hazim AZ, Ruan GJ, Khodadadi RB, Slusser JP, Marshall AL, Pruthi RK. A single-institution retrospective study of causes of prolonged prothrombin time and activated partial thromboplastin time in the outpatient setting. Int J Lab Hematol 2022;44(01):209–215
77 Neutze D, Roque J. Clinical evaluation of bleeding and bruising in primary care. Am Fam Physician 2016;93(04):279–286
78 Palla R, Peyvandi F, Shapiro AD. Congenital combined deficiency of the vitamin K-dependent clotting factors (VKCFD): a novel gamma-glutamyl carboxylase (GGCX) mutation. Blood 2015;125 (13):2052–2061
79 Mumford AD, Ackroyd S, Alikhan R, et al; BCSH Committee. Guideline for the diagnosis and management of the rare coagulation disorders: a United Kingdom Haemophilia Centre Doctors' Organization guideline on behalf of the British Committee for Standards in Haematology. Br J Haematol 2014;167(03):304–326
80 Tcherniantchouk O, Laposata M, Marques MB. The isolated prolonged PTT. Am J Hematol 2013;88(01):82–85
81 Mathews N, Tasneem S, Hayward CPM. Rare inherited coagulation and fibrinolytic defects that challenge diagnostic laboratories. Int J Lab Hematol 2023;45(Suppl 2):30–43
82 Suttie JW. Vitamin K and human nutrition. J Am Diet Assoc 1992; 92(05):585–590
83 Shearer M. The assessment of human vitamin K status from tissue measurements. In: Suttie JW, ed. Current Advances in Vitamin K Research. New York, NY1985
84 Fusaro M, Gallieni M, Rizzo MA, et al. Vitamin K plasma levels determination in human health. Clin Chem Lab Med 2017;55 (06):789–799
85 Card DJ, Gorska R, Harrington DJ. Laboratory assessment of vitamin K status. J Clin Pathol 2020;73(02):70–75
86 Sokoll LJ, Sadowski JA. Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population. Am J Clin Nutr 1996;63(04):566–573
87 Shaw MA, Liu A. Take the shot: a review of vitamin K deficiency. Pediatr Ann 2023;52(02):e42–e45
88 Loyal J, Shapiro ED. Refusal of intramuscular vitamin K by parents of newborns: a review. Hosp Pediatr 2020;10(03): 286–294
89 Shao X, Ren Y, Xie N, et al. Effect of cefoperazone/sulbactam on blood coagulation function in infected emergency department patients and the necessity of vitamin K1 (VK1) preventive intervention: a single-center, retrospective analysis. Med Sci Monit 2023;29:e939203
90 Tassew SF, Haile BA, Amera Birlie T. Outcome of rodenticide poisoning and its associated factors among adult patients admitted with rodenticide poisoning at the emergency unit of Debre Tabor Comprehensive Specialized Hospital, Debre Tabor, North Central Ethiopia. Open Access Emerg Med 2023; 15:189–197
91 van Breemen RB, Hafner JW, Nosal DG, Feinstein DL, Rubinstein I. Unmet clinical laboratory need in patients hospitalized for acute poisoning from long-acting anticoagulant rodenticides. Toxicol Commun 2021;5(01):93–96
92 Eden RE, Daley SF, Coviello JM. Vitamin K Deficiency. In: StatPearls. StatPearls Publishing; 2024 Accessed February 14, 2024 at: http://www.ncbi.nlm.nih.gov/books/NBK536983/
93 Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW. Identification of the gene for vitamin K epoxide reductase. Nature 2004; 427(6974):541–544
94 Wu SM, Stafford DW, Frazier LD, et al. Genomic sequence and transcription start site for the human gamma-glutamyl carboxylase. Blood 1997;89(11):4058–4062
95 Wu SM, Morris DP, Stafford DW. Identification and purification to near homogeneity of the vitamin K-dependent carboxylase. Proc Natl Acad Sci U S A 1991;88(06):2236–2240
96 Wu SM, Cheung WF, Frazier D, Stafford DW. Cloning and expression of the cDNA for human gamma-glutamyl carboxylase. Science 1991;254(5038):1634–1636
97 Hosseini MS, Shams M, Dorgalaleh A, Mansouritorghabeh H. Multiple Coagulation Factor Deficiency. In: Dorgalaleh A, ed. Congenital Bleeding Disorders. Springer International Publishing; 2018:219–238. Doi: 10.1007/978-3-319-76723-9_9
98 Chen HF, Wu TQ, Jin LJ, et al. Treatment of vitamin K-dependent coagulation factor deficiency and subarachnoid hemorrhage. World J Emerg Med 2011;2(01):73–76
99 Zeng L, Choonara I, Zhang L, Li Y, Shi J. Effectiveness of prothrombin complex concentrate (PCC) in neonates and infants with bleeding or risk of bleeding: a systematic review and meta-analysis. Eur J Pediatr 2017;176(05):581–589
100 World Health Organization. Global status report on blood safety and availability 2021. Accessed February 14, 2024 at: https://www.who.int/publications-detail-redirect/9789240051683
101 Lubetsky A, Hoffman R, Zimlichman R, et al. Efficacy and safety of a prothrombin complex concentrate (Octaplex) for rapid reversal of oral anticoagulation. Thromb Res 2004;113(06):371–378
102 Leissinger CA, Blatt PM, Hoots WK, Ewenstein B. Role of prothrombin complex concentrates in reversing warfarin anticoagulation: a review of the literature. Am J Hematol 2008;83(02): 137–143
103 Ng HJ, Lee LH. Recombinant activated clotting factor VII (rFVIIa) in the treatment of surgical and spontaneous bleeding episodes in hemophilic patients. Vasc Health Risk Manag 2006;2(04): 433–440
104 Ghosh S, Kraus K, Biswas A, 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(06):1412–1424
105 Halder M, Petsophonsakul P, Akbulut AC, et al. Vitamin K: double bonds beyond coagulation insights into differences between vitamin K1 and K2 in health and disease. Int J Mol Sci 2019;20 (04):896

 © 2024. Thieme. All rights reserved.

Author Query Form (STH/03283)

Special Instructions: Author please write responses to queries directly on proofs and then return back.

Q1: AU: Please confirm that given names (red), middle names (black) and surnames (green) have been identified correctly. Author names in bibliographic citations and online will appear as: Perrone S, Raso S, Napolitano M. Clinical, Laboratory, and Molecular Characteristics of Inherited Vitamin K–Dependent Coagulation Factors Deficiency. Please confirm if this is correct.

Q2: AU: Please check the corresponding details for accuracy.

Q3: AU: Patient's age and race have been mentioned in the article. Please check and confirm to ensure patient's safe identity.

Q4: AU: Patient's age and race have been mentioned in the article. Please check and confirm to ensure patient's safe identity.

Q5: AU: Medline reports only 5 authors, not 6. (Ref. 42 "Chen, Li, Shen, Huang, Hang, Vitamin, 2024")

THIEME