A novel factor V mutation causes a normal activated protein C ratio despite the presence of a heterozygous F5 R506Q (factor V Leiden) mutation

Activated protein C (APC) resistance (Dahlbäck et al, 1993) is a common risk factor for venous thromboembolism (Bucciarelli et al, 1999). This phenotype is highly associated with the F5 R506Q (F5 rs6025; factor V Leiden, FVL) mutation (Bertina et al, 1994). Heterozygous and homozygous discrepancies between F5 R506Q genotype and phenotype (APC resistance), known as pseudo-homozygosity, have been described (Castaman et al, 1997; Brugge et al, 2005). This incidental phenomenon suggests that, based on APC-resistance testing, there is a homozygous F5 R506Q mutation, yet, genetic testing only shows a heterozygous F5 R506Q mutation (Simioni et al, 1996). In such cases, polymorphisms located on the second factor V (FV) protein coding allele (i.e. wild-type for F5 R506Q) cause intracellular decreased FV protein production, leading to reduced FV activity (Castoldi et al, 1998). In contrast, the phenomenon “pseudo-wild-type” FVL, suggesting a wild-type F5 R506Q according to APC resistance testing but heterozygous F5 R506Q mutation by genetic testing, is infrequently described (Dargaud et al, 2003; Asselta et al, 2004). In these two studies, the polymorphisms responsible for the reduced FV protein levels were found to be located on the same FV protein coding allele, together with the F5 R506Q mutation.

In this study, four cases with such a “pseudo-wild-type” FVL, based on a novel detected polymorphism, were investigated. An adolescent, healthy female subject was advised by her physician to determine her F5 R506Q status before being prescribed hormonal contraceptives. She was a non-smoker with no personal or family history of any thromboembolic events. She was tested for both APC resistance and F5 R506Q mutation. Because there was a discrepancy between the functional APC resistance testing and the genetic F5 R506Q testing results, her family was tested and a pedigree was established. (Supporting Information Data S1) In the index subject, a heterozygous F5 R506Q (F5 rs6025; factor V Leiden, FVL) mutation, based on a novel detected polymorphism, were investigated. An adolescent, healthy female subject was advised by her physician to determine her F5 R506Q status before being prescribed hormonal contraceptives. She was a non-smoker with no personal or family history of any thromboembolic events. She was tested for both APC resistance and the F5 R506Q mutation. Because there was a discrepancy between the functional APC resistance testing and the genetic F5 R506Q testing results, her family was tested and a pedigree was established. (Supporting Information Data S1) In the index subject, a heterozygous F5 R506Q mutation but a normal APC ratio were detected (Fig 1). The APC ratio was cross-checked using three different assays based on different FV activation pathways, which yielded consistent, normal results (Table I).
mutation (F5 G1718S). Interestingly, this novel F5 G1718S mutation is located on the same allele as the heterozygous F5 R506Q mutation. Therefore, we hypothesized that this new F5 G1718S mutation is responsible for the low FV:Ag level. In this case, only a reduced amount of normal FV, which is coded by the second unaffected allele, is available and accounts for a wild-type-like normal APC ratio, suggesting a "pseudo-wild-type" for the F5 R506Q mutation (Fig 2A-c).

This infrequent phenomenon (Dargaud et al, 2003; Asselta et al, 2004) was explained by a missense or a frameshift mutation on the F5 gene, which causes amino acids to be exchanged or prematurely terminated, resulting in reduced FV protein levels. Published exchanges of amino acids in the region 1608 and 1745 of the F5 gene result in low FV protein levels (Fig 2B) (Castoldi et al, 2000). Based on these findings, our novel missense mutation, detected at position 1718 of the FV protein, lies within the described region 1608 – 1745, and therefore seems to be the reason for both the markedly reduced FV:Act and FV:Ag levels measured in our four study subjects. When comparing our findings of the "pseudo-wild-type" FVL to the well-described pseudo-homozygous FVL, the main difference exists in the location of the F5 R506Q mutation and the additional missense mutation on the F5 gene causing the reduced FV protein levels: For "pseudo-wild-type" FVL, both mutations have to be located on the same FV protein coding allele; whereas, for pseudo-homozygous FVL, the mutations have to be located on the different FV protein coding alleles, as demonstrated (Fig 2A, Table S1).

The medical history of the entire family was ascertained and considered when evaluating the "pseudo wild-type" FVL test results and determining the haemostasis risk burden. None of the subjects had a history of thromboembolic events or any bleeding complications. Therefore, it can be assumed that the thromboembolic risk that is usually associated with a heterozygous F5 R506Q mutation is no longer reliable if missense mutations, e.g., the F5 G1718S mutation, located on the same allele as the F5 R506Q mutation, are present.

In summary, the F5 G1718S mutation causes a normal APC ratio despite the presence of a heterozygous F5 R506Q mutation – described as a "pseudo-wild-type" FVL.

In these four cases, testing for only the F5 R506Q genotype would have failed to determine the individual's thromboembolic risk associated with the heterozygous F5 R506Q mutation. As numerous relevant missense mutations may exist on the F5 gene that have not yet been detected or described, clinicians should be aware of this possible discrepancy between genetic (F5 R506Q) and phenotypic (APC resistance) testing for FVL before drawing conclusions for thromboembolic prophylaxis and therapeutic management, especially when prescribing drugs such as combined oral contraceptives or hormonal replacement therapy.

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Table I. Activated protein C (APC) ratios in the plasma of all family members using three different test systems [Pefakit® APC Resistance (APC-R) Factor V Leiden assay, HEMOCLOT® Quanti V-L and COATEST® APC™ Resistance] in comparison with the F5 genotypes [F5 R506Q (FV Leiden) and F5 G1718S (FV Graz)].

| Family member | F5 R506Q (Factor V Leiden) | F5 G1718S (Factor V Graz) | Pefakit® [ratio] | HEMOCLOT® [%] | COATEST® [ratio] |
|---------------|-----------------------------|---------------------------|-----------------|--------------|-----------------|
| Cut off       |                              |                           | >2-9            | <10          | >2-2            |
| I.1           | HE                          | HE                        | 3-03            | 7-95         | 2-34            |
| I.2           | WT                          | WT                        | 4-20            | 3-15         | 2-87            |
| I.3           | WT                          | WT                        | 4-00            | 2-15         | 2-83            |
| I.4           | WT                          | WT                        | 4-36            | 2-30         | 2-50            |
| II.1          | HE                          | HE                        | 3-09            | 7-35         | 2-39            |
| II.2          | WT                          | WT                        | 4-20            | 2-95         | 3-00            |
| INDEX III.1   | HE                          | HE                        | 3-30            | 6-63         | 2-42            |
| III.2         | HE                          | HE                        | 3-13            | 7-45         | 2-52            |
| Controls F5 R506Q carriers | HE                          | WT                        | 1-4-2.5        | 14-86        | 1-3-2.2         |

HE, heterozygous; WT, wild-type.

Fig 2. (A, B) Co-inheritance of heterozygous factor V Leiden mutation (F5 1691G>A, F5 R506Q) and heterozygous factor V Graz (F5 5326G>A, F5 G1718S): The factor V Graz mutation is located in the A3 domain of the coagulation factor V; Mutations between the amino acid positions 1608 to 1745 are responsible for a premature factor V protein degradation and lead to the expression of a reduced amount of factor V protein (B). (a) Inheritance of heterozygous F5 R506Q mutation, leading to a normal amount of factor V protein; half of the protein is resistant to activated protein C (= APC resistance present). (b) The co-inheritance of F5 R506Q mutation and the F5 G1718S mutation on different alleles results in the expression of the FV Leiden protein only (= pseudo-homozygous APC resistance). (c) In the studied family, the co-inheritance of the F5 G1718S mutation and F5 R506Q mutation on the same allele leads to the expression of the non-FV Leiden protein only, resulting in a normal APC ratio (= “pseudo-wild-type” APC resistance).
Author contributions

FP, BH study design. FP, EMM, WR, HK performed analysis. FP, RBR, HK wrote paper. KLS edited paper. MTW, HM, ECW, GS supported discussion.

Conflict of interest

None declared.

Florian Prueller1,2
Reinhard B. Raggam1,2
Harald Mangge1,3
Martie Truscshing-Wilders1
Eva-Maria Matzhold4
Eva-Christine Weiss5
Barbara Hasiba6
Eva-Maria Matzhold4
Barbara Hasiba6

1Clinical Institute of Medical and Chemical Laboratory Diagnostics, 2Research Unit Perioperative Platelet Function, 3Research Unit Lifestyle- and Inflammation-associated Risk Biomarkers, 4University Clinic of Blood Group Serology and Transfusion Medicine, 5Department of Obstetrics and Gynaecology, 6Working Group for Family Medicine, 7Institute of Social Medicine and Epidemiology, Medical University of Graz, Graz, Austria and 8Institute of Clinical Chemistry and Laboratory Medicine, Technische Universität Dresden, Dresden, Germany

E-mail: florian.prueller@medunigraz.at

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods and materials.

Fig S1. Detection of the novel mutation 5326G>A (G1718S) in exon 16 of the F5 gene - Sequencing protocol and RFLP analysis of the female index patient.

Table S1. Phenotypic results of different co-inherited factor V Leiden heterozygous conditions in the presence of the novel heterozygous factor V Graz polymorphism and the prevalence of these conditions.

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