Discordance between phenotype and genotype study in Factor V Leiden carriers: a real life experience

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ABSTRACT

Factor V Leiden (FVL) mutation causes activated protein C (APC) resistance by decreasing the susceptibility of FVa to APC-mediated inactivation. In our Laboratory, the usual approach to the identification of FV deficit includes a combination of genetic and functional tests. The aim of this paper was to highlight the importance to investigate both the genotype and the phenotype in the diagnosis of FVL-APC resistance related thrombophilia. Among a group of 292 subjects examined for thrombophilia, we observed three patients with discordant results of genetic test for the detection of G1691A FV gene mutation and functional APCr assay: the first subject has a mild APC resistance with a wild type genotype; the second one, and her sister, shows a severe APC resistance with a heterozygous genotype. To determine the FV deficit, it is important to associate the molecular analysis of G1691A mutation with the APC resistance test.

CASE REPORT 1

A six year old boy was admitted to the hospital with a right-sided ilio-femoral deep-vein thrombosis (DVT) and severe pulmonary embolism. A standard protocol for evaluation of thrombophilia was applied (1-3). The results of the following tests were within the reference range: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), antithrombin (AT), protein C (PC), protein S (PS), factor VIII (FVIII) and factor IX (FIX). Lupus anticoagulant (LA) test was negative and the concentration of the antiphospholipid autoantibodies was also normal. Fibrinogen (FIB) concentration was slightly elevated (697 mg/dL, r.v. 200-450), D dimer (DD) was elevated (1679 ng/mL, r.v. <250). Activated protein C resistance (APCr) ratio was 0.92 (r.v. FVL homozygous <1.1, FVL heterozygous 1.1-1.9, FV wild type >2.0) consistent with the Factor V Leiden (FVL) homozygosis. The genetic test demonstrated a wild type homozygous for FII G20210A mutation and confirmed a homozygous FVL status. The family study is presented in Figure 1.

The Index Case [1] is homozygous for FVL, both mother [2] and father [3] were heterozygous for FVL. Maternal [7] and paternal grandfather [5] are wild type for FV and paternal grandmother [6] is FLV heterozygous. All these subjects show a negative clinical history for thrombophilia, and a full concordance between genotype and phenotype. Maternal grandmother [4] has a clinical history positive for previous thrombosis. This subject is wild type for FV, but has a APCr ratio of 1.31, consistent with a heterozygous status. Family history revealed that her mother [9] died of pulmonary embolism and her sister [8] was FVL heterozygous and had a clinical history positive for thrombosis. To clarify this discrepancy, first we repeated the laboratory tests, to confirm the results, then performed a careful anamnesis revealing that the patient had undergone, seven years earlier, an allogenic peripheral stem blood cells transplant (PBSCT) for a non-Hodgkin lymphoma. The pre-transplant data showed that the patient was heterozygous for FVL.

The methods for laboratory testing are listed in Table 1.

CASE REPORT 2

A 46 year old female was investigated one year after an episode of left sided lower limb thrombosis. Clinical history was positive for a previous left leg thrombosis

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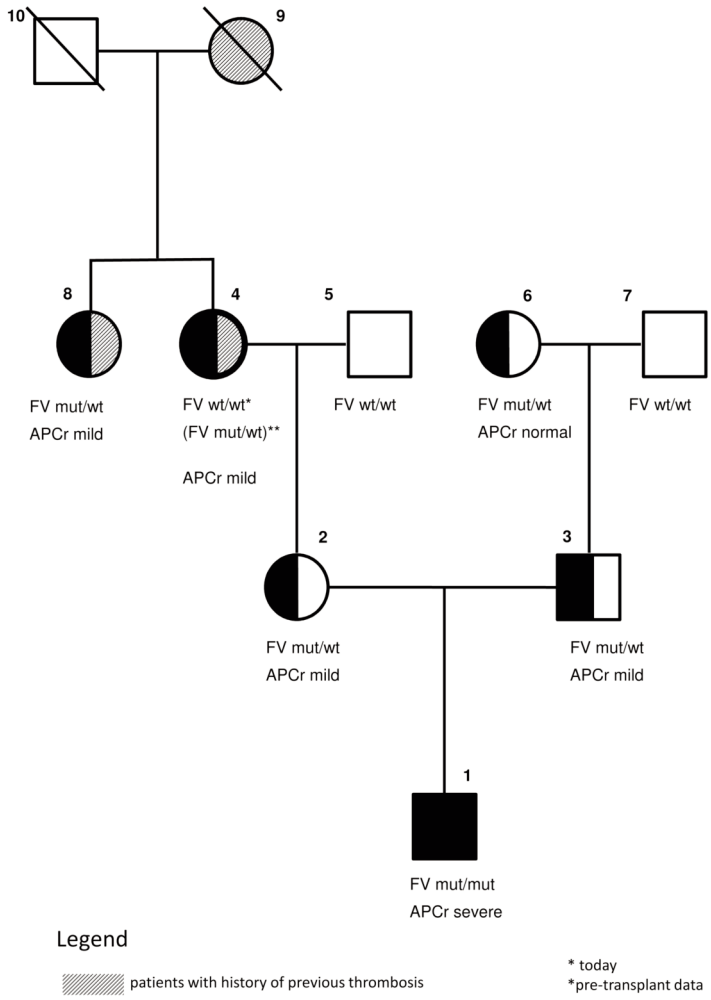

Figure 1

Case one, family study.

Index Case (1), Clinical history positive for thrombosis, FVL homozygous (genetic test), functional assay coherent with genetic test. Mother (2) and Father (3) Clinical history negative for thrombosis, FVL heterozygous (genetic test), functional assay coherent with genetic test. Grandmother (4) Clinical history positive for thrombosis, FV homozygous Wild Type (genetic test), functional assay not coherent with genetic test (APCr ratio 1.31, coherent with heterozygous). Sister (8) and Mother (9) of the Grandmother had a clinical history positive for thrombosis. Both Grandfathers (5 and 7), wild type for FV (genetic tests), functional assays coherent with genetic tests and clinical histories were negative for thrombosis.

APCr mild, Activated Protein C ratio consistent with a heterozygous status for Factor V Leiden; APCr severe, Activated Protein C ratio consistent with a homozygous status for Factor V Leiden; APCr Normal, Activated Protein C ratio normal; FVL mut/wt heterozygous status for Factor V Leiden; FVL mut/mut, homozygous status for Factor V Leiden; FV wt/wt, factor V wild type.

during the second pregnancy at the age of 29 years. During the first pregnancy, at 24 years of age, no thrombotic events occurred. PT, aPTT, TT, FIB, DD, AT, PC, PS were within the reference ranges. FVIII and FIX concentrations were also normal, LA detection was negative as it was the immunometric test for antiphospholipid autoantibodies. APCr ratio was 1.01 consistent with a diagnosis of FVL homozygosis. Molecular diagnosis demonstrated a wild type for FII and a heterozygous FVL status. To clarify the discrepancy, FV concentration was determined resulting rather low (45%). These results are consistent with a diagnosis of pseudo-homozygous APCr status. The family study of the case in presented in Figure 2. The index case [1] shows a compound heterozygous FVL/FV deficit, as her sister does [2]. They both had a clinical history positive for previous thrombosis. Their mother [10] was homozygous for FVL and had a clinical history positive for previous thrombosis; their father [11] had FV plasma levels consistent with a heterozygous deficit. Both sisters were married to wild type men for FV [3 and 4]. The index case [1] had two children, both FVL heterozygous [5 and 6]. The sister [3] had three children; two of them [7 and 8] are FVL heterozygous, the third one [9] shows a FV deficit.

The list of the methods used for laboratory testing is given in Table 1.

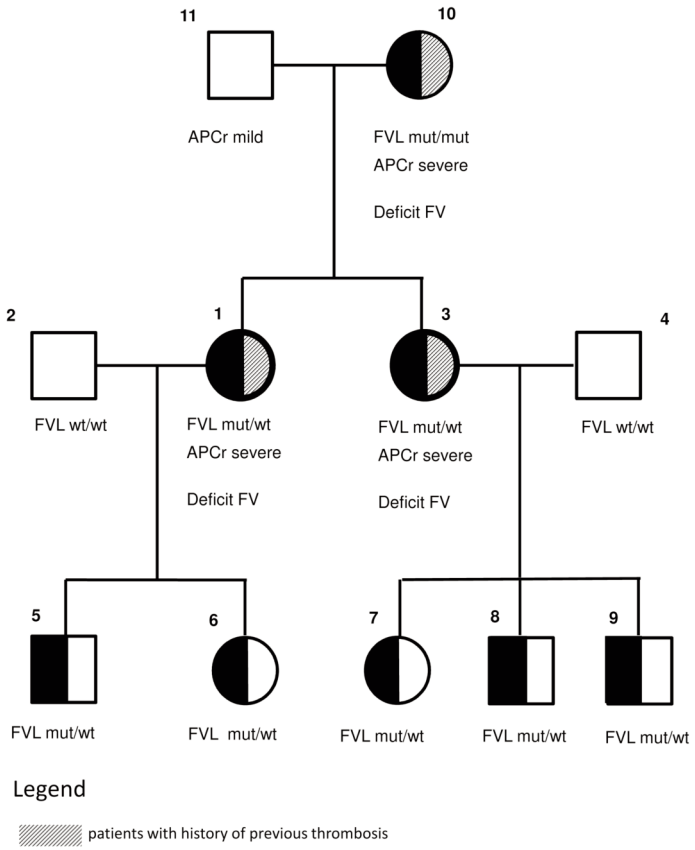

Figure 2
Case two, family study.
Index Case (1). Clinical history positive for thrombosis, FVL heterozygous (genetic test), functional assay coherent with a homozygous status; Factor V concentration was 45%. Picture suggestive of an APC pseudo homozygous status due a compound heterozygous status FVL + FV deficiency. Sister (3). Clinical history positive for thrombosis, FVL heterozygous (genetic test), functional assay coherent with a homozygous status; factor V concentration was 43%. Picture suggestive for pseudo an APC homozygous status due a compound heterozygous status FVL + FV deficiency. Husband (4). Clinical history negative for thrombosis, FV WT, functional assay coherent with genetic test, no FV deficit. Husband of subject 1 (2). Clinical history negative for thrombosis, FV WT (genetic test), functional assay coherent with genetic test, no FV deficit. Children of 1 and 2 (5 and 6). Clinical history negative for thrombosis, FVL heterozygous (genetic test), functional assay coherent with genetic test, no FV deficit. Children of 3 and 4 (7 and 8). Clinical history negative for thrombosis, FVL heterozygous (genetic test), functional assay coherent with genetic test, no FV deficit. Child of 3 and 4 (9). Clinical history negative for thrombosis, FVL WT (genetic test), functional assay coherent with genetic test, Factor V concentration was 51%. Mother of 1 and 2 (10). Clinical history positive for thrombosis, FVL homozygous (genetic test), functional assay coherent with genetic test, no FV deficit. Father of 1 and 3 (11). Clinical history negative for thrombosis, FVL WT (genetic test), functional assay coherent with genetic test, Factor V concentration was 48%.
APCr mild, Activated Protein C ratio consistent with a heterozygous status for Factor V Leiden; APCr severe, Activated Protein C ratio consistent with a homozygous status for Factor V Leiden; APCr Normal, Activated Protein C ratio normal; FVL mut/wt heterozygous status for Factor V Leiden; FVL mut/mut, homozygous status for Factor V Leiden; FV wt/wt, factor V wild type.

DISCUSSION

FV synthesis occurs in the liver as a large single-chain profactor (330Kd). Activated Factor V (FVa,) resulting from thrombin cleavage of FV, is composed of a heavy chain (105 Kd, residues 1 to 709); and a light chain (74 Kd residues 1546 to 2196) (4).

FVa inactivation by activated protein C (APC) is associated with three cleavages sites of the heavy chain at Arg^{306}, Arg^{506}, and Arg^{679}. Factor V Leiden (FVL) carriers show a G→A substitution at nucleotide 1691 in the factor V gene (resulting in an Arg^{506}→Gln mutation in the FV molecule). These subjects have a poor anticoagulant response to APC (the so called APC resistance, APCr), which is associated with a significant increase in risk for deep venous thrombosis, most likely because FVL is inactivated by APC at a slower rate than normal FV is, thus leading to prolonged thrombin generation (5).

FVL accounts for over 90% cases of APCr, but other acquired conditions such as pregnancy, oral contraceptive, lupus anticoagulant, elevated FVIII levels, and treatment with direct FXa inhibitors have been associated with APCr.

Table 1
Tests and analytical methods

Test	Analizer	Reagents
Prothrombin time	Sysmex CA 7000	Sclavo PT
Activated partial thromboplastin time	Sysmex CA 7000	Sclavo aPTT-S
Fibrinogen	Sysmex CA 7000	Sclavo Fibrinogeno
Thrombin Time	Sysmex CA 7000	Sclavo TT
D-Dimer	Sysmex CA 7000	Sclavo auto D-Dimer
Antithrombin III	Sysmex CA 7000	Sclavo ATIII (FXa)
Protein C	Sysmex CA 7000	Sclavo Proteina C cromogenica
Proptein S	Sysmex CA 7000	Stago Liatest Free protein S
Factor II	Sysmex CA 7000	Sclavo FII deficent plasma
Factor V	Sysmex CA 7000	Sclavo FV deficent plasma
Factor VII	Sysmex CA 7000	Sclavo FVII deficent plasma
Factor VIII	Sysmex CA 7000	Sclavo FVIII deficent plasma
Factor IX	Sysmex CA 7000	Sclavo FIX deficent plasma
Lupus anticoagulant	Sysmex CA 7000	Sclavo LA1 Screening reagent Sclavo LA2 Confirmatory reagent
Activated protein C resistance	Sysmex CA 7000	Pentapharm Pefakit APC-R FV Leiden
Anti CL IgG/IgM	Intrumentation Laboratory Bioflash	Quanta Flash aCL IgG/IgM
Anti B2GP1 IgG/IgM	Intrumentation Laboratory Bioflash	Quanta Flash B2GP1 IgG/IgM
FV G1691A FII G20210A	Cepheid GeneXpert	Cepheid Xpert FII e FV

Cepheid Italia, Milano Italy; Instrumentation Laboratory Italia, Milano Italy; Pentapharm, Basel Switzerland; Sclavo Diagnostic International, Siena Italy; Stago Italia, Milano Italy; Sysmex Europe, Nordstendt Germany supplied by DASIT Group, Milano Italy.

Factor V Leiden polymerase-chain-reaction–based genetic testing is usually considered a superior screening method for detecting the genetic defect. The presence of a DNA mutation is frequently used to define a disease or a risk state. Because DNA typing has become easy and convenient in contrast to protein characterization, it is generally assumed that a mutation if present (or not) at the DNA level, will be also present (or not) in the corresponding protein. However, discrepancies between phenotype and genotype can occur (6).

The two case reports here described are related to two patients with a discrepancy between phenotype (assay for detection of APCr) and genotype (assay for detection of G1691A mutation).

In the first case, we observed a functional assay consistent with a heterozygous status combined with a wild type genotype. This discrepancy was explained by evaluating the medical history of the patient, highlighting a previous allogenic peripheral blood stem cells transplant (PBSCT). In this case, the genetic test performed on leukocytes obtained from the peripheral blood, resulted negative (homozygous wild type), reflecting the status of the donor, whereas the functional test showed persistent hepatic synthesis of a mutated protein resulting from heterozygous status state of the patient (7-9). However, cases where, after a liver transplant from a donor homozygous for FVL, the recipient has developed a thrombotic disease, and acquired an APCR resistant phenotype, despite the presence of a genetic test negative for FVL have been reported (8, 9).

The second patient was classified as pseudo homozygous for APCr. This phenotype occurs when an individual heterozygous for FVL does not express also the "normal" (non-Leiden) factor V allele. Usually, in these subjects a low FV plasma concentration is observed and only circulating FVL is detected. Moreover these APCr pseudo-homozygotes subjects seem to present a more pronounced hypercoagulable state than factor V Leiden heterozygotes (10-13).

In our Laboratory, we adopted a prothrombinase-based assay to study APCr phenotype. There are evidences that this assay exhibits a higher sensitivity and specificity than classical aPTT-based assays or chromogenic FXa-based assays. Furthermore, prothrombinase-based assays show a satisfactory positive predictive value (99÷100%) (14, 15).

Overall, these data suggest the existence of heterogeneous genetic "lesions," which interfere with factor V expression, processing, secretion, and/or stability. Because the presence of the FVL molecule in plasma is directly related to pathology, identification and quantification of the circulating forms of Factor V in

plasma are required for the diagnosis of individuals with APCr besides the genetic test.

APCr is a complex phenotype resulting from the interaction between multiple genetic and acquired factors. More than 90% of the phenotypic variability of APCr is under genetic control, mainly due to Leiden mutation, but there are other known determinants of APCr. In our experience, we observed 3 subjects (1.0%) out of 292 investigated subjects (case 1, case 2 and her sister), showing discordant results between FVL genotype and APCr phenotype. The finding is therefore not very rare.

Since APCr seems an unifying mechanism by which various genetic and acquired factors increase the risk of venous thrombosis, a basic screening of risk of thrombosis could be appropriate. The APCr test lower cost, in comparison with the genetic analysis (about ten-fold lower), makes it more suitable to screen large number of subjects. Recently, a new prothrombinase-based activated APCr test to detect FVL provides improved discrimination between normal individuals and FVL carriers compared with the classical methods. Prothrombinase-based functional testing for APCr are probably more reliable and more cost-saving than genetic assays as first approach to thrombophylia risk factor assessment (4, 16, 17).

CONFLICTS OF INTEREST

None.

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