Research

Different outcome of six homozygotes for prothrombin A20210A gene variant

Pierpaolo Di Micco*1, Rosanna Di Fiore2, Alferio Niglio3, Sandro Quaranta2, Antonella Angiolillo4, Giuseppe Cardillo5 and Giuseppe Castaldo2,5

Address: 1Department of Internal Medicine, Fatebenefratelli Hospital of Naples, Naples, Italy, 2Department of Biochemistry and Medical Biotechnology, University of Naples "Federico II", Naples, Italy, 3Department of Internal Medicine, Second University of Naples, Naples, Italy, 4School of Sciences, University of Molise, Isernia, Italy and 5CEINGE-Advanced Biotechnologies, scarl; University of Naples “Federico II”, Naples, Italy

Email: Pierpaolo Di Micco* - pdimicco@libero.it; Rosanna Di Fiore - difiore@dbbm.unina.it; Alferio Niglio - alferio.niglio@unina2.it; Sandro Quaranta - quaranta@dbbm.unina.it; Antonella Angiolillo - angiolillo@unimol.it; Giuseppe Cardillo - cardillo@ceinge.unina.it; Giuseppe Castaldo - castaldo@dbbm.unina.it

* Corresponding author

Abstract

Prothrombin G20210A gene variant (FII G20210A) is a risk factor for venous thrombotic disease while conflicting results have been reported for the risk of arterial thrombotic events. However, vascular episodes were absent in up to 40% of the 67 homozygotes for the G20210A described so far, which indicates that the clinical expression depends on additional risk/trigger factors. We describe six homozygotes for the G20210A variant, among which the first pair of siblings (cases n. 3 and 4) reported so far that displayed a strongly heterogeneous clinical outcome. Case 1, a female of 27 years, developed a full thrombosis of common femoral, superficial and popliteal veins. She assumed oral contraceptives in the last two years. Case n. 2, 34 years old, suffered of recurrent pregnancy loss in absence of any causative alteration. Cases n. 3 and n. 5 experienced arterial thrombotic disease, i.e., juvenile myocardial infarction (40 years old) and stroke (48 years old), respectively, in absence of other risk factors. Finally, cases n. 4 and 6 identified as homozygotes for the FII G20210A variant being consanguineous of symptomatic subjects bearing the variant, did not experience any episode of venous nor arterial disease. Both of them have chronic liver disease with an impairment of the prothrombin time INR. Thus, homozygotes for the G20210A are at risk for arterial (in addition to venous) thrombotic events; chronic liver disease might modulate this risk.

Background

The G20210A variant in the 3’ UTR of prothrombin gene (FII G20201A) is associated with higher plasma prothrombin activity and has a prevalence of 1.2–4.6% in the general Caucasian population, and higher in patients affected by venous thromboembolism (VTE, [1,2]). Heterozygotes for the variant have an odds ratio for VTE ranging from 2 to 4 [3]. On the contrary, conflicting results have been reported for the risk of arterial thrombotic events in subjects bearing the G20210A variant [4-15]. The high incidence of the G20210A variant in the general population and a large-cohort meta-analysis suggest that heterozygosity for G20210A is a mild prothrombotic factor [10]. On the other hand, vascular episodes were absent in up to 40% of the 67 homozygotes for the G20210A described so far [3], which suggests that clinical expression in sub-
jects bearing inherited thrombophilia appear only in the presence of additional risk factors [3,11]. The clinical history of six homozygotes for the FII G20210A variant studied by our group, among which the first pair of siblings described so far, indicates that the clinical outcome of these subjects is strongly heterogeneous, and that both trigger and protective factors may be involved.

**Case reports**

**Case 1**
Female, current age: 60 years old. Both parents died when she was a child, the mother for AMI, the father for an unknown disease. Remote anamnesis was negative for thrombotic diseases and for atherothrombotic risk factors as smoking, alcohol, diabetes, dyslipidemia, hypertension and pharmacological and hormonal therapies. Similarly, risk factors for venous thrombosis as recent immobility, malignancy and recent surgery were absent. At the age of 48 years, she experienced an episode of left hemiparesis. Computerized tomography scan showed hypodensity in the talamus and in the motor cortical area. Carotidal and vertebral vascular ultrasound scan associated to color-Doppler, and ECG and echocardiography were negative. The patient was successfully treated with antithrombotic therapy. Laboratory demonstrated homozygosity for the FII G20210A gene variant, whereas other data (i.e., AT III, PC, PS, homocysteinemia, FVL and MTHFR variants) were normal. The antithrombotic therapy based on oral anticoagulation (INR range 2.0–3.0) is ongoing again and 12 years later (today), the patient did not refer any other thrombotic episode.

**Case 5**
Female, current age: 60 years old. Both parents died when she was a child, the mother for AMI, the father for an unknown disease. Remote anamnesis was negative for thrombotic diseases and for atherothrombotic risk factors as smoking, alcohol, diabetes, dyslipidemia, hypertension and pharmacological and hormonal therapies. Similarly, risk factors for venous thrombosis as recent immobility, malignancy and recent surgery were absent. At the age of 48 years, she experienced an episode of left hemiparesis. Computerized tomography scan showed hypodensity in the talamus and in the motor cortical area. Carotidal and vertebral vascular ultrasound scan associated to color-Doppler, and ECG and echocardiography were negative. The patient was successfully treated with antithrombotic therapy. Laboratory demonstrated homozygosity for the FII G20210A gene variant, whereas other data (i.e., AT III, PC, PS, homocysteinemia, FVL and MTHFR variants) were normal. The antithrombotic therapy based on oral anticoagulation (INR range 2.0–3.0) is ongoing again and 12 years later (today), the patient did not refer any other thrombotic episode.
Case 6

male, current age: 40 years. This patient came to our attention because his sister, who suffered from recurrent abortions, was heterozygous for the FII G20210A variant. After genetic counselling, the analysis was extended to all first-degree relatives and he resulted to be homozygous for G20210A. All other laboratory data (i.e., AT III, PC, PS, FVL and MTHFR C677T variant) were normal. His father died at 40 years from AMI; his mother (heterozygote for FII G20210A) had never suffered from vascular diseases. The patient is a non-smoker, without hypertension, diabetes and/or dyslipidaemia; thrombotic risk factors for venous thromboembolism were also absent as recent surgery as recent immobility as malignancy and its therapies as previous personal history of venous thrombosis. Remote and recent anamnesis are negative for vascular diseases and these data were confirmed by a clinical examination and additional tests (i.e. electrocardiography, echocardiography and vascular ultrasound scan of carotid and lower limbs). The patient has a 20-year history of intravenous drug assumption (mainly heroin) and is affected by chronic viral hepatitis C and Child-Pugh stage A cirrhosis with a INR < 1.7 [16]. No episode of vascular disease occurred during the last 4 years and none antithrombotic treatment was suggested for him.

Discussion

Two of the six described cases homozygotes for the G20210A prothrombin variant experienced a deep vein thrombosis during oral contraceptive use (one of them) and recurrent pregnancy loss (the other one). Two other had an acute thrombotic arterial event. The last two, bearing the same genotype, did not experience any thrombotic arterial nor venous disease, and both were affected by chronic liver disease with an altered prothrombin activity. The first two cases confirm the well known association between inherited thrombophilia and venous thromboembolism and/or recurrent pregnancy loss [17,18].

Case n. 1 was a young woman and had a deep vein thrombosis of lower limb after two years of oral contraceptive use. Indeed, several studies described the early onset of venous thromboembolism in subjects bearing inherited thrombophilia [17] and the trigger action of oral contraceptives in particular in subjects with already known thrombophilia [19]. Similarly, the relationships between inherited thrombophilia and pregnancy loss are well known [18]. Our patient experienced three consecutive pregnancy losses without any known clinical, anatomic or genetic alterations causing adverse pregnancy outcomes other than the homozygosity for FII G20210A variant. Interestingly, the patient suffered of two episodes of early recurrent pregnancy losses and one episode of late pregnancy loss that are more frequently associated to inherited thrombophilia [18]. Furthermore, our cases support that the homozygosis for the FII G20210A variant may be a risk factor also for arterial thrombotic events besides of venous thromboembolism. Both the patients who experienced arterial thrombotic diseases had a parent who died from AMI and thrombotic episodes occurred at a relatively young age (40 and 48 years, respectively). Furthermore, they did not have other common risk factors for thrombotic arterial disease such as smoking, hypertension, diabetes, dyslipidaemia [20]. Conflicting data have been reported so far on the relationships between inherited thrombophilia and arterial thrombotic diseases [4-15]. A higher risk of AMI in young women carrying FII G20210A variant and an association between G20210A and coronary heart disease were reported. Other retrospective and prospective studies excluded that the G20210A variant may predispose to arterial thrombotic diseases. G20210A was not associated to an increased risk of infarction or stroke in a prospective study of 15,000 males [15], while a meta-analysis involving 66,000 cases and 91,000 controls indicated that the G20210A variant was associated to a moderately increased risk of coronary diseases [10].

Finally, other two described cases suggest that chronic liver disease may mitigate the risk of vascular disease in patients bearing the G20210A genotype. This information may be found as an innovative point of view on the gene-environment relationships existing in the pathogenesis of thrombotic diseases. In fact, patients 4 and 6, who did not have a history of vascular diseases, had liver disease (i.e., Child B and Child A cirrhosis in cases 4 and 6 respectively). Liver cirrhosis is typically associated to impaired synthesis [21] and post-translational maturation [22,23] of clotting factors, which increases the trend to develop hemorrhagic complications as acute gastrointestinal bleedings [24]. Consequently, these biochemical alterations may counteract the increase of prothrombin levels and activity that are associated to the G20210A variant [25]. Indeed, patients 4 and 6 had reduced INR according to the hypocoagulability due to the impaired liver function. In this setting it must be underlined that patients 5 and 6 were identified as homozygotes for FII G20210A being consanguineous of other affected subjects, and this evidence reinforces the role of the genetic counselling to the families, and the concept to extend molecular analysis to consanguineous once a symptomatic subject bearing inherited thrombophilia is identified. In conclusion, our cases support the idea that the G20210A homozygote genotype associated to another thrombotic risk factor acting as trigger factor is sufficient to give vascular complications as venous thromboembolism and/or female infertility [17,18]; moreover, inherited thrombophilia and in particular the prothrombin A20210G variant is a relevant risk factor for recurrent pregnancy loss. On the other hand prothrombin A20210G may be also a risk factor for arterial disease, as AMI, in particular if early onset and familial anamnesis were positive also in absence of further risk fac-
itors for arterial disease. Furthermore, although data need to be confirmed on a larger population, we suggest also that concomitant hypoaggregation, due to impaired liver function, may reduce the risk of venous and arterial thrombotic disease in homozygotes for the G20210A prothrombin variant.

Conclusion
So, due to the lacking data available in the Literature, our case series underlined that the natural history of subjects carrying A20210G variant of prothrombin may be difficult to be evaluated in such situation. Relevant clinical aspect that should be considered are related to the strong association with repeated pregnancy loss and venous thromboembolism, in particular if further risk factor is present. On the other hand, other clinical aspects that should be considered also by further studies are the possible association with arterial thrombosis in presence/absence of further risk factor for atherothrombosis and the role of comorbidities with a trend toward hypoaggregation that may mitigate thrombotic trend as liver dysfunction in our cases.

Competing interests
The authors declare that they have no competing interests, nor any financial supports during patients’ selection, experimental tests and article extension.

Authors’ contributions
PDM and AN performed patients’ selection. RDF and SQ performed all biochemical tests described in the paper. GC and AA performed revision of the Literature. PDM and GCas performed paper extension.

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