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Prevalence of thrombophilia and thrombotic events in patients with Fabry disease in a reference center for lysosomal disorders in Southern Brazil

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ABSTRACT

Introduction: Venous thromboembolism (VTE) is a multifactorial genetic disorder that occurs in approximately one in a thousand adults per year. Because there is no laboratory test or clinical marker useful for predicting which patients with Fabry disease may develop thrombotic events, this study aimed to determine whether there is a hereditary predisposition to hypercoagulation in these patients.

Methods: The prevalence of p.R506Q mutation in the factor V gene and of c.G20210A mutation in Factor II (prothrombin) gene was evaluated in 39 patients with Fabry disease from Southern Brazil and correlated with clinical findings. The DNA analysis was performed by real-time polymerase chain reaction on genomic DNA using TaqMan probes.

Results: In this group of patients, the frequency of mutation in the prothrombin gene was 1.28%, whereas no patient showed mutation in the factor V gene; additionally, there was no correlation between these mutations and the incidence of thrombotic events.

Conclusion: Hereditary thrombophilia due to mutations in factor V and prothrombin genes does not seem to be related to thrombotic events in Fabry patients in our cohort, although studies in larger cohorts and the inclusion of additional factors may be required to determine if a correlation exists.

Keywords: *Fabry disease; rs1799963; rs6025; stroke; thrombotic event; real-time PCR*

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Venous thromboembolism (VTE) is a multifactorial disorder whose etiology involves the interaction of genetic and/or acquired risk factors that affect proteins of the coagulation system. Among the genetic factors, mutations in the factor V and prothrombin genes are the two main causes of hereditary thrombosis[1,2]. The p.R506Q mutation, also known as Factor V Leiden (FVL) mutation, refers to an alteration in the factor V gene that could result in activated protein C (APC) resistance and subsequently in the imbalance between hemostasis and thrombosis[1-4]. The prothrombin c.G20210A mutation is a point mutation in the 3'-untranslated region of the factor II gene, which can increase the synthesis of prothrombin and thereby increase its concentration[5]. In a meta-analysis Kim et al, showed that these two gene mutations, alone or in combination with other risk factors can increase the occurrence / recurrence of VTE[6].

Fabry disease (FD) is an X-linked disorder of glycosphingolipids that is caused by mutations in the GLA gene leading to a deficiency of α-galactosidase A (GLA) (EC 3.2.1.22) and is associated with dysfunctions of many cell types. As a result, patients have a markedly increased risk of developing small-fiber peripheral neuropathy, stroke, myriad cardiac manifestations, and chronic renal disease. Dysfunction of cerebrovascular circulation in FD has been shown in a number of studies using imaging end-points such as cerebral perfusion by

positron emission tomography and arterial spin tagging using MRI[7-9]. FD has historically been considered rare, with an incidence of one in 40,000 to 117,000 male births[10]; however, a recent newborn screening study (which did not include female subjects) demonstrated that up to one in 3,100 boys are affected[11]. Virtually all complications of FD are non-specific in nature and clinically indistinguishable from similar abnormalities that occur in the context of more common disorders in the general population. However, a high incidence of thrombotic events in FD has been postulated[12,13]. We therefore focused our research on the analysis of mutations in coagulation factors which may also be involved with such complications by changing the state of hemostasis.

FVL heterozygosity is present in around one in 20 people, and thus the expected concurrence of FVL and FD is from 1:62,000 to 1:2,340,000 male patients. This combination is comparatively underreported in the literature, with only three papers referring to human cases and a single mouse model examining FVL homozygosity concurrent with FD[14-17]. Each of these studies highlighted the considerable stroke risk associated with this genetic combination. One theoretical mechanism for this pathological association is through the accumulation of globotriaosylceramide (GL3) influencing the formation and function of antithrombotic lipid rafts, and by doing so altering the glycosphingolipid dependent inactivation of factor V by APC, a protein that has an important role on the regulation of hemostasis. In patients with FD and FVL, this would lead to a decreased anticoagulant response to APC and a procoagulant state (compared to patients with FD but without FVL), leading to cerebral white matter lesions and stroke[18]. Conversely, to date, no studies have investigated the association between prothrombin mutation and FD, even though the first is one of the most frequent mutations predisposing to thrombophilia.

METHODS

The study was approved by the local Ethics Committee (#08632). The sample comprised 39 patients with FD (20 males and 19 females) from 18 families, with a mean age of 47.82 years. All the patients were followed at the Medical Genetics Service of Hospital de Clínicas de Porto Alegre, Brazil (HCPA-SGM), which is considered to be the reference center for lysosomal disorders in the state of Rio Grande do Sul. Patient's clinical information was obtained from medical records.

DNA samples were extracted by the salting out technique and were kept frozen. DNA quantitation was performed using a spectrophotometer (NanoDrop®1000, Thermo Fisher Scientific,USA) and then samples were diluted to a standard concentration of 20 ng/μL.

For genotyping, real-time polymerase chain reaction (PCR) was performed using the TaqMan assay, which uses a pair of PCR primers and a TaqMan probe containing minor groove binder (MGS) at the 3' end and fluorescent dye (FAM™ or VIC®) at the 5 'end. The two different fluorescent dye probes are complementary to either the wild-type or the mutant allele. The reaction was performed in a 48-well plate using the StepOne™ equipment (Applied Biosystems™, California,USA). Negative, homozygous mutant, heterozygous and homozygous wild type standard controls were used in every assay in order to allow the clustering of DNA sequences into different genotype groups according to the TaqMan Genotyper software. Temperature cycles for real-time PCR were those pre-established by the manufacturer, with an extension temperature of 60 °C.

FV assay (ID _ 11975250 rs6025) contains a p.R506Q mutation associated FAM dye-labeled TaqMan probe, featuring the amino acid glutamine (Q), while VIC dye-labeled TaqMan probe corresponds to the normal amino acid, arginine (R). The prothrombin-assay (ID _ 87266802-10 rs1799963) with a c.G20210A mutation in the VIC probe carries adenine (A) and FAM probe carries the codon with guanine (G).

RESULTS

The allele frequency of the c.G20210A mutation was 1.28%, corresponding to only one individual heterozygous for this mutation (2.56%) and no homozygous mutant patients were identified. None of the patients were shown to be either heterozygous or homozygous for the FVL. The only patient who was heterozygous for c.G20210A mutation was a 48-year-old female with the 30delG mutation in the GLA gene. Until now, she had never experienced any thrombotic event.

Six patients (15.4%) had at least one thrombotic event as stroke or ischemic transient attack. The average age of stroke was 53.5 years. From the five patients who had an episode of stroke, three were women and two were men. Additionally, one man had an episode of transient ischemic attack (TIA). Hence, in this study 50% of thrombotic events occurred in females and 50% in males.

DISCUSSION AND CONCLUSION

Lenders et al. suggested that FVL may increase the risk of patients with FD to develop a thromboembolic event[19]. There is controversy whether storage in the endothelial cells and the prothrombotic state are the origin of arterial damage or whether smooth

muscle cell proliferation in the arterial media layer is the initiating step in the cascade that leads to FD vasculopathy[20,21].

Although hereditary thrombophilias are important causes of thrombotic events, they were not associated with an increased number of these events in our sample of patients with FD. The only patient heterozygous for the mutation in the prothrombin gene did not develop any thrombotic event, while six other patients without the analyzed mutations developed stroke or TIA. The incidence of hereditary thrombophilia varies between different regions and populations but is more common in Caucasians. The global prevalence of heterozygosity for the mutation in the prothrombin gene is 2% on average (1.7 to 2.4%)[22], while for the prevalence of FVL ranges from 0.45 to 5.2%[22]; therefore, we can consider that our findings are within the expected range.

Studies in mice demonstrated that the presence of FVL homozygosity and GLA deficiency greatly increases fibrin deposition and occlusive thrombus formation compared with either FVL homozygosity or GLA deficiency alone. This observation suggests that, under certain circumstances, GLA deficiency leads to increased propensity toward spontaneous thrombosis[15]. However, heterozygosity for FVL does not appear to increase the risk of arterial stroke, despite its clear propensity to cause recurrent VTE[23].

A published review of 388 patients with FD showed that 13% had suffered a stroke or TIA[12]. In our study, we found a similar percentage of events. The patients in our study had stroke with a mean age of 53.5 years while in the general population this event is more common after age 55, showing that Fabry patients are at risk of presenting this complication at an earlier age.

Although other studies have already reported a high incidence of thrombotic events in FD patients[12,13] with hereditary thrombophilia, this was not confirmed in our sample. Studies in larger cohorts and the evaluation of additional risk factors may be required to determine if any association exists.

Disclaimer

This work has been approved by the Ethical Committee of Hospital de Clínicas de Porto Alegre (# 08-632), which is recognized by the Office for Human Research Protections as an Institutional Review Board (IRB0000921).

Conflicts of interest

None to declare.

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