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Homocysteine and tissue factor pathway inhibitor levels in patients with Fabry's disease

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Cerebrovascular disorders are a frequent clinical manifestation of Fabry's disease, a rare inborn X-linked (locus Xq22) recessive deficiency of the lysosomal enzyme alpha-galactosidase A. The enzymatic defect results in progressive accumulation of neutral glycosphingolipids in the lysosomes of vascular endothelial and other cells throughout the body [1].

The incidence of classical disease has been estimated to be about 1:40000 and clinical features include involvement of heart, kidney, skin, nervous system, and the eye. Cerebrovascular manifestations consist of large-vessel ectasia, large-vessel occlusive disease, and small-vessel occlusive disease [2].

The occurrence of clinical manifestations shows phenotypic variance not only associated to different genetic mutations, but also within different members of affected families [2]. In the general population, increased susceptibility to cerebrovascular ischemic events has been described in relation to prothrombotic risk factors [3].

Aim of this study was to evaluate the possible role of prothrombotic risk factors in the clinical presentation of Fabry's disease.

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We investigated three unrelated Italian families for a total of nine Fabry patients (four hemizygotes and five heterozygotes, age ranges 21–55 years) compared with 27 Italian control subjects (12 males and 15 females; age ranges 21–55 years). Four of nine Fabry patients (two hemizygotes and two heterozygotes) had recurrent strokes and one of them suffers also from Parkinson symptoms. Magnetic resonance imaging was abnormal in all the patients and showed lacunar infarctions in the periventricular white matter, basal ganglia and pons [4]. The four patients with cerebrovascular complications did not present cardiovascular risk factors (e.g. smoking, dyslipidemia, diabetes, hypertension, cardiopathy, coagulapathies and vasculitis).

Citrated plasma for protein C (PC) activity (DADE-Behring, Dudingen, France), protein S (PS) activity (Instrumentation Laboratory, Milan, Italy), lipoprotein(a) [Lp(a)], tissue factor (TF) and tissue factor pathway inhibitor (TFPI) (TintElize Lp(a), Biopool, Umea, Sweden; IMUBIND Tissue Factor ELISA Kit; IMUBIND Total TFPI ELISA Kit, American Diagnostica Inc, Greenwich, CT, USA) were collected and stored at −80 °C. Anticardiolipin antibodies (aCL) IgG/IgM and anti-Beta2-Glycoprotein I (anti-Beta2-GPI) IgG/IgM (aCL: First Cardiolipin, Eurospital, Trieste, Italy; anti-Beta2-GPI: Arnika, Milan, Italy) were detected in serum samples. Homocysteine (Hcy) levels were detected in EDTA samples by the FPIA method (IMX Abbot Laboratories, Abbott Park, IL, USA). Factor V G1691A, Factor II G20210A and MTHFR C677T polymorphisms were identified as previously described [5–7].

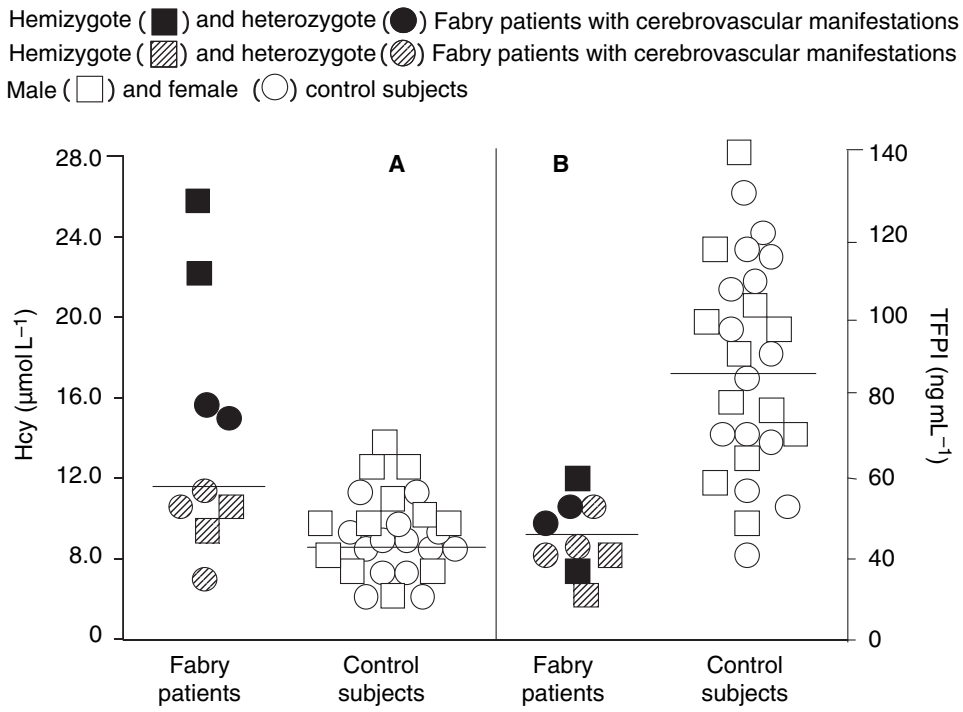

Fig. 1. Homocysteine plasma levels (1A) and TFPI plasma levels (1B) in Fabry's disease patients.

The non-parametric Mann–Whitney *U*-test for unpaired data was used for comparisons between single groups. For genetic analysis, Hardy–Weinberg equilibrium was assessed using χ^2analysis.

No patient showed alterations of blood coagulation inhibitors, Lp(a) and the presence of antiphospholipid antibodies and prothrombotic mutations (data not shown). Homocysteine median value was higher in patients than in controls [11 (6.5–25.6) μmol L^{-1} vs. 8.8 (4.8–13.8) μmol L^{-1}, $P = 0.005$] (Fig. 1A). Hyperhcy (above the 95th percentile cut-off of the control population; $M = 15.5$ μmol L^{-1}, $F = 12.5$ μmol L^{-1}) was found in the 4/4 patients with cerebrovascular disease. Median folic acid and vitamin B6 values were lower in patients than in controls [7.9 (3.2–18.8) nmol L^{-1} and 22.3 (11.7–50.5) nmol L^{-1} vs. 13.8 (7.0–24.5) nmol L^{-1} and 40.1 (15.8–68.4) nmol L^{-1} respectively $P < 0.05$], whereas no difference was found in vitamin B12 levels. Tissue factor and TFPI levels were higher and respectively lower in Fabry patients than controls [TF: 152 (46–274) pg mL^{-1} vs. 107 (22–313) pg mL^{-1}; $P = 0.18$; TFPI: 43.0 (35.0–60.0) ng mL^{-1} vs. 86.3 (38.2–151.0) ng mL^{-1}, $P < 0.0001$ (Fig. 1B)].

These results suggest that hyperhcy may contribute to the cerebral ischemic events in Fabry patients. Hyperhcy may contribute to the endothelial dysfunction, which has been demonstrated in Fabry's disease [8,9]. In cerebral blood vessels of patients with Fabry's disease, a chronic alteration of the nitric oxide (NO) pathway has been demonstrated [8,10] and this condition may be influenced by Hcy, which increases the oxidative degradation of NO [11]. Low folate and vitamin B6 levels may be low due to inadequate dietary intake, malabsorption, increased utilization or to effects of drugs [12–14]. In this study, gastrointestinal manifestations were reported in three of four patients who suffered from cerebrovascular events, but malabsorption may take place in the absence of clinical signs.

In Fabry's disease, extensive clinical heterogeneity has been related to different genetic mutations, of which more than 110 variants have been described [1,15]. Interestingly, we observed that, in one of the families investigated, the three out of six patients affected by Fabry's disease, who suffered from cerebrovascular ischemic events, were found to be hyperhcy, suggesting the possible role of hyperhcy as mechanism involved in increasing the risk of occurrence of cerebrovascular events.

In this study, TFPI levels have been found to be low in all the patients so suggesting a diffuse endothelial alterations associated to the disease *per se*. Tissue factor pathway inhibitor plays a primary role in regulating TF-induced coagulation and significantly contributes to the anticoagulant properties of the endothelium [16]. Tissue factor pathway inhibitor decrease has been reported in conditions in which blood clotting activation occurs [17]. Low levels of plasma TFPI in Fabry's disease may be attributed to the endothelial dysfunction and/or consumption of the endothelial cell-associated TFPI owing to the proinflammatory and procoagulant profile of the Fabry patients. TF may have a role in determining the procoagulant profile to which other mechanisms participate. In conclusion, the present study shows that Hcy may have a role in the occurrence of cerebrovascular events in Fabry patients and that reduced vitamin availability is involved. The possible utility of checking for Hcy and vitamin levels, in particular in the presence of gastrointestinal

events, and the opportunity of vitamin administration deserves confirmation in a larger group of patients with Fabry's disease.

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Further evidence that fibrillar collagen is unable to promote platelet shape change and aggregation in the absence of secondary agonists

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Platelet activation by collagen involves multiple receptors including glycoprotein (GP)VI and the integrin $\alpha_2\beta_1$ as primary receptors, secondary agonists such as released ADP acting on both $P2Y_1$ and $P2Y_{12}$ receptors and thromboxane A_2 (TXA_2) activating Tp receptor. Intracellularly, PLCγ2 downstream of GPVI, as well as the Gq and G_{13} α-subunits of the heterotrimeric G-proteins have been demonstrated to be key signaling elements during platelet activation by collagen. We recently have shown that simultaneous inhibition of TXA_2 formation by aspirin and blockade of the $P2Y_1$ receptor by gene targeting or using a selective $P2Y_1$ antagonist resulted in complete loss of shape change in platelets exposed to a high concentration (100 μg mL^{-1}) of fibrillar collagen [1]. Scanning electron microscopic (SEM) analysis provided clear images of platelets in direct contact with collagen fibers that had not undergone shape change and showed a typical resting discoid ultrastructure. These data led us to propose that the $P2Y_1$ receptor plays a key role in collagen-induced platelet

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