Homocysteine and Erythrocyte Sedimentation Rate Correlate with Cerebrovascular Disease in Fabry Disease

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Abstract

Background Cerebrovascular disease (CVD) is a common clinical problem in Fabry disease; however, expression of this disease manifestation is not uniform and risk factors for its development are not well studied. A number of common CVD risk factors are known in the general population, and these may also play a role in the development of CVD in Fabry disease.

Aim To evaluate the potential associations between various risk factors and CVD in patients with Fabry disease.

Methods and Results Thirty-two Fabry disease patients were studied, with 15 having evidence of CVD. *T*-tests were used to compare the positive and negative CVD groups and logistic regression was used to look for correlations with CVD history.

CVD-positive patients were older (49.73 vs. 37.59 years, $p < 0.001$) and had worse renal function (GFR 61.53 vs. 96.61 mL/min/1.73 m², $p < 0.005$), higher homocysteine (17.79 vs. 10.53 μmol/L, $p < 0.05$) and erythrocyte sedimentation rate (ESR) levels (23.8 vs. 7.64 mm/h, $p < 0.001$), and elevated Mainz Severity Score Index (MSSI) scores (23.8 vs. 11.8, $p < 0.001$).

Correlations were found between age (odds ratio (OR) 1.11), DTPA glomerular filtration rate (OR 0.95), homocysteine concentration (OR 1.22), ESR (OR 1.16) and the MSSI (OR 1.19) scores with a positive CVD history (all $p < 0.05$).

Conclusion Elevated homocysteine and ESR are independent risk factors for CVD in Fabry disease. This finding adds to our ability to predict those patients with Fabry disease who are at a higher risk of developing CVD, and may be an aid in deciding which patients should have primary CVD prevention therapies.

Introduction

Fabry disease is a rare X-linked dominant lysosomal storage disorder caused by mutations in the *GLA* gene leading to a deficiency in α-galactosidase A. This deficiency results in accumulation of globotriaosylceramide and secondary metabolic perturbations, such as elevated globotriaosylsphingosine (Aerts et al. 2008), which lead to the major clinical manifestations of renal failure, cardiac disease and cerebrovascular disease (CVD) in adulthood.

Fabry disease has historically been considered rare, with an incidence of one in 40,000–117,000 male births (Mehta and Ginsberg 2005); however, recent studies have demonstrated that it is an under-recognised cause of stroke in the young, with a 0.65–5.0% prevalence in a variety of cohorts (Rolfs et al. 2005; Baptista et al. 2010; Brouns et al. 2010; Wozniak et al. 2010). Data from the Fabry Outcome Survey (FOS) indicate that stroke or TIA occurs in about 13% of affected individuals overall (15% males, 11.5% females) (Mehta and Ginsberg 2005). T2-weighted hyperintense white matter lesions (WMLs) are commonly seen on the MRI brain scans of Fabry patients. These lesions increase with age and are found in almost all patients by the age of 50 (Ginsberg et al. 2006). While enzyme replacement therapy (ERT) has been shown to positively modify the cardiovascular and renal manifestations of Fabry disease, a reduction in TIAs or strokes has

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not been demonstrated in clinical trials (Testai and Gorelick 2010).

The underlying pathophysiological mechanisms of stroke in Fabry remain unclear; however, it is generally agreed that there are additional mechanisms that cause stroke other than simply glycosphingolipid storage. Patients with Fabry disease demonstrate abnormal neurovascular pathophysiology such as thickening of the common carotid artery intima and dysfunction of cerebral circulation (Testai and Gorelick 2010). There is increased cerebral blood flow, altered vascular reactivity, and cellular dysfunction (Moore et al. 2003), as well as altered nitrogen oxide metabolism, which plays a central role in regulation of cerebral blood flow (Shu et al. 2009). These changes are proposed to cause the cerebral tissues to become more metabolically vulnerable leading to demyelination, gliosis, increased interstitial water content and finally to WML formation (Moore et al. 2003). Thus, while the exact cellular mechanisms that result in vascular pathology are unknown, a vascular dysfunction is present (Mehta and Ginsberg 2005; Altarescu et al. 2008).

In the general population, prothrombotic and other risk factors have been described that increase susceptibility to CVD. We looked for correlations between these selected risk factors and the occurrence of either transient ischaemic attack (TIA), stroke or WMLs in our cohort. We included in our analysis a number of variables that are already known to be associated with disease severity.

Methods

Patients

Thirty-two patients (14 females and 18 males) with Fabry disease (median age 42, range 18–65) were recruited among patients seen at the Department of Genetic Medicine, Westmead Hospital, Australia.

Data

A review of the medical record of each patient was undertaken and age, blood pressure, enzyme replacement therapy (ERT) status, serum creatinine, diethylene-triamine-penta-acetic acid (DTPA) glomerular filtration rate (GFR), high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol, triglycerides, microalbumin, urinary albumin to creatinine ratio (ACR), urinary dipstick, echocardiogram, homocysteine, C-reactive protein (CRP), ESR, antithrombin III, protein C, protein S, p-ANCA (anti-neutrophil cytoplasmic antibody) and c-ANCA, anti-nuclear antibodies (ANA), complement C₃ and C₄, international normalised ratio (INR), factor V Leiden mutation and

prothrombin gene mutation were determined. Investigations to obtain missing data were subsequently arranged as required. Each patient's history of stroke or TIA was determined through the medical record. The presence of WMLs was determined by review of the radiology report. The MSSI was calculated according to the originally published report (Beck 2006).

Patients who did not consent to or were unable to have specific investigations were taken into account in the data and were designated as missing values in the statistical analysis.

Statistical Analyses

Demographic and clinical data between CVD-positive and CVD-negative groups were compared by student *t*-test. Correlations and odds ratios were calculated using logistic regression with a confidence interval of 95%. Statistical significance was established at $p < 0.05$. All statistics were calculated using SPSS (Release 18.0.0).

Ethical Approval

Ethics approval for this study was given by the human research ethics committee of the Children's Hospital at Westmead (project MR 2009-06-15). Written, informed consent was taken from all participants.

Results

Fifteen patients had a history of stroke/TIA or WMLs (CVD positive) and 17 patients did not (CVD negative). None of our patients had low antithrombin III activity, protein C or S deficiencies, or a prothrombin gene mutation (Tables 1 and 2).

Table 1 Baseline characteristics of patients during study period ($n = 32$)

Mean age (SD) (years)	43.28 (12.29)
Male	18 (56%)
Female	14 (44%)
Number of patients on ERT	16 (50%)
Median length of ERT (months)	54 (1,76)
<i>Conventional vascular risk factors</i>	
Current or former smoker	7 (22%)
Hypertension	11 (34%)
Atrial fibrillation	5 (16%)
Arrhythmias other	8 (25%)
Ischaemic heart disease	10 (31%)

Data shown as n (%) or median (minimum, maximum)

Table 2 Patient genotypes

Genotype	Prevalence (<i>n</i> = 32)
p.N215S	4
p.A37T	3
c.1114_1114INSTCCC	3
p.I91T	3
p.C202R	2
p.E48Q	2
p.Y365X	2
c.157_160D	1
p.C223Y	1
c.931DELC	1
c.988DELC	1
p.F169S	1
c.IVS4+861C>T	1
p.M187I	1
p.N215S/p.C202R compound heterozygote	1
p.N298K	1
p.Q111X	1
p.R220X	1
p.T141I	1
Data not available	1

The *t*-test comparisons between CVD-positive and CVD-negative patients are summarised in Table 3. CVD-positive patients were older and had significantly lower GFR, higher homocysteine and ESR levels, and elevated MSSI scores.

A binomial logistic regression model was then used to evaluate which clinical variables were independently associated with a positive history of CVD (Table 4). The analysis revealed that age, DTPA GFR, homocysteine concentration, ESR, and MSSI score correlated with a positive history of CVD.

Discussion

This study shows that age, serum creatinine, DTPA GFR, homocysteine concentration, ESR, and MSSI score are significantly and independently correlated with CVD history. Of these variables, we predicted that age, serum creatinine and DTPA GFR, and MSSI would be statistically significant as the natural history of Fabry disease (reflected in patient age) and disease severity (as defined by MSSI and described by deteriorating renal function) are already known to correlate with the appearance of CVD (Beck 2006; Mehta et al. 2010). Homocysteine and ESR have not previously been independently associated with CVD in Fabry disease.

In our study, homocysteine concentration had a significant and positive relationship with CVD history. None of

our patients with elevated homocysteine levels had low red cell folates and one patient had a vitamin B12 level just below the lower limit of normal (112 pmol/L, 125–780). Large studies in the general population have consistently demonstrated that homocysteine concentrations show a strong, positive, and dose-related correlation with stroke incidence and risk (Hankey and Eikelboom 2005). Homocysteine levels are known to be elevated in patients with Fabry disease (Demuth and Germain 2002; Fedi et al. 2005), but prior to our study have not been shown to correlate directly with clinical manifestations. The ability of folate and B vitamin supplementation to lower homocysteine concentrations suggests an obvious way of reducing CVD risk; however, recent meta-analyses of large trials in the general population have demonstrated that folic acid supplementation does not have a significant effect in averting stroke (Lee et al. 2010; Miller et al. 2010).

Friedman et al. (2001) demonstrated that the kidneys play an important role in the metabolism of homocysteine – for patients with CKD, every 1 µmol/L increase in homocysteine levels has been shown to be independently associated with a 1% risk of vascular events (Friedman et al. 2001). Logistic regression analysis accounts for confounding effects, such as renal impairment, in our study group; hence, our analysis demonstrates that homocysteine alone is a risk factor for CVD in Fabry patients.

ESR but not CRP was correlated with a positive CVD history in our study population. The lack of correlation with CRP echoes previous studies where CRP was not associated with the MSSI and clinical severity in Fabry disease (Altarescu et al. 2008; Vedder et al. 2009). The significant difference in ESR between CVD-positive and CVD-negative patients (23.8 vs. 7.64) and the correlation between ESR and positive CVD history is a novel finding. Inflammatory markers, such as the ESR and CRP, have been shown to be associated with coronary artery disease risk and stroke risk and outcome (Chamorro et al. 1995; Swartz et al. 2005). Why the ESR is raised in Fabry patients with CVD is unknown, although we hypothesise that the raised ESR is a marker of worsening abnormal glycosphingolipid deposition and red cell membrane dysfunction in patients with more severe disease.

A potential shortcoming of this study is the small number of patients in our study cohort. In general, logistic regression analysis overemphasises correlations in small study populations, thus raising the possibility that the homocysteine and ESR correlations are spurious. However, the positive correlations determined between proven risk factors (age, renal impairment and MSSI) and CVD act as internal positive controls, encouraging us that our results are true and relevant. Additionally, there were also significant differences as assessed by *t*-test between the CVD-positive and CVD-negative groups for each of these variants.

Table 3 *T*-test comparisons of means of clinical data between CVD-positive and CVD-negative groups

	CVD positive (<i>n</i> = 15)	CVD negative (<i>n</i> = 17)	<i>p</i> value
Age, years	49.73 ± 9.192	37.59 ± 12.052	< 0.001
Months since first infusion	33.93 ± 31.146	13.41 ± 25.600	0.05
Serum creatinine, μmol/L	174.07 ± 178.694	74.31 ± 17.054	0.056
DTPA GFR, mL/min/1.73 m ²	61.525 ± 32.382	96.613 ± 23.672	0.004
Systolic blood pressure, mmHg	116.730 ± 16.180	123.140 ± 15.027	0.280
Diastolic blood pressure, mmHg	74.670 ± 9.998	79.930 ± 10.623	0.181
Triglycerides, mmol/L	1.568 ± 0.651	1.805 ± 2.583	0.752
HDL, mmol/L	1.205 ± 0.511	1.338 ± 0.250	0.513
LDL, mmol/L	2.480 ± 0.835	2.714 ± 0.674	0.549
Total cholesterol, mmol/L	4.907 ± 1.334	4.777 ± 1.210	0.820
Microalbumin, mg/L	647.964 ± 1100.543	274.073 ± 562.462	0.255
ACR, mg/mmol	90.896 ± 114.242	28.811 ± 49.943	0.064
Protein dipstick, <i>n</i>	1.730 ± 1.272	2.000 ± 1.414	0.726
Proteinuria, mg/24 h	1615.000 ± 1732.412	375.000 ± 148.492	0.419
IV wall thickness, mm	12.867 ± 2.825	11.083 ± 3.942	0.183
Left posterior wall thickness, mm	12.933 ± 2.685	11.167 ± 3.298	0.137
Homocysteine, μmol/L	17.791 ± 8.169	10.525 ± 4.464	0.014
CRP, mg/L	5.180 ± 1.328	4.980 ± 4.544	0.887
ESR, mm/h	23.800 ± 13.975	7.640 ± 4.651	0.001
Protein S, %	0.000 ± 0	0.000 ± 0.408	NS
p-ANCA	0.000 ± 0	0.080 ± 0.289	NS
c-ANCA	0.000 ± 0	0.170 ± 0.577	NS
Complement C3	0.300 ± 0.483	0.270 ± 0.467	0.897
Complement C4	−0.100 ± 0.316	0.000 ± 0	0.306
Factor V Leiden	0.13	0.080	0.737
MSSI	23.770 ± 7.143	11.790 ± 9.33	0.001
Smoking history	0.55 ± 0.522	0.40 ± 0.548	0.63

P-values <0.05 are indicated in bold

Conclusion

In our cohort of Fabry disease patients, we found that in addition to age, renal function, and overall disease severity, elevated homocysteine and ESR correlated with CVD. These findings are of importance since our understanding of the exact pathophysiology of Fabry disease remains imperfect, and determining variables that are markers of disease risk is important in the clinical care of our patients. Larger studies into the role of homocysteine and ESR in Fabry disease are warranted. Patients with one or more of the above risk factors could be considered for primary prevention of CVD.

Author Contributions

Ron Cheung. Wrote the initial draft article and performed statistical analyses.

David Sillence. Revised the draft article and supervised enzyme replacement therapy.

Michel Tchan. Designed the study and revised the draft article. Supervised enzyme replacement therapy. Guarantor.

Competing Interest Statement

Dr Tchan reports having received reimbursement from Genzyme Corporation for travel expenses to attend educational meetings.

Details of Funding

No funding was obtained for this study.

Ethics Approval and Patient Consent

Ethics approval was obtained from the human research ethics committee of the Children's Hospital at Westmead

Table 4 Correlations between clinical data and CVD positive status

	OR	95% CI	<i>p</i> value
Age, years	1.110	1.024–1.202	0.011
Months since first infusion	1.026	0.999–1.053	0.057
Serum creatinine, $\mu\text{mol/L}$	1.056	1.003–1.110	0.036
DTPA GFR, mL/min/1.73 m^2	0.950	0.909–0.994	0.026
Systolic blood pressure, mmHg	0.972	0.925–1.022	0.274
Diastolic blood pressure, mmHg	0.948	0.877–1.025	0.182
Triglycerides, mmol/L	0.917	0.547–1.536	0.741
HDL, mmol/L	0.426	0.037–4.869	0.492
LDL, mmol/L	0.643	0.166–2.497	0.524
Total cholesterol, mmol/L	1.090	0.543–2.187	0.809
Microalbumin, mg/L	1.001	0.999–1.002	0.285
ACR, mg/mmol	1.010	0.998–1.022	0.105
Protein dipstick, <i>n</i>	0.835	0.329–2.116	0.704
Proteinuria, mg/24 h	1.003	0.987–1.019	0.727
IV wall thickness, mm	1.183	0.925–1.512	0.181
Left posterior wall thickness, mm	1.247	0.928–1.675	0.143
Homocysteine, $\mu\text{mol/L}$	1.218	1.017–1.459	0.032
CRP, mg/L	1.019	0.801–1.296	0.881
ESR, mm/h	1.164	1.029–1.316	0.016
Protein S, %	1.000	0.062–16.143	1.000
Complement C3	0.750	0.172–7.601	0.890
Factor V Leiden	1.665	0.097–28.470	0.725
MSSI, score	1.185	1.044–1.344	0.009
Smoking history	1.800	0.210–15.407	0.592

(project MR 2009-06-15). Written, informed consent was taken from all participants.

Synopsis

Elevated homocysteine concentrations and ESR are independent risk factors for CVD in Fabry disease.

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