Thromboembolic events in Fabry disease and the impact of factor V Leiden

ABSTRACT

Objectives: Although several reports suggest an increased thromboembolic event rate, especially regarding strokes and TIAs at early age in patients with Fabry disease (FD), the risk for patients with FD to experience these events, the clinical relevance of additional risk factors including the concurrence of factor V Leiden (FVL), and the benefit of enzyme replacement therapy (ERT) regarding these events remain unclear.

Methods: Three hundred four consecutively recruited patients with FD were evaluated for their lifetime occurrence of thromboembolic events such as stroke, TIA, deep vein thrombosis, and pulmonary embolism. The thromboembolic risk was determined in patients with FD and concurrent FVL, and the impact of ERT was assessed.

Results: The 304 patients with FD had a median age of 41 years and 53 (17.4%) had experienced at least one thromboembolic event during their lifetime. Among 226 patients with FD screened for FVL, 16 gene carriers were identified (7.1%). The occurrence of thromboembolic events in patients with FD and concurrent FVL was significantly increased compared to those without FVL (hazard ratio 5.45, 95% confidence interval 2.29–12.99; ρ < 0.001). Patients with FD receiving ERT had a significantly decreased risk of thromboembolic events compared to those without ERT (hazard ratio 0.362, 95% confidence interval 0.132–0.992; ρ = 0.0422).

Conclusion: This observational study confirms that patients with FD have a high risk of clinically relevant thromboembolic events, which could be aggravated by a concurrence of FVL. ERT might be of benefit in preventing vascular events in patients with FD. The latter observation needs confirmation, however, by randomized and controlled clinical trials.

Glossary

BMI = body mass index; CI = confidence interval; DVT = deep vein thrombosis; ERT = enzyme replacement therapy; FD = Fabry disease; FVL = factor V Leiden; GLA = α-galactosidase A; HR = hazard ratio; PE = pulmonary embolism; TOAST = Trial of ORG 10172 in Acute Stroke Treatment.

Fabry disease (FD; OMIM #301500) is an X-linked (Xq22.1) inborn error of glycosphingolipid catabolism resulting from deficient α-galactosidase A (GLA) (*300644) activity. Fabry-specific manifestations, such as early stroke, malignant arrhythmia, myocardial infarction, as well as progressive renal and cardiac failure, result from the differential systemic cellular accumulation of globotriaosylceramide (Gb3).1 FD manifestations and early death may be partly avoided or delayed by early enzyme replacement therapy (ERT).1,2

Vasculopathy has a compelling pathophysiologic impact on FD manifestations. Proposed mechanisms are abnormal blood flow and vessel architecture, endothelial dysfunction, and prothrombotic state.3,4 Several studies substantiated a high and early incidence of stroke and TIAs in patients with FD.3–8

This clinical peculiarity may be complicated by the concurrence of other prothrombotic risk factors such as factor V Leiden (FVL; c.1691G>A [R506Q]). FVL heterozygosity increases the
risk of thromboembolic events such as deep vein thrombosis (DVT) compared with non-carriers.9 Even if the FVL mutation as such does only slightly increase the risk of stroke in the general population,10,11 the concurrence of FD and FVL has been suggested to translate into increased ischemic cerebral lesions and a potentially higher stroke risk.12–14

In the current report, we investigated the risk of thromboembolic events with and without the concurrence of FVL in a large cohort of well-characterized, consecutively recruited patients with FD from 2 main German Fabry centers and analyzed the clinical impact of ERT in a retrospective study design.

METHODS Standard protocol approvals, registrations, and patient consents. All investigations were performed after approval of the Medical Association of Westfalian-Lippe and the ethical committees of the medical faculties of the University of Muenster (project 2011-347-E, date of report July 7, 2011) and the Johannes Gutenberg University of Mainz (project 010-308, date of report April 25, 2012). Written informed consent was obtained from all patients.

Study design and patients. Between 2000 and 2012, 304 patients with genetically confirmed FD were consecutively recruited at Fabry centers in Muenster and Mainz (Muenster, n = 123; Mainz, n = 181). The patient data were analyzed from the time of their recruitment to the study base.

Genotyping for GLA gene mutations was performed by direct sequencing of all 7 coding exons including adjacent intron-exon boundaries as reported previously.15 A detailed overview of detected GLA mutations (n = 96) and appropriate phenotype is provided in table e-1 on the Neurology® Web site at Neurology.org.

Genotyping for the FVL mutation (c.1691G>A [R560Q]) was performed by amplification and direct sequencing of a 267-base pair fragment in the DNA of 226 patients with FD (Muenster, n = 123; Mainz, n = 103) using the oligonucleotide combination FVL_sense (5'-TGCCCAATGCTTTAACAG-3') and FVL_antisense (5'-TGGTATACACTGGTGCTAA-3'). Subsequent sequencing of both DNA strands was performed. Seventy-eight patients with FD from Mainz were not genotyped for the FVL mutation because of unavailable DNA samples.

Diagnostic criteria for thromboembolic events and variables. According to clinical routine procedures, thromboembolic events were defined as stroke, TIA, DVT, and pulmonary embolism (PE). Stroke/TIA patients were classified according to Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria.16 The classification is based on documented data including clinical history with the assessment of main risk factors (hypertension, hyperlipidemia, diabetes mellitus, family history of vascular disease), blood tests including thrombophilic risk factors, Holter ECG, assessment of extracranial arteries (carotid ultrasound examination, or angiography), and cerebral CT/MRI according to Amarenco et al.17 Events that were not classifiable according to TOAST criteria were excluded from subsequent analyses. Risk factors and confounders for thromboembolic events included essential hypertension (defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or the use of antihypertensive drugs; secondary forms of hypertension were excluded where appropriate by standard laboratory and imaging techniques), type 2 diabetes mellitus (defined as fasting plasma glucose ≥7.0 mmol/L [≥126 mg/dL] or the use of antidiabetic drugs), hypercholesterolemia (defined as cholesterol level ≥200 mg/dL, or use of lipid-lowering drugs), smoking, body mass index (BMI), sex, and age. Blood pressure was measured with auscultatory sphygmomanometers at the upper arm with cuff and bladder dimensions adapted to the arm circumference in a quiet room after 5 minutes of rest, with the patient in a seated position, back and arm supported according to current European Society of Hypertension/European Society of Cardiology Guidelines.17 Duration of ERT (agalsidase-alfa or agalsidase-beta) was assessed as the time difference from start of ERT until the occurrence of a thromboembolic event or to the last visit (event-free censoring).

Statistical analysis. Three hundred four subjects from the 2 Fabry centers were included in the analysis for thromboembolic events. If not otherwise stated, continuous variables were expressed as median (range) and categorical data as numbers and relative frequencies. Differences between groups were analyzed with the Student t or Mann-Whitney U test for continuous data, and the Fisher exact test for categorical data. Statistical significance was considered at a 2-sided p < 0.05. Event rates were calculated per 100 person-years of observation as described above. Cox proportional hazard models were used to determine the influence of FVL on the hazard rate for thromboembolic events and adapted according to Krall et al.18 We adjusted for confounders such as age, sex, BMI, hypertension, type 2 diabetes mellitus, hypercholesterolemia, and smoking. The significance for additional thromboembolic events was calculated using the Fisher exact test. Furthermore, we applied stepwise Cox regression analyses with forward selection (level for entry: 0.10; level for stay: 0.10) to determine the influence of initiation of ERT on the risk of thromboembolic event rates according to Crowley and Hu.19 All results are reported with their respective 95% confidence intervals (CIs). SAS version 9.3 (SAS Institute Inc., Cary, NC) was used for all statistical analyses.

RESULTS Of the 304 patients with FD from 2 German Fabry centers, 123 patients were recruited at the Fabry center in Muenster and 181 at the Fabry center in Mainz between January 2000 and December 2012. Our analysis involved total lifetime events of patients with FD including stroke, TIA, DVT, and PE until the event or the beginning of ERT treatment to exclude the effect of ERT.

Table 1 demonstrates that the majority of the patient cohort was female and their average age was 41 years. We identified 96 different GLA mutations in the 304 patients. Mutations and respective clinical phenotypes are presented in table e-1. About 2 of 3 patients received ERT at the time point of data analysis. Among all patients with FD, 14.1% had experienced at least one stroke or TIA, while 3.3% experienced a DVT or PE. Sixty-seven percent of all neurologic events were of “undetermined origin” because of undetermined origin (46%) or several concurrent reasons (21%), mostly microangiopathic with a concurrent cardioembolic origin). Fourteen percent were of “microangiopathic origin.” Five percent were of “cardioembolic origin” due to atrial fibrillation or
Abbreviations: DVT = deep vein thrombosis; ERT = enzyme replacement therapy; FD = Fabry disease; PE = pulmonary embolism.

Patients with thromboembolic events include patients with stroke, TIA, DVT, and PE with at least one event per patient.

Table 1  Baseline clinical characteristics and parameters of the study population

<table>
<thead>
<tr>
<th>Patients with FD, n</th>
<th>Male, n ( % )</th>
<th>Age, y, median (range)</th>
<th>Different GLA mutations, n</th>
<th>With ERT, n ( % )</th>
<th>Lifetime stroke/TIA, n ( % )</th>
<th>Lifetime DVT/PE, n ( % )</th>
<th>Lifetime any thromboembolic event, n ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>304</td>
<td>126 (41.4)</td>
<td>41 (5–81)</td>
<td>96</td>
<td>209 (68.8)</td>
<td>43 (14.1)</td>
<td>10 (3.3)</td>
<td>53 (17.4)</td>
</tr>
</tbody>
</table>

Abbreviations: DVT = deep vein thrombosis; ERT = enzyme replacement therapy; FD = Fabry disease; PE = pulmonary embolism.

In a first step, the effect of ERT on thromboembolic events was analyzed in a descriptive analysis of...
187 patients with FD (table 4). During 2000 and 2012, we observed in total 23 thromboembolic events during a total observation time of 360,208 days. With ERT, 15 thromboembolic events occurred in 281,935 days, while 8 events occurred in 78,273 days without ERT, corresponding to a frequency of 3.73 events per 100 person-years without ERT and 1.94 events per 100 person-years with ERT, respectively (table 4). Subsequently, in a detailed Cox regression analysis, we determined the time-dependent status of ERT and its potential influence on thromboembolic events. Stepwise regression analysis revealed that age (HR = 1.037, 95% CI 1.010–1.065; \( p = 0.0153 \)) and the time-dependent ERT status (HR = 0.362, \( p = 0.0422 \); table 4) were associated with thromboembolic events, while further tested cofactors (sex, \( p = 0.2719 \); BMI, \( p = 0.7735 \); smoking, \( p = 0.1779 \); type 2 diabetes mellitus, \( p = 0.2500 \); hypercholesterolemia, \( p = 0.4011 \); hypertension, \( p = 0.6320 \)) did not meet the required significance level for entry into the model. The results of the Cox regression analysis indicate that patients with FD not receiving ERT have an approximately 2.8-fold increased risk of thromboembolic events compared with those receiving ERT.

**DISCUSSION** In the current retrospective study, we analyzed 304 well-characterized patients with FD for the risk of thromboembolic events in an observational period of 13 years (2000–2012). In brief, our main findings are as follows: (1) the thromboembolic event rate for patients with FD exceeded 0.17; (2) the incidence of thromboembolic events in patients with FD and the concurrence of FVL was 5.45-fold higher than in patients with FD without FVL; and (3) the risk of thromboembolic events in patients with FD not receiving ERT was approximately 2.8-fold higher compared with those receiving ERT.
Stroke and TIA are common and serious clinical manifestations in FD,\(^5\) with an overall prevalence of 13%,\(^8\) and a high risk of additional events.\(^6,20\) In our cohort of 304 patients with FD, we identified a comparable prevalence of approximately 14%. Several case studies exist, indicating that the concurrence of FD and FVL may lead to an increased formation of ischemic cerebral lesions and increased risk of stroke.\(^12-14,24\) In addition, double knockout mice with a deficiency for GLA and FVL showed increased tissue fibrin deposition and thrombosis in different tissues, underlining a synergistic effect of both inborn errors in Fabry vasculopathy.\(^25\) After correction for classic risk factors for cerebrovascular as well as thrombotic events, such as sex, smoking, BMI, hypercholesterolemia, hypertension, and type 2 diabetes mellitus, in our study, the risk of thromboembolic events in patients with FD and concurrent FVL was increased 5.45-fold compared to those without FVL. Of note, Heit et al.\(^26\) reported that the risk of venous thromboembolic events (DVT or PE) in heterozygous FVL carriers (patients without FD) increased 3.6-fold for patients older than 60 years, highlighting age as an important risk factor in patients with FVL. In our study, patients with FD and concurrent FVL had an earlier decrease of event-free survival, starting at the average age of 30 years (data not shown). This suggests that the deleterious and age-dependent effect of FVL\(^26\) may be observed at an even earlier age in FD. In the current study, when considering FVL carriers with stroke/TIA or DVT/PE separately, the analysis showed an approximately 3.4-fold increase for stroke/TIA and an approximately 14.8-fold increase for DVT/PE. Consequently, our results confirm former case reports\(^12-14\) and general clinical observations in FD centers (C. Kurschat, Fabry Center Cologne, and F. Weidemann, Fabry Center Wuerzburg, personal communication, 2014), in that the risk of stroke/TIA in patients with FD and concurrent FVL is significantly increased.

During the entire observation period, the relative risk for cumulative events was approximately 1.6-fold higher in FVL carriers. This indicates a clinically

<table>
<thead>
<tr>
<th>Event</th>
<th>All (n = 226)</th>
<th>FD/FVL(−) (n = 210)</th>
<th>FD/FVL(+) (n = 16)</th>
<th>Cox regression HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboembolic events</td>
<td>31 (13.7)</td>
<td>23 (11.0)</td>
<td>8 (50.0)</td>
<td>5.45 (2.29-12.99)(^a)</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>23 (10.2)</td>
<td>18 (8.6)</td>
<td>5 (31.3)</td>
<td>3.38 (1.15-9.96)(^b)</td>
</tr>
<tr>
<td>DVT/PE</td>
<td>8 (3.5)</td>
<td>5 (2.4)</td>
<td>3 (18.8)</td>
<td>14.76 (2.72-80.00)(^c)</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; DVT = deep vein thrombosis; FD = Fabry disease; FVL = factor V Leiden; HR = hazard ratio; PE = pulmonary embolism.

\(^a\)\(p < 0.001.\)

\(^b\)\(p < 0.05.\)

\(^c\)\(p < 0.01.\)

Data are n (%). Patients with thromboembolic events include patients with stroke, TIA, DVT, and PE with at least one event per patient. Cox regression analysis was adjusted for confounders (age, body mass index, hypercholesterolemia, hypertension, type 2 diabetes mellitus, smoking, and sex). To analyze the influence of FVL, only the observation time free of ERT was considered, that is, from birth until a thromboembolic event or the last visit (event-free).

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**Table 2** Clinical characteristics of the study population without (−) and with (+) FVL concurrence

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n = 226)</th>
<th>FD/FVL(−) (n = 210)</th>
<th>FD/FVL(+) (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>97 (42.9)</td>
<td>89 (42.4)</td>
<td>8 (50.0)</td>
</tr>
<tr>
<td>Age, y, median (range)</td>
<td>41.0 (11-81)</td>
<td>41.5 (10-79)</td>
<td>38.0 (14-63)</td>
</tr>
<tr>
<td>BMI, kg/m², median (range)</td>
<td>23.7 (14.6-38.8)</td>
<td>23.7 (14.6-38.8)</td>
<td>22.6 (14.6-27.9)</td>
</tr>
<tr>
<td>ERT, n (%)</td>
<td>160 (70.8)</td>
<td>147 (70.0)</td>
<td>13 (81.3)</td>
</tr>
<tr>
<td>ERT duration, y, mean (range)</td>
<td>6.1 [1-12]</td>
<td>6.2 [1-12]</td>
<td>4.5 [1-9]</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>76 (33.6)</td>
<td>73 (34.8)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>62 (27.4)</td>
<td>59 (28.1)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>7 (3.1)</td>
<td>7 (3.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>38 (16.8)</td>
<td>33 (15.7)</td>
<td>3 (18.8)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI = body mass index; ERT = enzyme replacement therapy; FD = Fabry disease; FVL = factor V Leiden. \(^*p < 0.05.\)
relevant increased risk of additional thromboembolic events in patients with FD and FVL and the need of close clinical monitoring.

Since an increased risk of thromboembolic events in general results in increased morbidity and mortality, we strongly recommend that in patients with these events, an additional diagnostic screening for thrombophilic risk markers such as the FVL mutation as well as FD is warranted. In the case of a concurrence of FD and an additional thrombophilic risk factor, such as the FVL mutation, we strongly recommend counseling the patient in avoiding additional risk factors for venous thromboembolism (e.g., use of combined oral contraceptives, hormone replacement therapy) and providing pharmacologic anticoagulant prophylaxis in thrombophilic risk situations (surgery, immobilization, long-distance flights, postpartum period, etc.). In addition, strict blood pressure and lipid monitoring and the use of renin-angiotensin-aldosterone system inhibitors or statins where appropriate should be performed to prevent progression of vasculopathy. According to current general guidelines for stroke prevention, acetylsalicylic acid treatment should only be considered for primary stroke prevention for persons whose risk is sufficiently high for the benefits to outweigh the risks associated with treatment (a 10-year risk of cardiovascular events of 6%–10%).

However, regarding our results and in accordance with the current Fabry expert opinions and German FD guidelines, primary prevention of thromboembolic events including antiplatelet therapy should be considered in patients with FD especially with a concurrence of FVL.

The benefit of ERT has already been shown for different Fabry-related symptoms and organ manifestations. ERT reduces neuropathic pain and gastrointestinal symptoms, leads to a stabilization of renal and cardiac function and structural involvement, reduces the frequency of clinically significant renal and cardiovascular events, and prolongs life expectancy. Several studies have shown the optimal benefit when ERT is started at an early disease stage before extensive fibrosis or other irreversible tissue damage occurs.

In our retrospective study, we demonstrate that patients with FD not receiving ERT had an approximately 2.8-fold higher risk of thromboembolic events compared with patients receiving ERT. However, the moderate p value associated with this risk makes the result sensitive to a small change in the events per analyzed group. Regarding the arterial and venous system, we could show that the risk of stroke and TIA as well as of DVT and PE in patients with FD not receiving ERT was increased. According to our observations, ERT might be of benefit in preventing vascular events.

The retrospective design of our study is a limitation since no conclusions should be drawn concerning the exact risk of future thromboembolic events once FD is diagnosed in the concurrence of FVL. Therefore, prospective and controlled clinical studies are warranted to quantify the increased risk of future thromboembolic events in patients with FD in a concurrence of FVL after diagnosis and the risk reduction of thromboembolic events by ERT.

In conclusion, patients with FD have a high risk of clinically relevant thromboembolic events, which might be prevented by ERT. In our retrospective study, the concurrence of FD and FVL led to an even higher risk of thromboembolic events. Future prospective studies are needed to clarify which patients with the combination of FD and other thrombophilic risk factors benefit from more intensive oral anticoagulant strategies.

**AUTHOR CONTRIBUTIONS**

M.L. and N.K. carried out the genotyping, participated in the experimental design and data analysis, and drafted the manuscript. M.L. and H.-W.H. were responsible for the statistical analyses. T.D., M.S., and B.S. participated in the data collection. T.D., R.M., and M.B. participated in the data analysis. S.-M.B. and E.B. designed and coordinated the study, drafted and finalized the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

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