Association of Factor V Leiden Mutation with Delayed Graft Function, Acute Rejection Episodes and Long-term Graft Dysfunction in Kidney Transplant Recipients

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Keywords

Factor V Leiden mutation, kidney transplantation

Summary

We analysed whether the factor V Leiden mutation – the most common hereditary predisposing factor for venous thrombosis – is associated with early and long-term graft dysfunction after kidney transplantation in 394 Caucasian kidney transplant recipients. The presence of factor V Leiden mutation was identified by allele specific PCR. The prevalence of the factor V Leiden mutation was compared to 32216 unselected neonates. The prevalence of the factor V Leiden mutation (GA genotype) was similar in 394 kidney transplant recipients and 32216 neonates. The frequency of known factors predicting long-term graft function were similar in patients with the GA genotype and with the normal factor V gene (GG genotype). The GA genotype was associated with the occurrence of no primary graft function (risk: 2.87; 95% confidence interval: 1.01-8.26; p < 0.05), the number of dialysis after transplantation in patients with no primary graft function until graft function (7.5 ± 2.06 dialysis in GA patients; 4.2 ± 0.36 dialysises in GG patients; p < 0.05), and the risk for at least one acute rejection episode (risk: 3.83; 95% confidence interval: 1.38-10.59; p < 0.02). The slope of 1/creatinine per year was significantly lower in patients with the GA genotype (GA patients: – 0.0204 ± 0.008 dl/mg per year; GG patients: 0.0104 ± 0.004 dl/mg per year; p < 0.02). The annual enhancement of the daily protein excretion rate was elevated in patients with the GA genotype (GA patients: 38.5 ± 16.6 mg/24 h per year; GG patients: 4.9 ± 4.4 mg/24 h per year; p < 0.02). Our study showed that the factor V Leiden mutation is associated with the occurrence of delayed graft function, acute rejection episodes and chronic graft dysfunction after kidney transplantation.

Introduction

One of the most important predictors of short and long term kidney graft function is represented by the degree of histocompatibility between donor and recipient. Alloantigen independent risk factors for a poorer outcome of kidney grafts include cold ischemia time, increasing donor age and donor ethnic background as well as recipient’s lipid disorders and blood pressure (1, 2, 3, 4). In biopsy specimens obtained from patients with rejections, intravascular fibrin deposits are frequently seen (5, 6). These findings have been considered as part of the rejection process on its own. The activation of the coagulation cascade may be an unspecific result of rejection, or it may be involved in the etiology under some circumstances such as coagulatory abnormalities caused by immunosuppressive therapy or recurrence of HUS as the primary kidney disease (7). It is conceivable, therefore, that a procoagulatory disposition of the recipient may represent a significant independent risk factor for poorer short and long term graft function and it has been shown in recent smaller studies that thrombophilia – regardless of the underlying thrombophilic disease – is associated with an increased rate of rejection episodes and early graft loss (8, 9). An abnormality of the coagulatory factor V (factor V Leiden) is the most common hereditary cause for thrombophilia and occurs with a heterozygote frequency of about 5% in most north-western European population (10). Factor V Leiden is characterised by an Arg 506 Glu amino acid substitution that results in a loss of efficient metabolism of the activated factor V by activated protein C (APC resistance) and in a less efficient degradation of activated factor VIII (11, 12, 13).

In the present study we tested the hypothesis whether factor V Leiden contributes to early and chronic graft dysfunction in 394 kidney transplant recipients.

Materials and Methods

Study Patients and Control Subjects

Study Patients

This study included 394 kidney transplant recipients – all Caucasians – with a functioning graft, who were transplanted between 1964 and 1996. Our study population represents all patients visiting the nephrology outpatient clinics at the University Hospitals of Berlin (Charité) from January 1998 until December 1998. The mean follow-up time after transplantation was 6.62 ± 3.08 years (mean ± SD) for patients with the factor V Leiden mutation and 6.79 ± 2.94 years (mean ± SD) for patients with the normal factor V gene. The following patients characteristics were documented: recipient gender and age, donor gender and age, recipient and donor CMV status, number of transplantations, duration of dialysis before transplantation, most recent percent of panel reactive antibodies at the time of transplantation, cold ischemia time, HLA mismatch, and number and time point of acute rejection episodes. The mean serum creatinine concentration and the mean protein excretion rate for every year after transplantation was calculated from all available serum creatine and protein excretion data measured at routine visits in the outpatient clinics. Linear
regression analysis was performed in order to calculate the slope of 1/serum creatinine per year (dl/mg/year) for each patient as a parameter of disease progression.

Primary graft function was assessed by the number of dialysis recorded after transplantation until sufficient graft function. Patients without any need for dialysis after transplantation were taken as cases with primary graft function.

Transplanted patients’ informed consent was obtained in each case, thus the study was in accordance with the ethical standards of the Helsinki declaration of 1975 (revised in 1983).

Controls

The control group for the frequency of the factor V Leiden mutation consisted of all 32,216 neonates who were anonymously analyzed during 1999 in the Berlin neonatal screening program. According to the official database of Berlin, less than 1% of all neonates in 1999 were not Caucasians. Genotyping for the factor V Leiden mutation from the babies screening filters was checked and recommended by the ethical committee of the University Hospital Charité.

Allele Specific PCR for the FVL Mutation

Genotyping was performed as recently described (14). Briefly, DNA was purified from blood obtained from the kidney graft recipients by standard protocols. For genotyping the controls, we used the 3 mm discs that we obtained from, the babies’ screening filter cards. These 3 mm discs were directly used for PCR analysis in a 96 well plate in a PTC-100 PCR cycler (MJ-Research). First, the samples are immersed in PCR buffer including the primers and are subjected to 3 pre-cycles (3’ 95° C, 3’ 60° C). Subsequently, the Taq polymerase is added and the allele specific PCR with 40 cycles is started (30’ 95° C; 1’30” 60,5° C; 30’ 72° C). This allele-specific PCR is carried out to identify the normal Gs at codon 506 of the FV gene or the FV Leiden mutation. The choice of the primers resulted in the generation of three different specific fragments of 390bp (positive control for the PCR reaction), 233bp (FV normal; primers: 5’-GCAGATCCCTGGACAGGCA-3’ and 5’-AATGTTATCACACTGGTGCTAA-3’), and 177bp (FV Leiden; primers: 5’-GGACAAATACCTGTATTCCTC-3’ and 5’-CTTTCAAGGCGAGACAACAC-3’).

Statistics

All data were analysed using SPSS for Windows, Version 9.0. Categorical data were compared using the Chi-square test. Continuous variables were assessed by Man-Whitney U test. The level of significance was set at p < 0.05. An initial univariate analysis compared the frequency of possible risk factors for rejection, primary graft function, or chronic graft dysfunction in the GA genotype group and the GG genotype group (recipient gender and age, donor gender and age, recipient and donor CMV status, percentage of first kidney transplantation, duration of dialysis before transplantation, panel reactive antibodies, cold ischemia time, and HLA mismatches) were similar in patients with the factor V Leiden mutation and those with the normal factor V gene (Table 2). There were no significant differences between the frequencies of underlying diseases leading to end-stage kidney disease in kidney transplant recipients with and without factor V Leiden mutation (Table 3).

Results

Patients Characteristics

The allele frequency for the factor V Leiden mutation was not statistically different in the 394 kidney transplant recipients and the 32216 consecutively new-born babies born in Berlin (Chi²-test: p > 0.5; see also Table 1) indicating that the factor V Leiden mutation is no risk factor for developing end stage kidney disease. A similar allele frequency for the factor V Leiden mutation in a Caucasian population was reported by others (10). Despite the similarity of the allele frequency for the factor V Leiden mutation in the 394 kidney transplant recipients and the 32216 consecutively new-born babies, we could not exclude that the factor V Leiden mutation might influence disease progression in specific kidney diseases (for example: HUS). 22 out of the 394 kidney transplant recipients were carries of the factor V Leiden mutation. Two of these 22 patients (9,09%) had vein thrombosis. 22 out of the 372 kidney transplant recipients without factor V Leiden mutation (5.9%) also had vein thrombosis. However, these differences were not significant (p > 0.5). None of the 24 thrombosis seen in our study affected the renal transplant vein.

The frequency of factors that might independently predict graft function like recipient gender and age, donor gender and age, recipient and donor CMV status, percentage of first kidney transplantation, duration of dialysis before transplantation, panel reactive antibodies, cold ischemia time, and HLA mismatches were similar in patients with the factor V Leiden mutation and those with the normal factor V gene (Table 2). There were no significant differences between the frequencies of underlying diseases leading to end-stage kidney disease in kidney transplant recipients with and without factor V Leiden mutation (Table 3).

Primary Graft Function

Data concerning primary graft function were available in 355 transplanted patients. 21 had the factor V Leiden mutation (GA genotype). 334 patients had the normal factor V gene. 16 patients out of 21 with factor V Leiden had no primary graft function (76.2%), whereas 199 patients out of 334 with the normal factor V gene had no primary graft function (59.4%); p < 0.05. The risk in patients with factor V Leiden mutation with respect to no primary graft function is 2.87; 95% confidence interval: 1.01-8.26; p < 0.05.

Table 1 Allele frequency in kidney transplant recipients and controls

<table>
<thead>
<tr>
<th>Factor V genotype</th>
<th>Transplant Recipients (n=384)</th>
<th>Controls (n=32216)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>372 (94.4%)</td>
<td>30191 (93.7%)</td>
</tr>
<tr>
<td>GA</td>
<td>22 (5.6%)</td>
<td>1984 (6.2%)</td>
</tr>
<tr>
<td>AA</td>
<td>0 (0 %)</td>
<td>41 (0.1%)</td>
</tr>
</tbody>
</table>

The allele frequency for the factor V Leiden mutation was similar in kidney transplant recipients and controls (consecutively newborn 32216 babies born in 1999 in Berlin). The percental frequency of the haplotypes are given in brackets.
The duration of cold ischemia time is one of the most important predictors of primary graft function. We thus analyzed the impact of the factor V Leiden mutation on the risk of having no primary graft function in patients with short (8-24 h) and long (25-48 h) cold ischemia time (CIT). Complete data concerning primary graft function matching with data concerning duration of cold ischemia time were available in 353 transplanted patients. Eight patients with GA genotype and a short CIT out of 13 (61.5%) had no primary graft function, whereas 99 patients out of 218 (45.4%) with the normal factor V gene and short CIT had no primary graft function (p = 0.22). A striking finding was that all 8 patients (100%) with the factor V Leiden mutation (GA genotype) and long CIT had no primary graft function, whereas 97 patients out of 135 patients (71.9%) with the normal factor V gene and long CIT had no primary graft function (p = 0.068).

**Number of Dialyses after Transplantation until Graft Function**

Data concerning the number of dialysis after transplantation until graft function in patients without primary graft function were documented in all 215 transplanted patients without primary graft function. 16 had the factor V Leiden mutation (GA genotype). 199 patients had the normal factor V gene.

The 16 patients with the factor V Leiden mutation needed 7.5 ± 2.06 (mean ± SEM) dialysis after transplantation until graft function, whereas the 199 patients without factor V Leiden mutation needed 4.2 ± 0.36 (mean ± SEM) dialysis after transplantation until graft function (p < 0.05).

**Rejection Episodes**

17 out of 22 patients (77.3%) with the factor V Leiden mutation had at least one acute rejection episode, whereas only 175 out of 377 patients (46.4%) without the factor V Leiden mutation had at least one acute rejection episode. Performing multiple logistic regression analysis, we could demonstrate that the GA genotype (factor V Leiden mutation) was associated with a much higher risk for at least one acute rejection episode (risk: 3.83; 95% confidence interval: 1.38-10.59; p < 0.02).

**Long-term Graft Function**

Long-term kidney function after renal transplantation was assessed by the slope of 1/creatinine. For this analysis we could include 372 patients with the normal factor V gene and 22 with the factor V Leiden mutation. The slope of 1/creatinine per year was significantly lower in patients with the GA genotype (GA patients: – 0.0204 ± 0.008 dl/mg per year; GG patients: 0.0104 ± 0.004 dl/mg per year; p < 0.02) (Fig. 1).

Increasing urinary protein excretion is a second parameter of chronic graft dysfunction. We had complete data concerning the progression of daily protein excretion rate from 363 patients without factor V Leiden mutation and 22 with factor V Leiden mutation. Baseline daily protein

### Table 2 Kidney transplant recipients characteristics

<table>
<thead>
<tr>
<th></th>
<th>GG genotype</th>
<th>GA genotype</th>
<th>Data available (patients)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient gender female (%)</td>
<td>35.5</td>
<td>36.4</td>
<td>394</td>
<td>0.93</td>
</tr>
<tr>
<td>Donor gender female (%)</td>
<td>32.2</td>
<td>40.0</td>
<td>362</td>
<td>0.47</td>
</tr>
<tr>
<td>Recipient CMV-IgG negativ (%)</td>
<td>34.7</td>
<td>23.1</td>
<td>261</td>
<td>0.39</td>
</tr>
<tr>
<td>Donor CMV-IgG negativ (%)</td>
<td>39.1</td>
<td>26.7</td>
<td>251</td>
<td>0.86</td>
</tr>
<tr>
<td>Recipient age (years)</td>
<td>37.3±12.7</td>
<td>34.5±11.5</td>
<td>394</td>
<td>0.32</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>32.6±13.9</td>
<td>32.1±11.7</td>
<td>363</td>
<td>0.94</td>
</tr>
<tr>
<td>First kidney transplantation (%)</td>
<td>90.3</td>
<td>86.4</td>
<td>394</td>
<td>0.55</td>
</tr>
<tr>
<td>on dialysis before kidney transplantation (years)</td>
<td>2.6±2.4</td>
<td>3.2±2.5</td>
<td>390</td>
<td>0.15</td>
</tr>
<tr>
<td>Panel reactive antibodies positiv (%)</td>
<td>21.6</td>
<td>21.1</td>
<td>352</td>
<td>0.95</td>
</tr>
<tr>
<td>Cold ischemia time (h)</td>
<td>21.4±8.1</td>
<td>22.5±4.6</td>
<td>390</td>
<td>0.50</td>
</tr>
<tr>
<td>HLA mismatch points</td>
<td>2.8±1.3</td>
<td>2.9±1.3</td>
<td>354</td>
<td>0.71</td>
</tr>
</tbody>
</table>

All data were obtained from the patients records. Especially for patients transplanted before 1989 data concerning CMV infection, HLA matching and panel reactive antibodies were sometimes not determined or not documented. Data are presented as means ± SD. Chi²-test or Mann-Whitney-test were used when appropriate. There were no significant differences in all tested parameters between patients with the GG genotype and the GA genotype.

### Table 3 Underlying kidney diseases

<table>
<thead>
<tr>
<th>Underlying kidney disease</th>
<th>GA genotype</th>
<th>GG genotype</th>
<th>p-value (X²-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic glomerulonephritis</td>
<td>6 (27.3)</td>
<td>156 (41.9)</td>
<td>0.17</td>
</tr>
<tr>
<td>Chronic pyelonephritis</td>
<td>5 (22.7)</td>
<td>80 (21.5)</td>
<td>0.89</td>
</tr>
<tr>
<td>Hypertensive nephrosclerosis</td>
<td>0 (0)</td>
<td>8 (2.2)</td>
<td>0.49</td>
</tr>
<tr>
<td>Allogenic nephrosclerosis</td>
<td>1 (4.6)</td>
<td>8 (2.2)</td>
<td>0.47</td>
</tr>
<tr>
<td>IgA-Nephropathy</td>
<td>0 (0)</td>
<td>6 (1.6)</td>
<td>0.55</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0)</td>
<td>5 (1.3)</td>
<td>0.58</td>
</tr>
<tr>
<td>SLE</td>
<td>1 (4.6)</td>
<td>4 (1.1)</td>
<td>0.16</td>
</tr>
<tr>
<td>Other</td>
<td>6 (27.3)</td>
<td>87 (23.4)</td>
<td>0.68</td>
</tr>
<tr>
<td>unknown</td>
<td>3 (13.6)</td>
<td>18 (4.8)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Data are presented as raw data and relative frequency (in %). There were no significant differences between the frequencies of underlying diseases leading to end-stage kidney disease in kidney transplant recipients with and without factor V Leiden mutation.
excretion 6 months after transplantation was similar in patients without factor V Leiden (540 ± 52 mg/day) and patients with factor V Leiden (432 ± 117 mg/day) (mean ± SEM). The protein excretion rate increased in patients without factor V Leiden with an annual progression rate of 4.9 ± 4.4 mg/24 h per year significantly slower (p < 0.02) as compared to patients with the factor V Leiden mutation (38.5 ± 16.6 mg/24 h per year).

Discussion

In the present study we tested the hypothesis whether the most frequent congenital coagulatory disorder, the factor V Leiden mutation, contributes to acute and especially long-term graft dysfunction in Caucasian kidney transplant recipients. Our study demonstrated an association of primary graft function, number of dialyses after transplantation, frequency of acute rejection episodes, and parameters of long-term graft function such as slope of $1/\text{creatinine}$ per year and annual enhancement of the daily protein excretion rate with the recipients’ factor V Leiden mutation. Beside the well known association of the Factor V Leiden mutation with a higher risk of early graft loss and kidney transplant vein thrombosis (9, 15), our study demonstrated for the first time that the factor V Leiden mutation is also an important risk factor for chronic graft dysfunction. Chronic graft dysfunction is the leading cause of graft loss during the last decade.

Delayed Graft Function and Factor V Leiden

Kidney graft recipients with the factor V Leiden mutation had a significantly lower rate of primary graft function. Moreover, patients with the factor V Leiden mutation needed significantly more dialysis after transplantation until graft function. These findings indicate that the factor V Leiden mutation is associated with delayed graft function. Delayed graft function on its own – on the other hand – is an independent predictor of long-term graft function (16-18). So far, the most important cause of delayed graft function is postischemic acute tubular necrosis (19). The incidence of this complication increases when the cold ischemia time exceeds 24 h (20). Our data suggest that both, prolonged cold ischemia time (> 24 h) and factor V Leiden mutation might exert important synergistic effects upon primary graft function. None of the patients with prolonged cold ischemia time and factor V Leiden mutation had primary graft function. However, this was only a statistical trend (p = 0.068) due to the small number of patients (eight, see results section) with prolonged cold ischemia time and factor V Leiden mutation. We suggest that tissue damage caused by prolonged cold ischemia time could lead to an enhanced risk for microthrombosis in the graft particularly when the transplant recipient is a carrier of the factor V Leiden mutation.

Rejection Episodes and Factor V Leiden

The present study revealed that the risk for acute rejection episodes in patients with the factor V Leiden mutation is 3.83. The factor V Leiden mutation is thus an important independent risk factor for acute rejection episodes after kidney transplantation. Our cohort is in agreement with a recent study showing in a smaller cohort that thrombophilia per se (due to either inherited or acquired thrombophilic disorders like protein S, protein C, and antithrombin deficiency and...
inherited factor V Leiden mutation) is associated with an increased risk for acute rejection episodes (8).

There are at least two possible mechanisms that might explain the association of factor V Leiden mutation with a markedly increased risk for acute rejection episodes:

i) Adhesion and/or chemotaxis of lymphocytes in the vascular beds of the graft might be enhanced in response to vascular clotting as a trigger for acute rejections.

ii) Clinically incipient rejections might be aggravated by the congenital hemostasis defect.

The factor V associated increased risk for acute rejection episodes and delayed graft function as seen in our study might also contribute to the early loss of renal transplants in patients with thrombophilia recently reported by Fischereder et al. (9).

**Chronic Graft Dysfunction and Factor V Leiden**

Kidney transplant recipients with a history of acute rejection episodes and delayed graft function are more likely to develop chronic graft dysfunction than those without such episodes (16-23). The factor V associated increased risk for acute rejection episodes and delayed graft function thus might be an important factor contributing to poorer long-term graft function. This concept is supported by the finding of a significant association of the slope of 1/serum creatinine per year and the annual enhancement of the daily protein excretion rate – two clinical parameters of chronic graft dysfunction – with the factor V Leiden mutation.

Besides the increased risk for acute rejection episodes and early graft dysfunction in patients with the GA genotype that might lead to chronic graft dysfunction (see above), it is furthermore quite possible that continuously occurring subclinical microthrombosis in the kidneys of patients with factor V Leiden mutation contribute to chronic graft dysfunction.

In conclusion, the present study indicates that recipients’ factor V Leiden mutation is associated with early graft dysfunction, acute rejection episodes and clinical signs of chronic graft dysfunction. In addition, our association study might stimulate clinical trials testing whether anticoagulants influence the grafts’ outcome in carriers of the factor V Leiden mutation.

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