The factor V Leiden mutation and risk of renal vein thrombosis in patients with nephrotic syndrome

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Abstract
Background: Acquired abnormalities of coagulation and fibrinolysis in nephrotic syndrome have been implicated in the pathogenesis of renal vein thrombosis (RVT). Whether resistance to activated Protein C due to a mutation in the gene for factor V (FV Leiden/FV506Q, the commonest inherited risk factor for venous thrombosis) could contribute to risk of RVT in patients with nephrotic syndrome is unknown.
Methods: Genotyping for the factor V Leiden mutation was undertaken in a retrospective study of 35 patients with a history of nephrotic syndrome, 10 of whom had suffered clinically significant and radiologically proven RVT.
Results: Two patients (6%) were heterozygous for the FV506Q mutation, a prevalence similar to studies within the general population. One heterozygote had suffered a RVT, whilst the other without a native RVT subsequently had a primary renal allograft thrombosis.
Conclusions: In a retrospective study the prevalence of the FV Leiden mutation was not increased in patients with nephrotic syndrome nor associated with prevalence of clinically significant RVT. Whilst this study was insufficiently powerful to fully exclude an association, it suggests acquired rather than inherited alterations in the coagulation/fibrinolytic balance associated with nephrosis may be of greater importance in venous thrombotic risk, and that routine screening of patients with nephrosis for this mutation will not identify the majority of patients at risk for RVT. Confirmation of these results and determining whether the natural history of thrombosis or underlying renal disease in carriers of the FV Leiden mutation differs from those without this mutation, will require a large prospective study.

Key words: Nephrotic syndrome; renal vein thrombosis; factor V Leiden mutation

Introduction
Renal Vein Thrombosis (RVT) is a well recognised thrombotic complication of nephrotic syndrome. Its prevalence is uncertain but has been reported to vary from 5% when only clinical criteria are used for further evaluation, to as high as 50% if evidence for thrombus is actively sought in asymptomatic patients [1]. Intravascular volume depletion, immobilisation, abnormalities of pro-coagulant and anticoagulant mechanisms including elevated fibrinogen and its deposition as fibrin, increased urinary loss of anticoagulant factors such as antithrombin III and free Protein S may all contribute to risk of RVT [2–5]. Evidence for a procoagulant state is also shown by raised indices of the coagulation activation peptide Prothrombin fragment F1+2 and thrombin-antithrombin complexes [6]. In adults, membranous nephropathy appears to be particularly associated with risk of renal vein thrombosis [2,5]. Proteins C and S provide a major endothelial anticoagulant defence mechanism and inherited deficiencies due to genetic defects are associated with increased risk of venous thrombosis in the general population [7]. These mutations however are heterogeneous and uncommon, occurring in only 0.1–0.5% of the population. Activated protein C (APC) resistance has recently been found to be strongly associated with inherited risk of venous thrombosis [8]. A major contributor to the impairment of the anti-coagulant effect of activated Protein C is due to a G/A substitution at base pair 1691 in factor V (FV) resulting in the substitution of glutamine (Q) for arginine (R) at amino acid 306 [9]. FV506Q (FV Leiden) retards the degradation of activated factor V (Va) by activated protein C ~10 fold compared with FV506R, and promotes continuing thrombin generation leading to a hypercoagulable state [10]. APC resistance due to FV506Q has been shown to be associated with increased risk of venous thrombosis in the general population [8], oral contraceptive users [11], and the risk of primary venous thrombosis in healthy men [12]. These studies suggest that ~5% of the European and North American population are carriers for FV Leiden making it the most common
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Subjects and methods

Ten adult patients with a diagnosis of renal vein thrombosis and nephrotic syndrome were identified from the records of the Oxford Renal Unit, which services a population of ~2 million, over the last 15 years. 25 other patients who had a diagnosis of nephrotic syndrome without a history of renal venous thrombosis during this period were used as controls. It was not possible to precisely match the severity or duration of nephrotic syndrome between the two groups but histological diagnosis, disease progression and sex matching were closely approximated. No patient had a familial history of venous thrombosis. All patients had undergone renal biopsy for histological diagnosis except two patients with extensive thrombosis requiring anticoagulation where biopsy was considered too hazardous. Nephrotic syndrome was defined as plasma albumin <30g/l and urinary protein excretion >3.5g/day. Oedema was usually but not always present. The period of nephrotic syndrome varied from 3 months to >10 years. Renal Vein thrombosis was definitively diagnosed by any or several of the following techniques; renal vein venography, MRI or contrast enhanced CT scanning. Eight patients with RVT were diagnosed at presentation with nephrotic syndrome, one patient had RVT diagnosed in the context of a sudden deterioration in renal function 12 months after initial presentation, and another patient had the diagnosis delayed until 4 months following presentation. Nine had evidence of pulmonary embolism and all were anti-coagulated either for life, or for the duration of their nephrotic syndrome. Prophylactic anti-coagulation was not used and in the absence of clinical or radiological evidence for RVT (haematuria, enlarged or poorly functioning kidney on imaging, or evidence of peripheral venous thrombosis or pulmonary embolism) this diagnosis was not actively sought by venography. Patients were not routinely screened for indices of a hypercoagulable state. No patient was diabetic but two patients had subsequent diagnoses of malignancy (adenocarcinoma of the stomach 8 months post renal biopsy without evidence for RVT and primary mesothelioma of the lung diagnosed >1 year after original presentation, RVT present). This study was approved by the regional ethics committee. An additional 321 patients attending the same renal unit (61% male, median age 50) with chronic renal disease (33% with membranous nephropathy of the nephrotic syndrome [13]. The present study was 12 h at 37°C and the digestion products were separated by electrophoresis through a 2% agarose gel containing ethidium bromide and visualised under UV light. Internal controls of a homozygote for FV506Q and a heterozygote, previously genotyped using different PCR primers, were included on each run. All patients positive for the FV mutation were confirmed by repeating the PCR and digestion at least twice.

Statistics

Categorical data were compared by Chi-square or Fisher's exact test, means by unpaired t-test and medians by Mann Whitney U-test using Instat Computer Software. Assuming that the inheritance of one FV506Q allele in the general population is reported to give an odds ratio (OR) for venous thrombotic risk of between 5–10, at a z of 0.05 and b of 0.1, we would require 57 (OR = 5) or 19 (OR = 10) patients in each group respectively to have a 90% probability of avoiding a type II error.

Results

Demographic details of the two groups are recorded in Table 1. The two patient groups did not differ according to age, sex, diagnosis, or progression to end stage renal failure. Two patients were heterozygous for the FV506Q mutation (prevalence 6%), both of whom had membranous nephropathy. The distribution of FV Leiden genotype and RVT is shown in Table 2. There was no significant difference in the distribution of the FV506Q mutation according to presence or absence of

<table>
<thead>
<tr>
<th>Sex (M:F)</th>
<th>RVT+</th>
<th>RVT–</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54 ± 12</td>
<td>51 ± 13</td>
</tr>
<tr>
<td>FSGS¹</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Not biopsied</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>ESRF²</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

¹FSGS = Focal and segmental hyalinosis and sclerosis.
²ESRF = End-stage renal failure.

Table 2. Prevalence of factor V Leiden mutation (FV506Q) and presence (+) or absence (−) of renal vein thrombosis (RVT) in 35 patients with nephrotic syndrome

<table>
<thead>
<tr>
<th>FV506Q</th>
<th>RVT+</th>
<th>RVT–</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV506Q</td>
<td>1</td>
<td>1¹</td>
<td>2</td>
</tr>
<tr>
<td>FV506R</td>
<td>9</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>Prevalence</td>
<td>10%</td>
<td>4%</td>
<td>6%</td>
</tr>
</tbody>
</table>

¹This patient suffered a separate venous thrombotic event. P = 0.5 Fisher’s exact test.
Discussion

This retrospective study shows that the prevalence of the FV Leiden mutation in a selected group of patients with nephrotic syndrome was 6%, consistent with the prevalence reported in other studies drawn from European and North American populations, and with a further large cohort of patients with chronic renal disease attending the same renal unit. The prevalence did not differ significantly between the patients with or without renal vein thrombosis. The FV Leiden mutation is the most common genetic risk for venous thrombosis and in selected populations with deep venous thrombosis or pulmonary embolism, heterozygosity for this mutation confers an increased risk of venous thrombosis of between 5–10 fold, and homozygosity 50–100 fold [10]. In healthy men followed in a prospective study the FV Leiden mutation conferred an increased risk of primary but not secondary venous thrombosis and predicted increased risk of recurrent thrombosis [15]. The majority of patients heterozygous for this mutation may however, suffer no adverse event unless an environmental precipitant is encountered such as surgery, pregnancy or the oral contraceptive [16]. The present study does not suggest that the FV Leiden mutation was significantly associated with the prevalence of renal vein thrombosis associated with nephrotic syndrome. Although one case report has identified the occurrence of lower limb venous thrombosis in a carrier of the mutation who presented with nephrotic syndrome [13], the relatively high prevalence of the FV Leiden mutation in the general population suggests that this association will be likely to occur by chance alone. The finding that both patients who carried the FV Leiden mutation suffered a venous thrombosis (native or renal allograft vein thrombosis), is consistent with evidence suggesting heterozygosity for the FV Leiden mutation is a risk factor for venous thrombosis, but the lack of an association with the majority of thrombotic events in this study argues that the acquired abnormalities of coagulation and fibrinolysis associated with nephrotic syndrome are of greater relevance. However, several limitations of this study require caution in interpretation of these results. It is possible that in a retrospective study, selective loss of patients with the FV506Q may underestimate this risk, however no study in the general population has identified this mutation as associated with increased risk of death, indeed the high prevalence of this mutation suggests otherwise [17]. It is possible a true association was not detected because the power of this study was insufficient, or because subclinical thrombosis, (which was not actively sought) was not detected and patients were misclassified. Only a larger (probably multicentre) study can address the issue of power, but misclassification appears unlikely to influence the results because the prevalence of the FV506Q allele was not increased in either patient group when compared with a large chronic renal disease control group nor reported prevalence studies. Whilst precise matching of severity and duration of nephrosis was impossible, a similar proportion developed end-stage renal failure suggesting severity of disease was approximated, and nine of the patients had evidence of RVT at presentation, suggesting risk of clinical events was maximal early in the disease course.

Screening for additional coagulation abnormalities, and correlation with functional evaluation for APC resistance was not undertaken in this study due to the uncertainty regarding potential confounding by the heterogeneity of current clinical status including continuous anticoagulation, resolution or progression of disease status, or great temporal dislocation from the clinical event which would not allow meaningful retrospective comparison between patients. Co-inheritance of associated mutations in the genes for protein C or S for example has been shown to significantly increase the risk of clinical events in familial thrombosis in patients with APC resistance [10], but as none of the patients in this study had a familial history of venous thrombosis, the presence of additional mutations would be unlikely. In this study, genotyping for the factor V506Q mutation had the advantage of independence from transient and changing environmental factors measured at varying intervals from the clinical event and indicated a life-long estimate of venous thrombotic risk.

Whether patients with venous thrombosis, or populations at risk, should be screened for the FV Leiden mutation is being vigorously debated [18,19]. Evidence is accumulating that this mutation contributes significantly to venous thrombotic risk in the general population. Information derived from screening gives a lifelong index of thrombotic risk, may assist in decision making regarding length of anti-coagulation and risk of recurrence, and can be undertaken whilst the patient is anti-coagulated. Additional studies examining the FV506Q mutation in differing disease states associated with thrombosis, and in case reports [20], will continue to generate information regarding its pathological consequences, indications for screening and therapeutic interventions under different circumstances. Whilst this study does not suggest a significant role for the FV506Q mutation and risk of RVT in nephrotic syndrome, screening patients may still be worthwhile because it is relatively simple, inexpensive and may
give additional information regarding risk of recurrence and continuing risk after resolution of nephrosis for example. Screening selected patients such as those with a family history of venous thrombosis, those with progressive renal failure where transplantation is considered, or those with recurrent thrombosis may also be important. Confirmation of these results and determination of additional information such as whether carriers of this mutation may have a more severe thrombotic state, earlier presentation, greater complications or a worse outcome requires evaluation within a larger prospective study.

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References


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