of intron 4 leads to the formation of a new acceptor splice site. Among the previously unreported mutations that we found, the Y-31 nonsense mutation, the TT insertion at L-12 and R164, the C deletion at L81, and the T-G transversion at K176. The deletion at L81 in exon 2 (delC) leads to a change of the corresponding amino acid and to the formation of a premature stop codon (CCC-CTG-AGT→CCC-TGA-GTA). The heterozygous insertion at L-12 found in exon 2 (T2430insTT) is predicted to change all corresponding amino acids encoded by the subsequent portion of the exon 2 leading to a premature stop codon within the exon 2 at position -2. Although the K176T-G transversion causes a lysine to asparagine substitution, this occurs at the invariant AG of the exon at the acceptor site and is likely to affect correct mRNA splicing as in the related AAG to AAA mutation.

In family 6 the L81delC occurred within a codon in which two different small deletions (2706delT and 2705-6delCT) have been previously reported. This codon is bridged by a homonucleotide tract (CCCCC), a well-known recognized hot spot consensus sequence for point mutations. The other 2 unrecognized mutations were missense substitutions, which occurred in exon 4 (P321S) and in exon 5 (V364D). The P321 is located in the turn between strand 2b and strand 3b and is a highly conserved residue among serpins. The V364 residue is located in the z-sheet A, at the bottom of strand 5A, and, in serpins, may be occupied by valine or leucine, all non-polar, aliphatic amino acids. In contrast, aspartic acid is a charged amino acid, which disrupts secondary structures, the H-bonding side chain competing directly with backbone H-bonds. Thus, it is conceivable that both substitutions impair correct AT folding and, in turn, structural integrity. The number of different mutations found in this mutation screen of only 8 families confirms the marked genetic heterogeneity in this group of patients and also suggests that the AT gene is hypermutable, most mutations arising randomly and often being responsible for truncated proteins.

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Key words: antithrombin, gene, mutations, thrombosis.

Letters to the Editor

Thrombosis

High factor VIII plasma levels as a risk factor for venous thrombosis: no evidence of inheritance from a family cohort study

We assessed inheritance of high factor VIII:C (FVIII:C ≥150%) levels in 52 probands carrying factor V Leiden and 176 first-degree relatives. Although age-adjusted FVIII:C levels aggregated within families, relatives of probands with FVIII:C levels ≥150% and <150% were comparable regarding median FVIII:C levels, cumulative distribution curves, and annual incidence of venous thromboembolism. Aggregation of high FVIII:C levels could not be explained by inheritance and was not predictable from FVIII:C levels in probands.

References


Table 1. Characteristics of 52 probands with factor V Leiden and FVIII:C levels ≥ 150% and < 150%, and their 176 first-degree relatives.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 probands (FVIII:C ≥150%)</th>
<th>Group 2 probands (FVIII:C &lt;150%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. of probands</td>
<td>24</td>
<td>28</td>
<td>0.58</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>11 (46)</td>
<td>21 (75)</td>
<td>0.03</td>
</tr>
<tr>
<td>Median age at study entry, yrs (range)</td>
<td>46 (22-70)</td>
<td>38 (19-78)</td>
<td>0.13</td>
</tr>
<tr>
<td>FVIII:C (%), median (range)</td>
<td>189 (150-270)</td>
<td>125 (49-146)</td>
<td>-</td>
</tr>
<tr>
<td>N. of relatives</td>
<td>88</td>
<td>88</td>
<td>1.0</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>46 (52)</td>
<td>45 (51)</td>
<td>0.88</td>
</tr>
<tr>
<td>Women on oral contraceptives, n (%)</td>
<td>13 (28)</td>
<td>12 (27)</td>
<td>0.87</td>
</tr>
<tr>
<td>FV Leiden carriers n (%)</td>
<td>59 (67)</td>
<td>59 (67)</td>
<td>1.0</td>
</tr>
<tr>
<td>Median age at study entry, yrs (range)</td>
<td>42 (15-78)</td>
<td>45 (14-78)</td>
<td>0.47</td>
</tr>
<tr>
<td>FVIII:C ≥150%, n (%)</td>
<td>36 (41)</td>
<td>36 (41)</td>
<td>1.0</td>
</tr>
<tr>
<td>FVIII:C [%], median (range)</td>
<td>138 (64-318)</td>
<td>136 (47-275)</td>
<td>0.34</td>
</tr>
<tr>
<td>in parents</td>
<td>146 (104-300)</td>
<td>159 (47-236)</td>
<td>0.95</td>
</tr>
<tr>
<td>in brothers/sisters</td>
<td>136 (64-240)</td>
<td>132 (76-275)</td>
<td>0.51</td>
</tr>
<tr>
<td>in children</td>
<td>135 (94-318)</td>
<td>126 (62-176)</td>
<td>0.23</td>
</tr>
<tr>
<td>in relatives with VTE</td>
<td>160 (82-300)</td>
<td>132 (47-244)</td>
<td>0.40</td>
</tr>
<tr>
<td>in relatives without VTE</td>
<td>136 (64-318)</td>
<td>136 (62-275)</td>
<td>0.47</td>
</tr>
<tr>
<td>VTE, n (%)</td>
<td>8 (9)</td>
<td>11 (13)</td>
<td>0.48</td>
</tr>
<tr>
<td>Annual incidence of VTE, % (95% CI)</td>
<td>0.35 (0.15-0.70)</td>
<td>0.48 (0.24-0.86)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

A delayed decline in probands with high FVIII:C levels may be supposed, as measurements were not repeated, but does not explain high levels in asymptomatic relatives. Comorbidity, possibly associated with high FVIII:C levels, was reported in 3/52 probands and 8/176 relatives. Only one of these probands and one relative had FVIII:C levels ≥150%.

Selection bias is a potential source of selection bias. Since 67% of relatives carried factor V Leiden, whereas the expected percentage would be 50%, some selection or referral bias cannot be excluded. However, the prevalence of VTE was comparable in included (10.8%) and excluded relatives (8.6%) (p = 0.44), factor V Leiden was equally distributed among the two groups of enrolled relatives, and median FVIII:C levels were comparable in factor V Leiden carrier-relatives (136%, 47-318) and non-carriers (146%, 82-290) (p = 0.14). Moreover, selection would have influenced the estimated incidence of VTE rather than the assessment of inheritability. Adjustment of FVIII:C levels for ABO blood groups (not tested) might have changed the results, but variations related to blood group cannot explain the observed wide range of FVIII:C levels within families. Besides, a previous study showed that the risk of VTE associated with high FVIII:C levels is blood group independent.

![Figure 1. Cumulative distribution curves of FVIII:C levels in first-degree relatives of probands with factor V Leiden and FVIII:C levels ≥150% (— — —) and relatives of probands with factor V Leiden and FVIII:C levels < 150% (••••).](image-url)
Letters to the Editor

In conclusion, our data suggest familial aggregation of high FVIII:C levels, but provides no evidence of inheritance. FVIII:C levels in symptomatic GV Leiden carriers did not enable us to select families in which screening would identify relatives at high risk, due to the concomitance of GV Leiden and high FVIII:C levels.

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Key words: inheritance, factor VIII, venous thrombosis, factor V Leiden.

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Stem Cell Transplantation

Immunological reconstitution after autologous peripheral blood stem cell transplantation in patients with chronic lymphocytic leukemia. Comparison with an historical non-Hodgkin’s lymphoma group

The kinetics of immune reconstitution after autologous peripheral blood stem cell transplantation (APBSCT) was examined in a group of 6 patients with chronic lymphocytic leukemia (CLL) and compared to that in an historical group of 12 patients with non–Hodgkin’s lymphoma (NHL). Lymphocyte analysis included total lymphocyte count, CD3, CD4, CD8, CD4/8 ratio, CD19, and CD16/56 and 1 year before and after transplantation. Immunological recovery in the CLL group was similar to that in the NHL group.

References


Immunological reconstitution after autologous peripheral blood stem cell transplantation in patients with chronic lymphocytic leukemia. Comparison with an historical non-Hodgkin’s lymphoma group

T-cell dysfunction has been described in CLL and could contribute to both the etiology and the progression of the disease.1 High dose therapy and stem cell transplantation is increasingly being used for the treatment of CLL.2 Despite the fact that many data about lymphocyte recovery after unselected PBSCT or selected CD34+ PBSCT have been published so far in lymphoproliferative disorders such as Hodgkin’s lymphoma, non-Hodgkin’s lymphoma, and multiple myeloma, no data are so far available about lymphocyte recovery after unselected PBSCT for chronic lymphocytic leukemia.3-5

Between May 1999 and April 2002, six pretreated adult patients affected by chronic lymphocytic leukemia underwent APBSCT. These patients were compared to 12 patients affected by NHL who were autografted at our institution during the same period. All patients received unselected peripheral blood autografts.

CLL patients were conditioned with MitMel (mitoxantrone 60 mg/m² on day –5 and melphalan 180 mg/m² on day –2). The median number of CD34+ cell infused was 2.57×10⁶/kg. Patients received granulocyte colony-stimulating factor (G-CSF) starting on day 7, until a stable ANC (ANC > 0.5×10⁹/L) was achieved for 3 consecutive days. In the control group eight patients were conditioned with BuMel (busulfan 16 mg/kg, from day –6 to –3 and melphalan 140 mg/m² on day –2), two patients with BuCy2 (busulfan 16 mg/kg, from day –7 to –4 and cyclophosphamide 60 mg/kg on day –3 and –2) and two with BEAM (BCNU 300mg/m² on day –7, etoposide and aracyn 200 mg/m² on days –6 to –3, and melphalan 140 mg/m² on day –2). The median number of CD34+ cells infused was 5.8×10⁶/kg. Only eight patients received G-CSF, which was started on day +7, and continued until a stable ANC > 0.5×10⁹/L was achieved for 3 consecutive days.

All results are expressed as median values. A difference was defined as statistically significant when p < 0.05. Differences between the study groups were analyzed using the Mann-Whitney U-test. A median of 22×10⁶/kg and 31.4×10⁶/kg (p = ns) of CD3+ T lymphocytes, and a median of 2.48×10⁶/kg and 0.84×10⁶/kg (p = ns) of CD19+ B lymphocytes were reinfused into the CLL group and the NHL group, respectively. The CLL patients achieved a stable ANC > 0.5×10⁹/L and platelet count > 200×10⁹/L at day 13 and day 14, respectively. In the NHL group neutrophil and platelet engraftment was achieved on day 13 and day 12, respectively. An absolute lymphocyte count (> 0.5×10⁹/L) was obtained on day 30 in the CLL group and on day 18 in the NHL group (p = 0.04). Table 1 shows the patients’ characteristics and clinical outcome.

The absolute lymphocyte count increased thereafter in