of intron 4 leads to the formation of a new acceptor splice site.[5] Among the previously unreported mutations that we found, 4 are likely to give rise to null alleles: 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.[7]

In family 6 the L81delC occurred within a codon in which two different small deletions (2706delT and 2705-6delCT) have been previously reported.[8,9] This codon is bridged by a homonucleotide tract (CCCC), a well-known recognized hot spot consensus sequence for point mutations.[10] 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 β-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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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.

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High FVIII:C levels have been recognized as a risk factor for venous thromboembolism (VTE). Only little is known about their inheritability. Polymorphisms have not been identified. Differences in FVIII:C levels related to ABO blood groups support a genetic contribution.[1] In a female twin study this contribution was 61%.[2]

We analyzed data from a previous family cohort study, designed to estimate the absolute risk of VTE in factor V Leiden carriers.[3] Consecutive patients with VTE and factor V Leiden (probands) and their first-degree relatives older than 15 years were enrolled.

Genetic effects on FVIII:C levels were assessed by calculation of Pearson correlations between residuals (i.e. differences between observed levels and expected levels as esti-

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

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

mated from a multiple regression model with age as an independent variable) in various pairs of relatives. Familial aggregation was established if the weighted sum of correlations (Q) between pairs of relatives within families was larger than its expected value.[4,5] Relatives of probands with FVIII:C levels ≥150% and <150%, were compared with respect to the proportion of high FVIII:C levels, FVIII:C distribution and annual incidence of VTE.

Of 52 probands, 24 had FVIII:C levels ≥150% (group 1) and 28 levels <150% (group 2). Each group contained 88 first-degree relatives (Table 1). Age, sex, women on oral contraceptives, factor V Leiden, and FVIII:C levels ≥150% were equally distributed among relatives in both groups. FVIII:C levels, overall and in subgroups, were comparable, as was the annual incidence of VTE. Cumulative distribution curves of FVIII:C levels in both groups overlapped almost completely (Figure 1).

Age-adjusted FVIII:C levels were correlated in 37 father-son pairs (r=0.41, $p = 0.01$) and 69 sister-sister pairs (r=0.37; $p = 0.002$), but not in 13 other pairs. These levels aggregated within families; the calculated and expected Q values were 289 and 223, respectively ($p = 0.01$). Both correlations of FVIII:C levels in some pairs of relatives, though weak, and familial aggregation of FVIII:C levels in this study support possible inheritability of FVIII:C. Similar findings were reported from a previous study in 12 factor V Leiden families, containing 182 first- and second-degree relatives.[6] Familial aggregation remained after adjustment for ABO-blood groups and age. Another study showed familial clustering of high FVIII:C levels in 77 relatives of 17 symptomatic patients with FVIII:C levels > 200%.[7] However, aggregation was not assessed in relatives of symptomatic patients with lower FVIII:C levels. High FVIII:C levels in our probands did not enable us to identify families with high FVIII:C levels. Actually, high FVIII:C levels were equally distributed among relatives of probands with FVIII:C levels ≥150% and relatives of probands who had lower FVIII:C levels. Because FVIII:C levels were measured at least 3 months after VTE, it is less likely that the division of probands into two groups reflected differences in acute phase response rather than their FVIII:C baseline levels. 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%.

A limitation of our study is the incompleteness of families, 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 bloodgroups (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.[8]

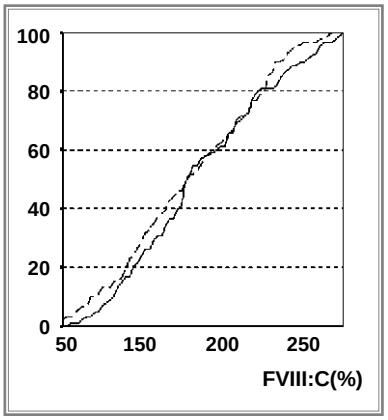

Figure 1. Cumulative distribution curves of FVIII:C levels in first-degree relatives of probands with factor V Leiden and FVIII:C levels ≥150% (— — —) and relatives of probands with factor V Leiden and FVIII:C levels < 150% (- - - -).

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

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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 counts before, on day +15, +30, +60, +90, and +120 and 1 year after transplantation. Immunological recovery in the CLL group was similar to that in the NHL group.

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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.[3-7]

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/m^2 on day –5 and melphalan 180 mg/m^2 on day –2). The median number of $CD34^+$ cell infused was 2.57×10^6/kg. Patients received granulocyte colony-stimulating factor (G-CSF) starting on day +7, until a stable absolute neutrophil count (ANC) > 0.5×10^9/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/m^2 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/m^2 on day –7, etoposide and aracytin 200 mg/m^2 on days –6 to-3, and melphalan 140 mg/m^2 on day –2). The median number of $CD34^+$ cells infused was 5.8×10^6/kg. Only eight patients received G-CSF, which was started on day +7, and continued until a stable ANC > 0.5×10^9/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 22×10^6/kg and 31.4×10^6/kg (p = ns) of $CD3^+$ T lymphocytes, and a median of 2.48×10^6/kg and 0.84×10^6/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 > 0.5×10^9/L and platelet count > 20×10^9/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 (> 0.5×10^9/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