Evidence for Genetic Homogeneity in a Familial Platelet Disorder With Predisposition to Acute Myelogenous Leukemia (FPD/AML)

To the Editor:

Although familial thrombocytopenias have been described in the literature since the early 1960s, they are a rare and heterogeneous group of disorders characterized by varying modes of inheritance. A number of autosomal dominant thrombocytopenias have been described, but their rarity has limited their detailed clinical and genetic analyses. In three kindreds to date, a unique autosomal dominant thrombocytopenia has been described with a predisposition to acute myelocytic leukemia termed familial platelet disorder-acute myelocytic leukemia (FPD-AML). A potential FPD-AML gene locus was recently mapped to the long arm of chromosome (21q22.1–2) by linkage analysis in one family. We now extend these observations by reporting a new kindred with a similar phenotype, which maps to an overlapping chromosomal region, suggesting that a defect in a single gene may underlie the FPD-AML phenotype.

The FPD-AML pedigree involves three generations of family with five affected individuals of mixed Czechoslovakian and Hungarian background (see Fig 1). Clinical history and blood specimens were available from 11 available family members, each of whom consented to participate in this study. There was no history of consanguinity in this family.

The proband (III-1) presented at the age of 4 years for hypospadias repair. He had easy bruising since infancy and was found preoperatively to have a platelet count of 106,000/µL. His family history was notable for his mother (II-2), at 41 years of age, developed progressive anemia and neutropenia and was shown to have a myelodysplastic syndrome (refractory anemia). Within 6 months of diagnosis of myelodysplasia, increased bone marrow blast counts above 30% were noted. Cytogenetics were normal, as were fluorescent in situ hybridization (FISH) studies for monosomy 5 and 7. Immunohistochemical typing of her blasts confirmed acute myelogenous leukemia, FAB-M1.

Because of the previously reported linkage of FPD-AML to chromosome 21q22 in one family, candidate locus linkage analyses were performed. Genotyping and two-point lod scores at 10 precisely mapped chromosome 21 polymorphic marker loci showed evidence for...
linkage at marker loci overlapping the previously defined FPD-AML region (eg, maximum two-point lod score at D21S65 = 1.682). These findings were extended by multipoint analyses, where the putative disease gene locus was placed at each map interval between D21S263 and D21S211. Maximum lod scores greater than 3 were obtained at each map interval (Table 1). Lastly, there was no evidence for allele sharing between this kindred and the French-Canadian pedigree previously reported, confirming distinct ancestry.

These results support the hypothesis that an inherited mutation in a gene mapping to the previously defined FPD-AML locus caused inherited thrombocytopenia and predisposition to AML in this kindred. Therefore, these data provide additional evidence for a singular genetic defect underlying the clinical phenotype of FPD-AML. Significantly, our results corroborate clinical and genetic data for the existence of a gene on chromosome 21q that is critical to normal hematopoiesis, and when dysregulated, may result in acquired hematologic disorders, such as myelodysplasia or AML. Candidate genes in the FPD-AML locus include: acute myelogenous leukemia (AML1), the interferon-α/β receptor (IFNAR), the Down syndrome critical region 1 (DSCR1), phosphoribosylglycinamide formyltransferase gene (GART), SON, and the cytokine receptor 2-4 (CRF2-4) (http://www.ncbi.nih.gov/Science 96). The AML-1 gene and DSCR1 region are of particular interest because of well-established associations with translocations (chromosome 8;21) in acute myelogenous leukemia, FAB-M2, involving the AML-1 gene, and childhood megakaryocytic leukemia, FAB-M7, in Down syndrome.6,7

Presently, the concurrent risk factors for developing AML in affected individuals is unknown. The risk of developing leukemia from this disorder appears to increase with age, and carries an overall lifetime risk of ~30%.5 It may well be that a single defective FPD-AML allele results in thrombocytopenia, while accumulation of a defect in the second allele results in the development of the myelogenous leukemic state. Because our findings support a unique genetic locus linked to a predisposition for AML in a subset of families with inherited thrombocytopenia, we believe that genetic screening evaluating linkage patterns and counseling should be offered for kindreds with familial autosomal dominant thrombocytopenia. Thorough family histories should be recorded in patients carrying the diagnosis of childhood chronic ITP and

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<th>Table 1. Multipoint Linkage Analysis</th>
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<td>Gene Order</td>
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<tr>
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<tr>
<td>D21S263-FPD-AML</td>
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<td>D21S263-D21S1413-FPD-AML</td>
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<td>D21S263-D21S1413-D21S216-AML</td>
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<td>D21S263-D21S1413-D21S216-D21S65-AML</td>
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All linkage analyses were undertaken assuming that FPD-AML is caused by a mutation in a single diallelic autosomal dominant gene with a population frequency of 0.0001, and that the phenotype is incompletely penetrant (90% lifetime probability of manifesting disease in carriers, and no chance of manifesting disease in noncarriers). The map-specific multipoint lod scores above were calculated at each marker interval between D21S263 and D21S211 using the MLINK and LINK-MAP programs as implemented in FASTLINK v 2.3P.5 These analyses assumed 1 cM distance between markers (except 2 cM between D21S65 and D21S211) and that there was no interference or sex differences in the recombination fractions. The five recombination fractions were estimated from the genotype data and indicate that there was only one recombinant haplotype (in one unaffected individual).
in patients with myelogenous leukemia to identify additional families with FPD-AML with its risk of AML, so that these families can be monitored for the risk of developing myelodysplasia or AML.

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REFERENCES

Human RhD\(\text{el}\) Is Caused by a Deletion of 1,013 bp Between Introns 8 and 9 Including Exon 9 of RHD Gene

To the Editor:

The Rh system is genetically controlled by two different but highly homologous genes on chromosome 1p34-36. The RHCE gene encodes a different RHCEe peptide and the RHD gene encodes the D polypeptide, and there are a large number of antigenic polymorphisms between these two peptides. Of these, the RhD\(\text{el}\) is characterized as RhD by using a conventional serological test, but it does show absorption and elution of anti-D. The molecular basis of RhD\(\text{el}\) is not known.

The blood of 21 D\(\text{el}\) (21.6%) of 102 serological RHD patients was obtained after an absorption and elution test. A modified polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method based on the polymorphisms between RHCE and RHD genes was used to analyze the D\(\text{el}\) gene structure, and the results showed that there was no difference between the RhD and RhD\(\text{el}\) gene except that at the BspHI site of exon 9, the D\(\text{el}\) gene lacked the BspHI site that was similar with the RHCE gene. Haplotyping by \(\text{SpI}\) bands showed no gross difference between RHD and RhD\(\text{el}\) genes (data not shown).

To further characterize the D\(\text{el}\) gene and its expression, a nested reverse transcriptase-polymerase chain reaction (RT-PCR) method was used to amplify the different region between RHD and D\(\text{el}\) genes. The RNA was extracted from red blood cells and white blood cells, and the RT-PCR was performed as described. The first PCR amplified the RHD or D\(\text{el}\) gene from exon 7 to exon 10 region (using upstream primer \(\text{P}_{1}: 5’-\text{TCCCAACGGCTCACTAGGG-3’}\) and downstream primer \(\text{P}_{2}: 5’-\text{GATACATGACTGACATATAAAATGGTG-3’}\), both are RHD gene-specific primers). The PCR products were subjected to nested-PCR: using an RHD specific upstream primer \(\text{P}_{1}: 5’-\text{GATACATGACTGACATATAAAATGGTG-3’}\), and downstream primer \(\text{P}_{2}: 5’-\text{CTGTCCAGGAGACACAGCGTG-3’}\) to amplify part of exon 7 and exon 8; using \(\text{P}_{1}\) and downstream primer \(\text{P}_{3}: 5’-\text{CTTCCAGAAAACCTTGTCATC-3’}\) to amplify from exon 7 to exon 9; using primer \(\text{P}_{1}\) and \(\text{P}_{4}\) to amplify from exon 7 to exon 10. The results showed that the D and D\(\text{el}\) genes were similar at exon 7 and 8, but there was no nested RT-PCR product for D\(\text{el}\) gene for the primers \(\text{P}_{1}\) and \(\text{P}_{8}\), and the nested PT-PCR product of D\(\text{el}\) gene for the primers \(\text{P}_{1}\) and \(\text{P}_{8}\) was shorter than the product of normal D gene. Direct sequencing of the nested RT-PCR products of D and D\(\text{el}\) genes showed that there was an exon 9 deletion of D\(\text{el}\) gene (Fig 1A).

To characterize the breakpoint of D\(\text{el}\) gene, a nested PCR method was used to amplify the breakpoint region. For the first PCR, an RHD gene-specific downstream primer (primer \(\text{P}_{8}\) ) and a nonspecific upstream primer (5’-GATTGGCTTCAGGTCCTCC-3’) were used to amplify part of the RhD or RhD\(\text{el}\) gene from exon 8 to 3’ noncoding region using genomic DNA. For the second PCR, two nonspecific Rh gene primers were used (upstream primer 5’-TCAGCATTGGGAACT-CAGC-3’, and downstream primer: 5’-GCTTTGCTTCTTCTGGATG-3’) to amplify from part of exon 8 to exon 10. The PCR products were subjected to direct sequencing or subcloning sequencing analysis. The results showed that the D\(\text{el}\) had a 1,013 bp deletion between introns 8 and 9, including whole exon 9 (Fig 1B).

The D\(\text{el}\) gene transcript maintains a normal open reading frame and thus should encode a protein with 463 amino acid residues with a new C-terminal extension from codon 384 as compared with the normal D protein of 417 amino acid residues (Fig 2). Although the D\(\text{el}\) show some D activity after an absorption and elution test, a case of Rh- with D\(\text{el}\) activity patient was transfused with RhD blood did develop anti-D antibody by a traditional serological test several weeks after transfusion. From this point of view, RhD\(\text{el}\) should be recognized as a type of RhD, and whether the D\(\text{el}\) blood transfused to RhD\(\text{el}\) or other types of RhD cases will develop anti-D\(\text{el}\) antibody needs further study.

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