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Human RhD *el* Is Caused by a Deletion of 1,013 bp Between Introns 8 and 9 Including Exon 9 of RHD Gene

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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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Human RhD^{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 RhCcEe polypeptide 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^{el} is characterized as RhD$^-$ by using a conventional serological test, but it does show absorption and elution of anti-D.[1] The molecular basis of RhD^{el} is not known.

The blood of 21 D^{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^{el} gene structure,[2] and the results showed that there was no difference between the RhD and RhD^{el} gene except that at the *Bsp*HI site of exon 9, the D^{el} gene lacked the *Bsp*HI site that was similar with the RHCE gene. Haplotyping by *Sph* I bands showed no gross difference between RHD and RHD^{el} genes[3] (data not shown).

To further characterize the D^{el} gene and its expression, a nested reverse tranascriptase-polymerase chain reaction (RT-PCR) method was used to amplify the different region between RHD and D^{el} genes.[4] The RNA was extracted from red blood cells and white blood cells, and the RT-PCR was performed as described.[5] The first PCR amplified the RHD or D^{el} gene from the exon 7 to exon 10 region (using upstream primer P_0: 5′-TCCCCACAGCTCCATCATGGG-3′ and downstream primer P_4: 5′-GTATTCTACAGTGCATAATAAATGGTG-3′, both are RHD gene-specific primers). The PCR products were subjected to nested-PCR: using an RHD specific upstream primer P_1: 5′-CATTGTGCTGC-TGGTGCTTG-3′, and downstream primer P_2: 5′-CTGTCAGGAGAC-CAGACGTG-3′ to amplify part of exon 7 and exon 8; using P_1 and downstream primer P_3: 5′-CTTCCAGAAAACTTGGTCATC-3′ to amplify from exon 7 to exon 9; using primer P_1 and P_4 to amplify from exon 7 to exon 10. The results showed that the D and D^{el} genes were similar at exon 7 and 8, but there was no nested RT-PCR product for D^{el} gene for the primers P_1 and P_3, and the nested PT-PCR product of D^{el} gene for the primers P_1 and P_4 was shorter than the product of normal D gene. Direct sequencing of the nested RT-PCR products of D and D^{el} genes showed that there was an exon 9 deletion of D^{el} gene (Fig 1A).

To characterize the breakpoint of D^{el} gene, a nested PCR method was used to amplify the breakpoint region. For the first PCR, an RHD gene-specific downstream primer (primer P_4) and a nonspecific upstream primer (5′-GATTGGCTTCCAGGTCCTCC-3′) were used to amplify part of the RhD or RhD^{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′-TCAGCATTGGGGAACT-CAGC-3′, and downstream primer: 5′-GCCTTGTTTTTCTTGGATG-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^{el} had a 1,013-bp deletion between introns 8 and 9, including whole exon 9 (Fig 1B).

The D^{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^{el} show some D activity after an absorption and elution test, a case of Rh$^-$ with D^{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^{el} should be recognized as a type of RhD$^-$, and whether the D^{el} blood transfused to RhD$^+$ or other types of RhD$^-$ cases will develop anti-D^{el} antibody needs further study.

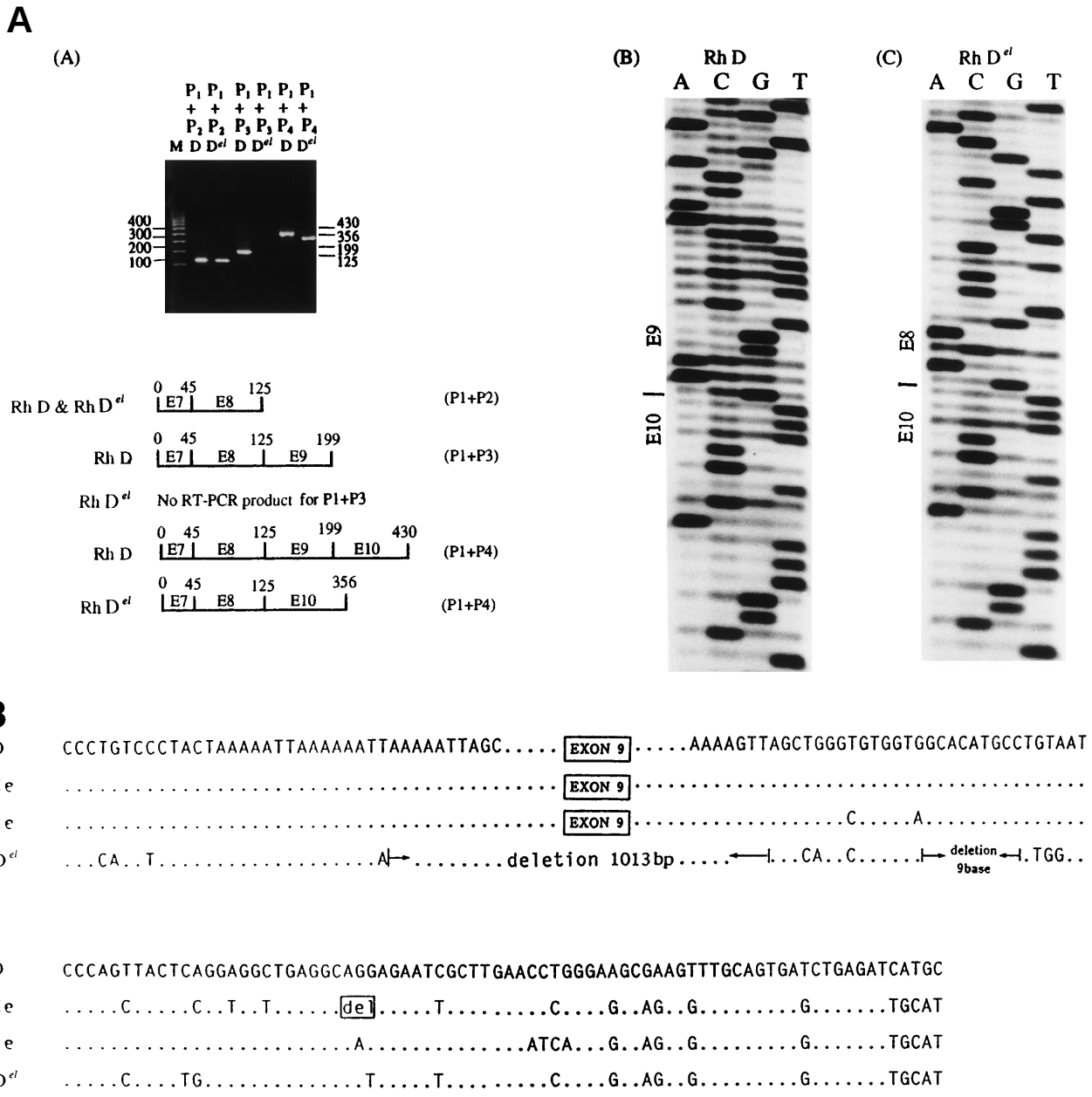

Fig 1. **(A) By using different pairs of RH D specific primers and nested RT-PCR to amplify the RH Del gene, there was a deletion of exon 9 of RH Del gene (A). The results were further confirmed by direct sequencing of the nested RT-PCR product (B) and (C). The sequences of primers P1, P2, P3 and P4 are shown in the text. (B) The breakpoint region sequence of Del in comparison with D, Ce, and ce alleles.**

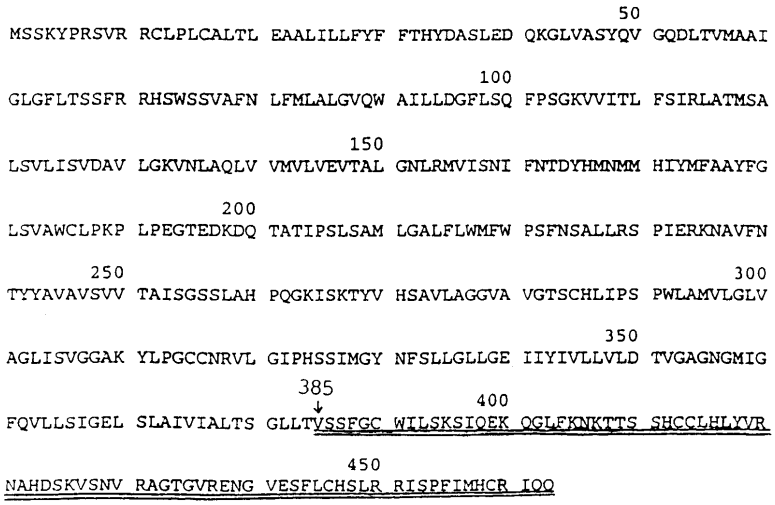

Fig 2. Amino acid sequence of RH Del are shown. The amino acids of Rh Del are different from RH D gene after codon 384, and there are 46 amino acids more in Rh Del protein.

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