Human RhD<sup>e</sup> 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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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 RhD2 polypeptide, and there are a large number of antigenic polymorphisms, a different RhCcEe polypeptide and the RHD gene encodes the D 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.

The gross difference between RHD and D el genes is caused by a deletion of 1,013 bp between introns 8 and 9 including exon 9 of RHD gene.

To further characterize the 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 P1) and a nonspecific upstream primer (5’-GA TTGGCTTCCAGGTCCTCC-3’), and the nested RT-PCR product of D el gene lacked the BspHI site that was similar with the RHCE gene. Haplotyping by SpH I bands showed no gross difference between RHD and RHD el genes (data not shown).

To further characterize the D 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 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 el gene from exon 7 to exon 10 region (using upstream primer P1: 5’-CTTCCAGAAACCTGTGCATC-3’ to amplify from exon 7 to exon 9; using primer P1 and P4 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 P1 and P3, and the nested PT-PCR product of D el gene for the primers P1 and P2 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 P1) and a nonspecific upstream primer (5’-GATTGGGTCCTCCAGTGTCCTC-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 RHD gene primers were used (upstream primer 5’-TCAGCATGAGGAACTCAGC-3’, and downstream primer: 5’-GCCTGTITITTTCTTGGATG-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).

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 RhD2 is characterized as RhD el by using a conventional serological test, but it does show absorption and elution of anti-D. The molecular basis of 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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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.
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