Molecular genetic analysis for the $B^3$ allele

Lung-Chih Yu, Yuh-Ching Twu, Ming-Lun Chou, Ching-Yi Chang, Chia-Ying Wu, and Marie Lin

Molecular genetic analysis of 14 samples from unrelated individuals with the $B^3$ phenotype is reported here. Two different molecular changes in the blood group $B$ gene were observed. One case was demonstrated to possess a $247G \rightarrow T$ mutation, which predicts an Asp37Tyr alteration. The $B$ genes of the other 13 cases were shown to have a $G \rightarrow A$ mutation at the $+5$ nucleotide of intron 3 (intervening sequence 3 [IVS3] + 5G $\rightarrow$ A). Reverse transcription polymerase chain reaction analysis showed that the complete exon 1–exon 7 $B$ transcript was absent, and transcripts that skipped exon 3 were instead present in the RNA sample from the $B_3$ individual with the IVS3 + 5G $\rightarrow$ A mutation. The result shows that the IVS3 + 5G $\rightarrow$ A mutation destroys the conserved sequence of the splice donor site and leads to the skipping of exon 3 during messenger RNA processing. The $B^3$ transcript without exon 3 predicts a $B$-transferase product that lacks 19 amino acids in the N-terminal segment. (Blood. 2002;100:1490-1492)

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individuals (Figure 2, lanes 2-13) had one allele with the IVS3 + 5G → A mutation at their ABO loci, as did the B3 propositus (lane 1), while none of the 30 group B individuals possessed the mutation (one of the results is shown in Figure 2, lane B). Further analysis demonstrated that all of the 12 B3 individuals with the IVS3 + 5G → A mutation were heterozygotes with one O allele as in the B3 propositus (data not shown). These results show that 13 of the 14 B3 individuals possess the B gene with the IVS3 + 5G → A mutation, while the mutation is virtually absent in the general group B population. One B3 individual did not possess the mutation in the B gene (Figure 2, lane 14).

One B3 individual possesses the B gene with 247G → T missense mutation

The ABO gene of the B3 individual without the IVS3 + 5G → A mutation was analyzed as described above. This B3 individual was shown to have a B/O phenotype, and a nucleotide change of 247G → T (translation initiation codon of ABO cDNA as nucleotides 1 to 3) was identified in the B gene. The 247 position locates in the exon 6 region, and the G → T mutation predicts an Asp83Tyr amino acid alteration. The nucleotide 247 position of the ABO genes of 30 group B individuals was inspected through PCR amplification and sequencing; none of them possessed a G → T mutation.

Exon 3 is skipped in the transcripts encoded from the B allele with the IVS3 + 5G → A mutation

As the IVS3 + 5G → A mutation changes the consensus sequence of a splice donor site (GT AGT),15-18 the transcript structures encoded from the B allele with the splice site mutation were inspected by reverse transcription PCR (RT-PCR). Two fragments (559 and 424 bp) were obtained from the RNA sample from the group B individual (Figure 3A, lane B). Direct sequencing of the products revealed that the larger fragment was composed of the complete B exon 2–exon 7 cDNA structure, while the smaller one had the same structure but without the exon 6 region. RT-PCR of the RNA of the B3 individual gave 2 smaller products (502 and 367 bp) (Figure 3A, lane B3). The 502-bp fragment was demonstrated to be the B exon 2–exon 7 structure with exon 3 skipped (Figure 3B), and the 367-bp fragment was the same structure without the exon 3 and exon 6 regions.

Although the B3 individual possesses a normal O3 allele, the O1 allele transcript was not detected in this RT-PCR analysis. This phenomenon is believed to result from a decreased stability of the O allele transcript.19 The presence of the transcripts without exon 6
The complete exon 1–exon 7 transcript of the B gene was shown to be virtually absent in the RNA of the B3 individual with the IVS3 + 5G → A mutation, and instead, both of the transcripts encoded from the B3 allele with the splice site mutation skipped exon 3. These results show that the IVS3 + 5G → A mutation in the B gene destroys the consensus of the splice donor site and thus leads to the skipping of exon 3 during mRNA splicing processes (Figure 4).

Exon 3 of the ABO gene comprises 57 bp, and the B3 transcript without exon 3 still retains the reading frame and predicts a protein product that lacks 19 amino acid residues in the N-terminal portion (Figure 5). The deleted segment of the 19 amino acids includes several residues of the predicted transmembrane domain of a normal B transferase. Whether this affects or changes the enzyme characteristic of the transferase is worth further investigation.

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References

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