Identification of a novel O allele in the Taiwanese population*

Ding-Ping Chen,1 Ching-Ping Tseng,2 Wei-Ting Wang,1 and Chien-Feng Sun1

1 Department of Clinical Pathology, Linkou Medical Center, Chang Gung Memorial Hospital, Taoyuan, and 2 Graduate Institute of Medical Biotechnology and School of Medical Technology, Chang Gung University, Taoyuan, Taiwan

Abstract. The ABO system is one of the major blood groups that have significant impact on blood transfusion and paternity testing. We have found a new ABO allele by analyses of the ABO genotype of the Taiwanese population. Exons 6 and 7 of the ABO gene were amplified by the polymerase chain reaction and analyzed by direct sequencing. The results indicated that the ABO gene in the Taiwanese population consists mainly of the A1, A1v, B, O1, and O1v alleles. In addition, a novel O allele designated as OTaiwan was identified that has G → T substitution at the nucleotide 801 of the O1 allele. The OTaiwan allele is inheritable, since it is also present in an offspring of the OTaiwan-carrying individual. The information presented herein is valuable for population research and for analyses of evolutionary lineage. (received 2 September 2003; accepted 21 November 2003)

Keywords: ABO gene, OTaiwan allele, direct sequencing, polymerase chain reaction

Introduction.

The ABO gene encodes a specific glycosyltransferase that plays an important role in the synthesis of ABO blood antigen [1]. Genomic analysis reveals that the ABO locus spans over 18 kilobases (kb) and consists of 7 exons, which range in size from 26 to 688 base pairs, with most of the coding sequences lying in exons 6 and 7 [2]. Several ABO genotyping methods, including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [3], allele-specific PCR [4], and PCR-based single-strand confirmation polymorphism [5], have been employed to facilitate analysis of ABO genetic polymorphism.

According to reports from several countries and populations, the distribution and frequencies of ABO alleles vary significantly [6-9]. Many rare alleles have been described, in addition to the common ABO alleles such as A1, A1v, B, O1, and O1v. In this study, we report a novel OTaiwan allele that was identified during the ABO genotype analysis of the Taiwanese population.

Materials and Methods

Specimens. Peripheral blood samples (N = 168) were drawn by venipuncture into EDTA tubes from blood donors at Chang Gung Memorial Hospital, Kweishen, Taoyuan, Taiwan.

PCR amplification and direct sequencing. Genomic DNA was extracted from each blood sample using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Because 91% of the ABO coding sequences lie in exons 6 and 7 [2], PCR-based gene analyses were performed on these two exons. Briefly, PCR was set up in a reaction volume of 50 µl containing 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, and 1.5 mM MgCl2), 10 nmol of dNTP, 6 pmol of forward and reverse primers (Table 1), 300 ng of genomic DNA, and 1 U of AmpliTaq Gold DNA polymerase (Applied

* The nucleotide sequences reported in this paper have been submitted to GenBank (accession number AY373436).
Biosystems, Foster City, CA). The reaction was performed in the GeneAmp PCR system 9600 (Applied Biosystems) with the following conditions: 1 cycle of 95°C for 10 min, 35 cycles of 94°C for 20 sec and 62°C for 30 sec, and 1 cycle of 72°C for 1 min. The final elongation step was 10 min at 72°C. Subsequently, 10 µl of PCR products were fractionated on 1.5% agarose gel and visualized by ethidium bromide staining. The remaining PCR product was used to carry out direct sequencing using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) and an ABI PRISM 377 Genetic Analyzer (Applied Biosystems) according to the manufacturer’s instructions.

Cloning of PCR product. The gel-purified PCR product was cloned into the pCRRII-TOPO vector by a Zero Blunt TOPO PCR Cloning kit (Invitrogen, Groningen, Netherlands). Briefly, 4 µl of fresh PCR product was mixed with 1 µl of TOPO vector for 5 min. Of the reaction mixture, 2 µl was used to transform the E. coli competent cells, as described by the manufacturer. DNA sequences of the PCR inserts were then determined using the Sequencing Kit (Applied Biosystems).

Results

To analyze the ABO genotype, we randomly selected 168 blood samples according to the ABO distribution ratios of the Taiwanese population. Hence, 40, 40, 80, and 8 samples of blood groups A, B, O, and AB, respectively, were included in this study. With leukocyte DNA as the template, exons 6 and 7 of the ABO gene were PCR amplified in 3 different reactions and subjected to DNA sequencing with the primers listed in Table 1.

The 168 samples were mainly homozygous or heterozygous for the 5 common ABO alleles (A1, A1v, B, O, and O1v). In one individual, a rare O allele with 1096G → A was identified, which has been reported previously [6]. In another individual of group O, a novel O allele (designated as OTaiwan) was identified when exon 7 was amplified by PCR (Fig. 1). To delineate the nature of OTaiwan, the PCR product was cloned into the Zero Blunt TOPO PCR cloning vector for sequencing.

DNA sequence analyses revealed that the pair of haplotypes for the two chromosomes/alleles under study consisted of OTaiwan and O1v. The O1v allele differed from O1 in five positions at nt 297, 646, 681, 771, and 829 [10]. The OTaiwan allele shared higher sequence homology with O1 and differed from O1 only at nt 801 with a G → T substitution (Fig. 2).

To confirm our finding, we obtained leukocyte DNA from an offspring of the OTaiwan allele-carrying individual for ABO genotyping. Sequence analysis revealed that the OTaiwan and B alleles were present in the ABO locus of this individual. These data indicate that the OTaiwan allele is inheritable and rule out possible PCR artifact or somatic mutation as the cause of the OTaiwan 801G → T nucleotide change.

Discussion

The ABO blood group has significant impact on medical practices such as blood transfusion and paternity testing. Understanding the ABO gene structure has facilitated the discovery of a variety of common and rare ABO alleles. For instance, molecular polymorphism of O alleles has been reported from 5 different ethnic groups in France [11]. A novel O(1) variant allele that is a combination

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences</th>
<th>Location</th>
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<tbody>
<tr>
<td>Exon 6F</td>
<td>5’-CCATGTTCAGAGGAACTGTC-3’</td>
<td>IVS5 +545 → Exon 6 +10</td>
</tr>
<tr>
<td>Exon 6R</td>
<td>5’-CATCTGGAGCGTTTGAATG-3’</td>
<td>IVS6 +323 → IVS6 +294</td>
</tr>
<tr>
<td>Exon 7F</td>
<td>5’-CGCCCTCAAGCTGCGT-3’</td>
<td>IVS6 +832 → IVS6 +839</td>
</tr>
<tr>
<td>Exon 7R</td>
<td>5’-AACACGGAGTGCTGAC-3’</td>
<td>Exon 7 +312 → Exon 7 +294</td>
</tr>
<tr>
<td>Exon 7-1F</td>
<td>5’-CGGTCTCCAACTATGTCCTGC-3’</td>
<td>Exon 7 +61 → Exon 7 +85</td>
</tr>
<tr>
<td>Exon 7-2R</td>
<td>5’-ACAACAGGACCAAAGGAAC-3’</td>
<td>IVS7 +104 → IVS7 +80</td>
</tr>
</tbody>
</table>
Fig. 1. Partial DNA sequences for the individual with $O_{Taiwan}$ alleles. Leukocyte DNA was PCR amplified with the primer pair exon 7-2F and exon 7-2R followed by sequencing with primer exon 7-2R. The graph for nt 789 to 813 of the ABO gene is shown. Arrow indicates the presence of double peaks: C/A at nt 801. Note that the DNA sequence is in reverse complimentary orientation.

Fig. 2. Partial DNA sequences for the 2 haplotypes of the individual with the novel O allele. The PCR product obtained as described in Fig. 1 was cloned into the Zero Blunt TOPO PCR cloning vector. Individual clone was selected for sequencing with primer exon 7-2R. The graph for nt 795 to 835 of the ABO gene is shown. Note that $O_{Taiwan}$ differed from $O_1$ at the nt 801 with a G to T substitution, and that $O_{1v}$ differed from $O_1$ at 5 positions, including nt 829 as the arrows indicates.
of exon 6 from an O(1v-3) allele and exon 7 from a B(1-1) allele has been identified and characterized [12]. Analyses of various phenotypes of the ABO blood group system in healthy Japanese individuals revealed more than 8 newly sequenced alleles [13].

In the present study, we identified a novel OTaiwan allele that has a G → T substitution at nt 801 in relation to the sequence of the O1 allele. We have shown that OTaiwan is inheritable and is not derived from PCR artifact, based on the analysis of the ABO locus of an offspring of the individual with OTaiwan allele. Similar to most reported O alleles [11,13], the 801G → T substitution of OTaiwan is downstream from the 261 delG site. It is not responsible for the formation of the resulting non-functional transferase. Hence, OTaiwan may constitute a polymorphic variant of the O allele. It is noteworthy that the OTaiwan mutation arises around the critical mutations known for enzyme specificity in the A and B alleles, and hence has potential importance for the development of genomic typing assays.

In this study, only 1 of the 168 blood samples has OTaiwan, so our results indicate that OTaiwan is uncommon in the Taiwanese population. The exact allele frequency of OTaiwan in the Taiwanese population and whether this allele is also present in other populations are matters that need to be ascertained. An RFLP method of rapid screening for the OTaiwan allele is under development.

In summary, this study has documented a novel O allele in the ABO blood group. Such information is valuable for population research and for analyses of evolutionary lineage among different populations.

Acknowledgments

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References