Molecular Polymorphism of O Alleles in the Chinese Han Population

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Abstract. The ABO blood group system is the most important in transfusion medicine. O blood group is common in Chinese Han people, but the distribution of various O alleles is unknown. Sequences of exon6 and exon7 of the O allele at the ABO gene locus were studied in 100 individuals of the O phenotype randomly selected from the Chinese Han population. Some samples, when required, were cloned and sequenced spanning exon6 and exon7. Eight O alleles were found in the Chinese population. Most have the 2 common O01 or O02 alleles. The allele frequency of ABO*O01 was 0.47, and that of ABO*O02 was 0.495. One individual was found to have O05 allele. Five alleles were found to differ from all alleles reported to date. Four of these alleles differed from either the O01 allele (1 out of 4) or O02 allele (3 out of 4) by 1 point mutation at A468G, G489A, T526C, or T1104G. The fifth allele differed from the O01 allele since it does not have nt261G deletion but has C467T mutation. This novel allele occurred in 2 individuals. O genetic analysis suggests that the O01 allele prevails, with O1v accounting for about 97% of these in the Chinese Han population. The O03 allele that has been shown to occur with a frequency of <5% in other populations was not detected. But the novel O allele without 261G deletion has been found in Chinese for the first time. Surely more O alleles will be found in the Chinese population.

Keywords: ABO blood-group system, O allele, genetic polymorphisms

Introduction

The ABO blood group is one of the main blood group systems that play an important role in transfusion medicine, transplantation practices, and paternity testing. To date, at least 56 different alleles O have been characterized (http://www.ncbi.nlm.nih.gov/projects/mhc/xslcgi.fcgi?cmd=bgmut/systems_info&system=abo). Exon6 and exon7 of the ABO gene encode 77% of the protein and 91% of the catalytically active part [1]. Because of that, over 40 of the alleles have been defined on the basis of exon6 and exon7 sequences, but not always for intron6. The mechanisms involved in the diversification of O alleles include interallelic exchange, nucleotide(nt) deletion, nt insertion, and nt substitution. The major O allele (O01) has a single nucleotide deletion at position 261 compared to the A101 allele, which results in a frame shift to create a different amino acid sequence and a stop codon at amino acid position 118, and thus the O allele encodes a truncated, catalytically inactive protein [2]. Another major O allele (O02) contains 9 mutations: nt106G>T, 188G>A, 189C>T, 220C>T, 297A>G, 646T>A, 681G>A, 771C>T and 829G>A, compared to the O01 background [3]. Another O allele (O03) lacks the G261 deletion but has a mutation (G802A) that presumably inactivates the enzyme by altering its sugar-binding site [4,5]. Most O alleles are derived by point mutations from the 2 worldwide alleles, O01 and O02, and share the guanine deletion at nt position 261.
Theoretically, an $O$ allele constitutes any allele with a mutation that causes total loss of the translated protein’s ability to transfer N-acetyl-galactosamine or galactose to its oligosaccharide acceptor.

It is instructive to study O allele polymorphisms. Different ethnic groups have their own O allele genetic character [6,7]. Studying the high diversity of inactive alleles in the ABO gene offers a precious opportunity to elucidate the evolution of the gene and the role of selection in the maintenance of such diversity in populations [8]. Recent data provide evidence that nondeletional $O$ alleles can produce detectable amounts of A antigens.

O phenotype is common in the Chinese Han people, and incidence of $O$ alleles is 64% [9], but the distribution of the various $O$ alleles is unknown. Through analyzing sequences of exon6 and exon7 of the $O$ allele of the ABO gene of 100 individuals, we identified 5 novel $O$ alleles in the Chinese.

Materials and Methods

Subjects. One hundred O phenotype donors from the Chinese Han population were randomly selected at ShenZhen Blood Center. The 100 subjects were members of 22 Chinese subgroups including 18 Guangdong individuals, 11 Heibeies, 11 Jianxies, 7 Henans, 7 Hunans, 4 Fujians, 4 Heilongjians, 2 Shachings, 2 Liannies, 2 Jilings, 2 Guizhous, 2 Shangdongs, 2 Anhuies, 1 Yunan, 1 Hebei, 1 Gansu, 1 Guizhou, 1 Hannan, and 1 Shanxi. Venous blood samples (5 ml) were drawn into EDTA tubes.

Serological methods. ABO typing included direct and reverse blood grouping, according to standard serologic methods. The antisera were commercial monoclonal anti-A and anti-B (Bioscot, Livingston, UK).

Direct sequencing of exon6 and exon7. PCR-based gene analyses were performed on exon6 and exon7. Primer pairs mol-46/mol-57 and mol-71/mol-101 described in a previous study were used to amplify the two exons [10]. The PCR fragment sizes for exon6 and exon7 were 252 bp (251 bp for $O01$) and 843 bp respectively. PCR amplification was carried out in a reaction volume of 50 μl containing PCR buffer, 400 μM each dNTP, 0.2 μM each primer, 300-500 ng of genomic DNA, and 0.44 U of Taq DNA polymerase (Promega, Madison WI, USA). Amplification was carried out under the following conditions: 95°C for 10 min; 10 cycles at 94°C for 60 sec, 63°C for 90 sec, and 72°C for 60 sec; 25 cycles of 94°C for 60 sec, 61°C for 90 sec, and 72°C for 60 sec; followed by final elongation at 72°C for 10 min. The PCR products were purified with a DNA fragment purification kit (Takara, Dalian, China) according to the manufacturer’s instructions. The purified products were sequenced using ABI PRISM BigDye terminator cycle sequencing reaction kit (Applied Biosystems) and the sequenced samples were analyzed by electrophoresis using an ABI PRISM 3100 DNA Sequencer.

Cloning and haplotype sequencing of exon6, intron6 and exon7. To determine the location of certain mutations at the ABO gene locus, a fragment of 2170 bp spanning exon6, intron6, and exon7 was amplified by the following primer pair: 5'-CTG GAA GGG TGG TCA GAG GA-3', and 5' -GTT ACT CAC AAC AGG ACG GAC-3'. The amplification was carried out in a volume of 50 μl containing 2×GC buffer I/II, 100 μM each dNTP, 0.1 μM each of the 2 primers, 500 ng of genomic DNA, and 1U of LA Taq polymerase (Takara, Dalian, China). The PCR conditions were: 1 cycle of 95°C for 10 min; 30 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 150 sec; followed by final elongation at 72°C for 10 min. The gel-purified PCR product was cloned into the pCRII vector with TOPO cloning kit (Invitrogen, Groningen, Netherlands). Specific sequencing reaction was performed in a final volume of 10 μl with the following 5 forward primers: F1, 5’-GGG CGT GGT CGT CAA GC-3; F2, 5’-TGT AAC AAC AGG AGG CCG-3; F3, 5’-CAA CCG CAG ACA CAT ACT TGA-3; F4, 5’-CAG GAC GGG CCT CCT GCA-3; F5, 5’-CCA GTG CCA GCC CTA CAT-3. Products of direct PCR and clone PCR that were simultaneously sequenced separated any PCR-induced errors from actual sequence polymorphisms.

Table 1. Distribution of O genotypes in 100 Han Chinese subjects.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of subjects</th>
<th>Genotype frequency (%)</th>
<th>Expected Value</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O01/O01$</td>
<td>18</td>
<td>18</td>
<td>22.09</td>
<td>1.0869</td>
</tr>
<tr>
<td>$O01/O02$</td>
<td>53</td>
<td>53</td>
<td>46.53</td>
<td>0.8997</td>
</tr>
<tr>
<td>$O02/O02$</td>
<td>22</td>
<td>22</td>
<td>24.5025</td>
<td>0.2256</td>
</tr>
<tr>
<td>$O02/O05$</td>
<td>1</td>
<td>1</td>
<td>$O02/OO$: 3.465</td>
<td>0.6194</td>
</tr>
<tr>
<td>$O02/O02 (T1104G)$</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$O01/O02 (G489A)$</td>
<td>1</td>
<td>1</td>
<td>$O01/Ow$: 3.29</td>
<td>0.8888</td>
</tr>
<tr>
<td>$O01/O01 (A468A)$</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$O01/O02 (T526C)$</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$O01/Ov (no206G-, C467T)$</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Ov/Ov$</td>
<td>0</td>
<td>0</td>
<td>0.1225</td>
<td>0.1225</td>
</tr>
</tbody>
</table>
Results

All O alleles were named following the unofficial nomenclature as described by the Blood Group Antigen Gene Mutation Database. Nucleotide sequences analysis of the 100 individuals studied allocated them into 8 genotypes. Table 1 lists the distribution of O genotypes of these Han Chinese subjects. Genotype frequencies within O individuals were in Hardy-Weinberg equilibrium.

Eight O alleles were found in the Chinese population. Ninety-three individuals have the two common O01 or O02 alleles. The allele frequency of ABO*O01 was 0.47 and that of ABO*O02 was 0.495. One individual was found to have O05 allele, which harbors nt297G on the basis of O01 allele. Five O alleles were found to differ from all alleles reported to date. Four of these alleles differed from either the O01 allele (1 out of 4) or O02 allele (3 out of 4) by 1 point mutation at A468G, G489A, T526C, or T1104G. The fifth allele differed from the O01 allele that does not have nt261G deletion but does have C467T mutation. This novel allele concurred in 2 individuals who came from Henan and Hunan, respectively. Relative frequencies were 0.005 for the O05 allele and 0.03 for the novel O allele.

Discussion

The O1 allele prevails, with O02 accounting for about 97% of alleles in the Chinese Han population. Four O variant alleles with nt261G deletion differed from either the O01 allele or O02 allele by 1 point mutation at A468G, 489 G>A, 526 T>C, or 1104 T>G, respectively. Nt468, 489, 526, and 1104 mutations located in non-coding regions and behind nt352 position do not affect translation of the O allele. Like other O1 alleles, the novel alleles encode a truncated, nonfunctional enzyme. The biological and evolutionary significance of the novel O alleles remains to be elucidated.

The O05 allele was first found in a Guangdong individual and it shares sequence characteristics of O01 alleles (G261 deletion) and B101 alleles (297G) [11]. The O03 allele, which has been shown to occur with a frequency of <5% in other populations [12], was not detected in this study. But a novel O allele without 261G deletion was found for the first time in this study.

Until now, only 5 O alleles have been found in the Chinese population among all 56 reported variable O alleles. According to the Blood Group Antigen Gene Mutation Database, they were described as O09 allele with nt261delG, 318C>T and 467C>T, O10 allele with nt261delG and 657C>T, O12 allele with nt261delG, 297A>G, 595C>T, 646T>A, 681G>A, 771C>T, 829G>A, and some mutations in intron3, intron4, and intron5, O13 allele with nt261delG, 297A>G, 646T>A, 681G>A, 771C>T, 829G>A, and O56 allele with 261delG, 496delA, as characterized by us [9,13]. All 5 alleles have the common nt261G deletion. In earlier reports, no O allele with absence of nt261G deletion has been found in the Chinese.

Twelve of all reported O alleles demonstrate absence of nt261G deletion but presence of another mutation. Among these 12, there are only two O alleles that have 467C>T mutation. The O08 allele shares sequence characteristics of A2 alleles (C467T polymorphism and C1060 deletion) and Ael alleles (G800 insertion) [14]. The O14 and O15 alleles have single nonsynonymous substitutions compared with A101 or A102 (467C>T), responsible for the A1 phenotype. O19 and O20 alleles have chimeric sequences of A-O02 and B-O02, respectively, from exon6 to exon7, of which the recombination-breakpoints are within or near intron6 [15].

Six O alleles without G261 deletion have been reported [16]. The O48 and O49 alleles were shown to have mutations resulting in Gly229Asp(nt G800 insertion) [14]. The O50 allele was described as an amino acid change from threonine(Thr) to methionine(Met) at amino acid 163 position, which results from nt488C>T. The O51, O52, and O53 alleles are A1-like alleles having nonsense mutations causing premature truncation at nt88, nt322, and nt542 positions, respectively.

Among the Han Chinese previously studied, only 2 alleles (ie, O01 and O02) were found; their frequencies were 0.59 O01 and 0.41 O02 in Putien and 0.51 O01 and 0.49 O02 in Fujiou; only 3 genotypes were found: O01/O01, O02/O02, and O01/O02 [9]. Our data indicate that the maximum frequency of alleles other than O01 or O02 is 0.03,
which is near to the reported 0.033, but different from the frequency of 0.008 found by Yip [9].

In sequences of exon6 and exon7 of the O allele at the ABO gene locus in 100 individuals of the O phenotype randomly selected from the Chinese Han population, we found 2 individuals who showed an absence of nt261G deletion but who had a missense mutation at nt467. The mutation C>T at nt467 results from Pro to Leu substitution at amino acid 156 position. The mutation is known to have little effect on decreasing the enzymatic activity because the polymorphism is found in the A102 and A103 alleles, which commonly occur in Chinese individuals.

Our results indicate that the molecular genetic background of O phenotype is heterogeneous in the Chinese population. The 5 rare alleles reported here are isolated cases. Surely more O alleles will be found in the Chinese.

Acknowledgements

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References