J Hum Genet (2005) 50:671–673
DOI 10.1007/s10038-005-0308-y

SHORT COMMUNICATION

Qiong Yu · Zhi-Hui Deng · Guo-Guang Wu
Yan-Lian Lian · Yu-Qing Su

Molecular genetic analysis for a novel Ael allele of the ABO blood group system

Received: 30 June 2005 / Accepted: 24 August 2005 / Published online: 8 October 2005

Abstract The ABO blood group is the most important system in clinical transfusion medicine. Previous studies on the genetic base of the common ABO group and some rare ABO subgroups have suggested that the molecular genetic background of the ABO gene in the Chinese population has specific character. In this study, we carried out a molecular genetic analysis of a family with an individual diagnosed as Ael subgroup by serological tests. A novel allele was identified in our A subgroup cases.

Keywords ABO · Allele · Ael subgroup

Introduction

The ABO blood group is the most important system in clinical transfusion medicine. The ABO gene contains seven exons and 1,062 base pair (bp) sequence codes. It is well known that product encoded from the *A* allele is an α-1,3- *N*-acetylgalactosaminyltransferase (A transferase), which can add GalNAc to the H structure (fuca1-2Galβ1-R). The product encoded from the *B* allele is an α-1,3-galactosyltransferase (B transferase), which can add Gal to the same H precursor. Because most *O* alleles have a single nucleotide deletion (nt261G) in exon six with premature termination of translation after amino acid 117, they cannot produce catalysis function transferase, which explains the recessive nature of the *O* allele.

Since Yamamoto et al. (1990) described ABO at the molecular level and identified the molecular basis of the ABO polymorphism in 1990, numerous novel *ABO* alleles coming from different ethnic groups or various areas have been elucidated and collected in the Blood Group Antigen Gene Mutation Database, http://www.bioc.aecom.yu.edu/bgmut/index.php.

Previous studies on the genetic base of the common ABO group and some rare ABO subgroups have suggested that the molecular genetic background of the ABO gene in the Chinese population has specific character (Yu et al. 2004; Deng et al. 2005). In this study, we carried out a molecular genetic analysis of a family with an individual diagnosed as Ael subgroup by serological tests. A novel allele was identified in our A subgroup cases.

Q. Yu (✉) · Z.-H. Deng · G.-G. Wu · Y.-L. Lian · Y.-Q. Su
ShenZhen Institute of Transfusion Medicine,
ShenZhen, GuangDong, China
E-mail: yuqiong7867@yahoo.com.cn

Materials and methods

A discrepancy between red blood cell (RBCs; forward) and plasma (reverse) ABO grouping results was observed in a 38-year-old Chinese donor man from FuZhou, FuJian, PR China. His direct family members, including his father, mother, and son, were subsequently analyzed; all four family members were recruited with informed consent. Venous blood samples (6 ml) were drawn into EDTA tubes.

Serological methods

ABO typing included direct and reverse blood grouping, adsorption-elution testing, and salivary blood group substances, according to serological standards described previously. Antisera used included monoclonal anti-A and anti-B (Bioscot, Livingston, UK), polyclonal anti-A (blend of human serum), anti-A1 from *Dolichos biflorus* (lectin; Dominion, Nova Scotia, Canada), monoclonal anti-AB (Immucor, Norcross, GA, USA), and polyclonal anti-AB (blend of human serum). Lectins were used for anti-H from *Ulex europaeus* (Dominion, Nova Scotia, Canada). All reagents were used according to the manufacturers' instructions. Their subjects' ABO groups were determined according to current practice (Daniels 2002).

PCR amplification of the ABO gene for DNA direct sequencing

DNA was prepared using a simple salting-out method. As 91% of the ABO coding sequences lie in exons 6 and 7 (Yamamoto et al. 1995), polymerase chain reaction (PCR)-based gene analyses were performed on the two exons for all four family members. Primer pairs mol-46/mol-57 and mol-71/mol-101 described previously (Olsson and Chester 1996) were used to amplify exons 6 and 7. The PCR fragment sizes for exons 6 and 7 were 252 bp (251 bp for *O1*) and 843 bp, respectively. PCR amplification was carried out in a reaction volume of 50 μl containing one time PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$), 400 μM each of dNTP, 0.1 μM each of primer pair, 300–500 ng of genomic DNA, and 2.5 U of Taq DNA polymerase (Applied Biosystems, Foster City, CA, USA). Amplification was carried out under the following conditions: 95°C for 10 min; 10 cycles at 94°C for 60 s, 63°C for 90 s, and 72°C for 60 s; 25 cycles at 94°C for 60 s, 61°C for 90 s, and 72°C for 60 s; followed by a final elongation of 72°C for 10 min. The PCR products were purified using the Takara DNA Fragment Purification Kit (Takara, Dalian, China) according to the manufacturer's instruction. The purified PCR products were directly sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and were analyzed with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Cloning sequencing of exon 6, intron 6, and exon 7 for the prophetic

To determine the haploid type of the *ABO* gene for the prophetic, a fragment of 2,170 bp spanning exon 6, intron 6, and exon 7 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 two times GC buffer I/II, 100 μM each dNTP, 0.1 μM each of two primers, 500 ng of genomic DNA, and 1 U of LA Taq polymerase (Takara, Dalian, China). The PCR conditions were as follows: 1 cycle of 95°C for 10 min, 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 150 s; 72°C for 10 min. The gel-purified PCR product was cloned into the pCR II vector with the TOPO Cloning Kit (Invitrogen, Groningen, The Netherlands). A total of seven clones containing the *A* allele were identified in a screen of 15 colonies. Seven clones were performed to be sequenced in a final volume of 10 μl used the following five forward primers: AF1, 5′-GGC GGC CGT GTG CCA GA-3′; AF2, 5′-TTG TCC TCC CAG AGG GTA GA-3′; AF3, 5′-CAA CCG CAG ACA CAT ACT TGA-3′; AF4, 5′-CAG GAC GGG CCT CCT GCA-3′; AF5, 5′-CCA GTC CCA GGC CTA CAT-3′.

Results

Serologic phenotype

We discovered an Ael phenotype by discrepancies between forward and reverse typing in routine ABO grouping. The RBCs of the prophetic were not agglutinated by monoclonal and polyclonal anti-A, anti-B, and anti-A, B regent at room temperature. Adsorption-elution tests performed by testing the individual's RBCs against anti-A produced elutes reacting moderately with A_1 cells in antihuman globulin medium. The serum samples contained anti-A activity with agglutinating A standard RBCs in 2+ reaction and anti-B activity with agglutinating B standard RBCs in 3+ reaction with few nonagglutinated cells. Only H substance was found in the saliva.

In the man's family, his father was serological type common A, and his mother and son were common O. ABO phenotypes of all members studied in the family are shown in Fig. 1.

Analysis results of the ABO gene sequence

We defined *ABO* allele using the unofficial nomination described by the Blood Group Antigen Gene Mutation Database.

The result of direct sequencing of the PCR products amplified from the four samples was compared with the consensus sequence of the *A101* allele. The prophetic harbored nt261G deletion with one haplotype, 467C/T and 425T/C heterozygous mutations on the basis of *A101* allele. The individual's father had 425T/C heterozygous and 467T homozygous mutations on the background of *A101* allele. His mother had 297G/A, 646T/A, 681G/A, 771C/T, and 829G/A heterozygous mutations comparing to *O01* allele with nt261G-deletion. According to knowledge of the Mendelian inheritance law, we can

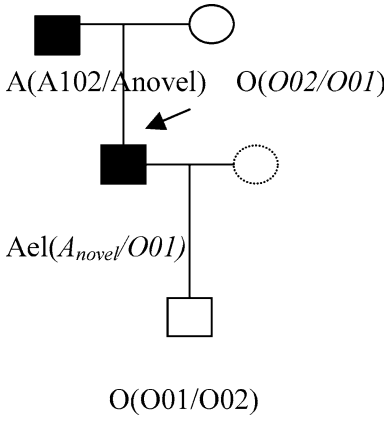

Fig. 1 Schematic diagram of the inheritance of Ael in a family. The phenotype and genotype determined are indicated below each symbol. *Arrows* indicate propositions. Samples shown in *dashed circle* were not studied

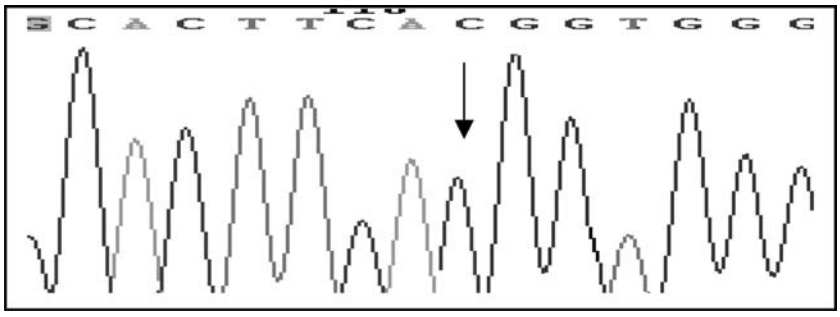

Fig. 2 Sequence of the new Ael allele at nt418–430. *Arrow* indicates the nucleotide 425 positions

conclude the ABO haplotype type of every sample. The mother and son were all genotyped as *O01*/*O02*. One haplotype of the prophetic was an *O01* allele, and another was an A102-like allele with single nt425T > C mutation in exon 7. We defined the gene as novel *ABO*Ael* allele. One haplotype of the man's father was *A102* allele, and another harbored the novel *Ael* allele with the nt425T > C in the A102 background. According to the cloning sequencing result, we can determine the haplotype sequence of the prophetic to be *A102*-like allele with single nt425C mutation in exon 7 once again. The sequence of the new *Ael* allele at nt418-430 is listed in Fig. 2.

Discussion

The Ael group is associated with a kind of weak *ABO* blood group gene. These red cells show no agglutinate at all by anti-A or anti-A, B test sera, but A antigens on RBCs could be detected only by adsorption-elution, which is known to be the most sensitive method for detecting blood group antigens on the surface of RBCs.

Up to now, five novel *Ael* alleles have been identified and characterized (Olsson et al. 1995; Ogasawara et al. 1996a, 1996b; Seltsam et al. 2003; Sun et al. 2003; Wu et al. 2005). The *Ael* allele described herein was also transmitted in a straightforward manner through two generations. The nucleotide sequences of Ael alleles of the father and prophetic had two point mutations at positions 425 (T > C) and 467 (C > T) compared with an *A101* allele. The 467C > T mutation is thought to have little effect on decreasing the enzymatic activity level because the mutation is a well-known polymorphism found in the *A102* and *A103* alleles (Ogasawara et al. 1996a, 1996b). The initial mutation has not yet been reported elsewhere. No known ABO transferase coded from human alleles has been found to harbor an amino acid alteration at position 142.

The new single base substitution resulted in an amino acid substitution (methionine to threonine at position 142). The sequence of the novel *Ael* allele in exon 7 has been deposited in GenBank with the accession number DQ092381. We conclude that the mutation can explain the serological observation of the weak A antigen. The detection of new alleles has proven helpful for determining the functional relevance of the different amino acid positions for substrate binding in the glycosyltransferase family. The mutation at the 425 positions was expected to diminish A transferase activity.

It first indicates that the alteration of amino acid at position 142 is critical to the activity of glycosyltransferases. The new *Ael* allele with a change in a residue in the blood group A glycosyltransferase is probably responsible for this variant A subgroup.

Acknowledgements This work was supported by the Research Fund of ShenZhen Bureau of Science and Technology (project no. 200304217), China.

References

Daniels G (2002) Human blood groups, 2nd edn. Blackwell, Oxford

Deng Z-H et al. (2005) Molecular genetic analysis for Ax phenotype of the ABO blood group system in Chinese. Vox Sang [in press]

Ogasawara K, Yabe R, Uchikawa M, Saitou N, Bannai M, Nakata K, Takenaka M, Fujisawa K, Ishikawa Y, Juji T, Tokunaga K (1996a) Molecular genetic analysis of variant phenotypes of the ABO blood group system. Blood 88:2732–2337

Ogasawara K, Bannai M, Saitou N, Yabe R, Nakata K, Takenaka M, Fujisawa K, Uchikawa M, Ishikawa Y, Juji T, Tokunaga K (1996b) Extensive polymorphism of ABO blood group gene: three major lineages of the alleles for the common ABO phenotypes. Hum Genet 97:777–783

Olsson ML, Chester MA (1996) Polymorphisms at the ABO locus in subgroup A individuals. Transfusion 36:309–313

Olsson ML, Hosseini-Maaf B, Chester MA (1995) An *Ael* allele specific nucleotide insertion at the blood group ABO locus and its detection using a sequence-specific polymerase chain reaction. Biochem Biophys Res Commun 216:642–647

Seltsam A, Hallensleben M, Kollmann A, Burkhart J, Blasczyk R (2003) Systematic analysis of the ABO gene diversity within exon 6 and exon 7 by PCR screening reveals new ABO alleles. Transfusion 43:428–439

Sun CF, Yu LC, Chen DP, Chou ML, Twu YC, Wang WT, Lin M (2003) Molecular genetic analysis for the *Ael* and A3 alleles. Transfusion 43(8):1138–1144

Wu GG, Yu Q, Su YQ, Deng ZH, Liang YL (2005) Novel ABO blood group allele with a 767T > C substitution in three generations of a Chinese family. Transfusion 45:645–646

Yamamoto F, Marken J, Tsuji T, White T, Clausen H, Hakomori S (1990) Cloning and characterization of DNA complementary to human UDP-GalNac: fucal2GalNac transferase mRNA. J Biochem 256:1146–1151

Yamamoto F, McNeil PD, Hakomori S (1995) Genomic organization of human histo-blood group ABO gene. Glycobiology 5:51–58

Yu Q, Wu GG, Deng ZH, Su YQ, Liang YL, Wei TL (2004) Study on the molecular genetic background of A2 subgroup in Chinese Han population. Chin J Blood Transfus 17:83–86