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Gene diversity of chimpanzee ABO blood group genes elucidated from exon 7 sequences

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Abstract

Human and non-human primate ABO blood group genes show relatively large numbers of nucleotide differences. In this study, we determined exon 7 sequences for 10 individuals of common chimpanzee and for four individuals of bonobo to estimate nucleotide diversities among them. Sequence data showed the existence of chimpanzee specific 9-base deletion in the beginning of the exon 7 coding region. From a phylogenetic network of exon 7 sequences of ABO blood group genes for human, common chimpanzee, bonobo and gorilla, effects of parallel substitutions and/or some kinds of convergent events are inferred in the chimpanzee lineage. We also estimated nucleotide diversities for common chimpanzee and bonobo ABO blood group genes, and these values were 0.4% and 0.2%, respectively. These values are higher than that of most human genes.

Keywords: Glycosyltransferases; Hominoids; Nucleotide diversity; Phylogenetic network

1. Introduction

The ABO alleles A and B code for terminal glycosyltransferases, which transfer N-acetylgalactosamine and galactose, respectively, to a common precursor (H substance). Yamamoto et al. (1990) determined the cDNA sequences of the three common human alleles A^1, B, and O, and Yamamoto et al. (1995) determined the genomic organization of the gene. Critical sites for the distinction between A and B activities of the glycosyltransferase have been identified (Yamamoto and Hakomori, 1990) in exon 7, and many non-human ABO blood group exons 7 were sequenced (e.g. Kominato et al., 1992; Martinko et al., 1993; Kermarrec et al., 1999). Saitou and Yamamoto (1997) compared nucleotide sequences of primate ABO blood group genes, and relatively large numbers of nucleotide differences were found among them. The number of differences is unusually large for human allelic divergence under neutral evolution.

In this study, we determine and analyze the nucleotide sequences of exon 7 for two *Pan* species to compare and elucidate the gene diversity among the hominoid ABO blood group genes.

2. Materials and methods

2.1. Sequencing of genomic DNA

Genomic DNAs of 10 unrelated common chimpanzees (*Pan troglodytes*) and four unrelated bonobo (*Pan paniscus*) were used for DNA sequencing. PCR reaction was performed containing 1 × Gene Taq Universal Buffer (Mg^{2+} free) (Nippon Gene), 1.5 mM $MgCl_2$, 0.2 mM dNTP, 10 pmol of each primer, and 1 unit of AmpliTaq Gold (PE Biosystems). PCR primers were designed based on database sequences including human, chimpanzee and gorilla as follows. SN-1: 5′-GT-

Abbreviations: PCR, polymerase chain reaction; SSCP, single strand conformational polymorphism.

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TAACCCAATGGTGGTGTTCT-3′, SN-2: 5′-GAGACGGCGGAGAAGCACTT-3′, SN-3: 5′-TGGGTCGCGGAACTCCATGTC-3′, SN-4: 5′-ACGTCCTGCCAGCGCTTGTA-3′, SN-5: 5′-AAGCGCTGGCAGGACGTGT-3′, SN-6: 5′-GTGGGCGTGGAGATCCTGACT-3′, SN-7: 5′-CCCTCGTCCTTGGGGATGTAG-3′, SN-8: 5′-GGGCCGGCGCTCGTAGGT-3′, SN-9: 5′-CCCCAGTCCCAGGCCTACAT-3′, SN-10: 5′-CGATGCCGTTGGCCTGGTC-3′, SN-11: 5′-GGTGGCAGGCCCTGGTGAG-3′.

Amplification was carried out in DNA GeneAmp PCR System 2400 (PE Biosystems) with the following temperature parameters: 10 min at 95°C followed by 40 cycles of 95°C for 30 s, 60–65°C for 15 s, and 72°C for 1 min. PCR products were purified using MicroSpin Columns S-300 HR (Amersham Pharmacia Biotech). At first, PCR products were sequenced in both strands using the direct sequencing method. Heterogeneous PCR products were then cloned in the TA cloning vector pCRII (Invitrogen). DNA sequencing was performed on double-stranded plasmid DNA and PCR products using Dye Terminator Cycle Sequencing Kit and ABI prism 377 DNA sequencer (PE Biosystems).

2.2. PCR-SSCP analysis

PCR-SSCP analysis following the method of Hayashi (1991) was used to identify heterozygous sites. PCR primers were end-labeled by gamma-32P-ATP, and PCR was done using the conditions described above. We used 0.5 × Tris–Borate–EDTA buffer and 5% acrylamide gel containing 5% glycerol for electrophoresis. During electrophoresis, the temperature was always kept at 16°C.

Table 1
Variant positions of ABO blood group genes in exon 7[a]

Sequence	Nucleotide position 3344444444445555555555555566666667777777777888888 7735666778881111222222233781244589000177899001222 5680789140791389012345634992926715234117316233569	Reference
Human (*Homo sapiens*)		
Hu-A1	ATTTTGGCGCAGTATGGAGGTGCACTGGTGTCGTCGGCCCCACGGGAGG	1
Hu-A2	....C.....	1
Hu-B	....C.....G.....T...A.....A.C....	1
Hu-O1	....C.....A.....	1
Hu-O2	....C.....A.....T.....A	1
Common chimpanzee (*Pan troglodytes*)		
Ch-A1	**..CA..A....G.....C.....T....T.....A...	2
Ch-A2	**..CA..A....G.....C.....T.....A...	2
Ch-A3	***.C.....G.....C.....C.....T.....	3
Ch-A4	***.....G.....C.....T.....A...	3
Ch-A5	***.C.C....C.G.....C.....T.....A...	3
Ch-I	....CA..A....G.....C.....T....T.....A...	This study
Ch-II	....CA..A....G.....C.....T.....A...	This study
Ch-III	....CA..A....G.....C.....T.....G...A...	This study
Ch-IV	....CA..A....G.....C.....T....T.G...A...	This study
Ch-V	....CA..A....G---------C.....T....T.....A...	This study
Bonobo (*Pan paniscus*)		
Bo-1	....CA..A...AG.....T.....A...	This study
Bo-2	....CA..A....G.....A.....T.....A...	This study
Bo-3	....CA..A....G.....T.....A...	This study
Gorilla (*Gorilla gorilla*)		
Go-B1	***.C.....G.....A.C....	2
Go-B2	***.C.....G.....A.C..A.	3
Go-B3	***.C.....G.....A.C....	3
Go-B4	***.C.....G.....A.C....	3
Go-B5	***.C.....G.....T.....A.C....	3
Baboon (*Papio cynocephalus*)		
Ba-B	**CCC..T.GG..G.T.G.....TC..C...A...CT..T.A.C.G..	2

[a] 1: Yamamoto et al. (1990), 2: Kominato et al. (1992), 3: Martinko et al. (1993). Dots, asterisks, and hyphens mean identical nucleotides as the human-A1 (Hu-A1) sequence, unavailable positions, and deletions, respectively.

2.3. Sequence analyses

CLUSTAL W version 1.6 (Thompson et al., 1994) was used for multiple alignments. Phylogenetic networks were constructed following the procedure of Bandelt (1994) and Saitou and Yamamoto (1997). The nucdiv program of the ODEN package (Ina, 1994) was used for estimation of nucleotide diversities.

3. Results and discussion

3.1. Comparison of sequences

We sequenced exon 7 for five ABO blood group alleles for common chimpanzee and three alleles for bonobo (DDBJ/EMBL/GenBank International Nucleotide Sequence Database accession numbers are AB031368–AB031372). Table 1 shows the variant sites of the exon 7 ABO blood group alleles. Type Ch-I and Ch-II are identical to reported chimpanzee A allele sequences, while others (types Ch-III, Ch-IV, and Ch-V) are novel. A 9-base deletion was found in position 518 to 526 of the type Ch-V sequence. This deletion does not cause frame shift mutation, and has previously been reported by Kermarrec et al. (1999).

We used PCR-SSCP analysis to test for heterozygosity in two overlapping fragments covering the entire exon 7 sequence. Fig. 1 shows a typical result of PCR-SSCP analysis, and shows the two distinct bands caused by heterozygosity of the amplified region. Each chimpanzee genotype gave a unique PCR-SSCP banding pattern, and those typings were used to verify the results obtained from sequencing of the cloned exon 7 PCR product.

3.2. The phylogenetic network of hominoid ABO blood group genes

Fig. 2 shows the phylogenetic network of exon 7 sequences of ABO blood group genes for human, common chimpanzee, bonobo, and gorilla. This phylogenetic network was constructed from sequence data of Table 1. The HCG node indicates possible ancestral position for all human–chimpanzees–gorilla ABO blood group genes. Sites 796 and 813 separate B type alleles of human and gorilla from others.

This network contains several parallelograms which indicate incompatible sites. Those structures can be generated by parallel substitutions and/or by some types of convergent events. For example, position 777 separates [Ch-II, Ch-III, Bo-1, Bo-2, Bo-3] and [Ch-I, Ch-IV, Ch-V], but this partition is incompatible with that for position 791 which separates [Ch-I, Ch-II, Ch-V, Bo-1, Bo-2, Bo-3] and [Ch-III, Ch-IV]. This incompatibility is possibly the result of recombination between chimpanzee ABO alleles.

Allele Ch-V has G in position 589, while all other chimpanzee alleles have C in this position. Because all other primates also have G in this position, it is possible that this is a reflection of ancestral polymorphism, or parallel mutation. A similar situation was also observed in position 467 for Ch-A4.

Positions 468 and 474 make a partition between [Ch-A3, Ch-A4, Ch-A5] and others. This means that

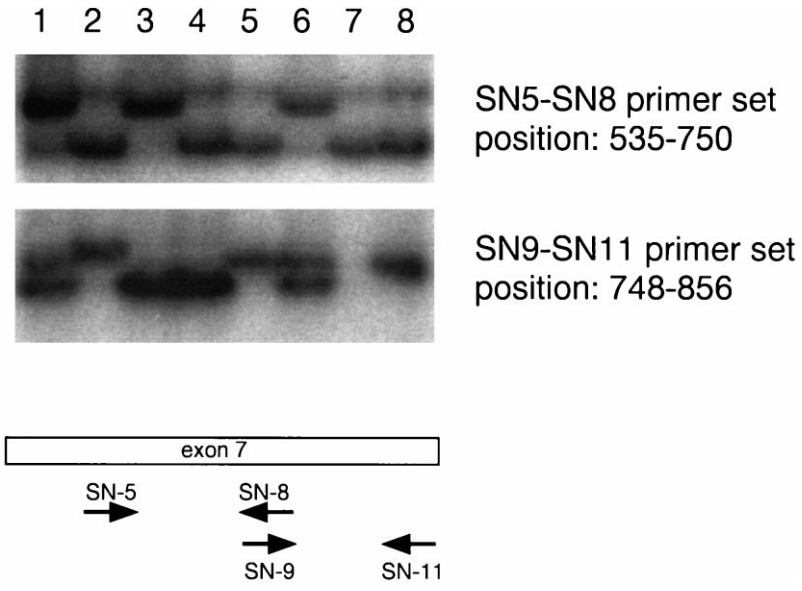

Fig. 1. Two examples of PCR-SSCP. Lane 1: CH-36, lane 2: CH-46, lane 3: CH-206, lane 4: CH-75, lane 5: CH-83, lane 6: CH-80, lane 7: CH-76, and lane 8: CH-86. In the case of the fragment amplified by using SN-5 and SN-8, lanes 1, 3 and 6 share the same sequence, while lane 1 and others share another sequence. In the case of the fragment amplified by using SN-9 and SN-11, lanes 1 and 6 show typical heterozygous pattern. Lane 7 was not amplified effectively (a weaker band appeared here).

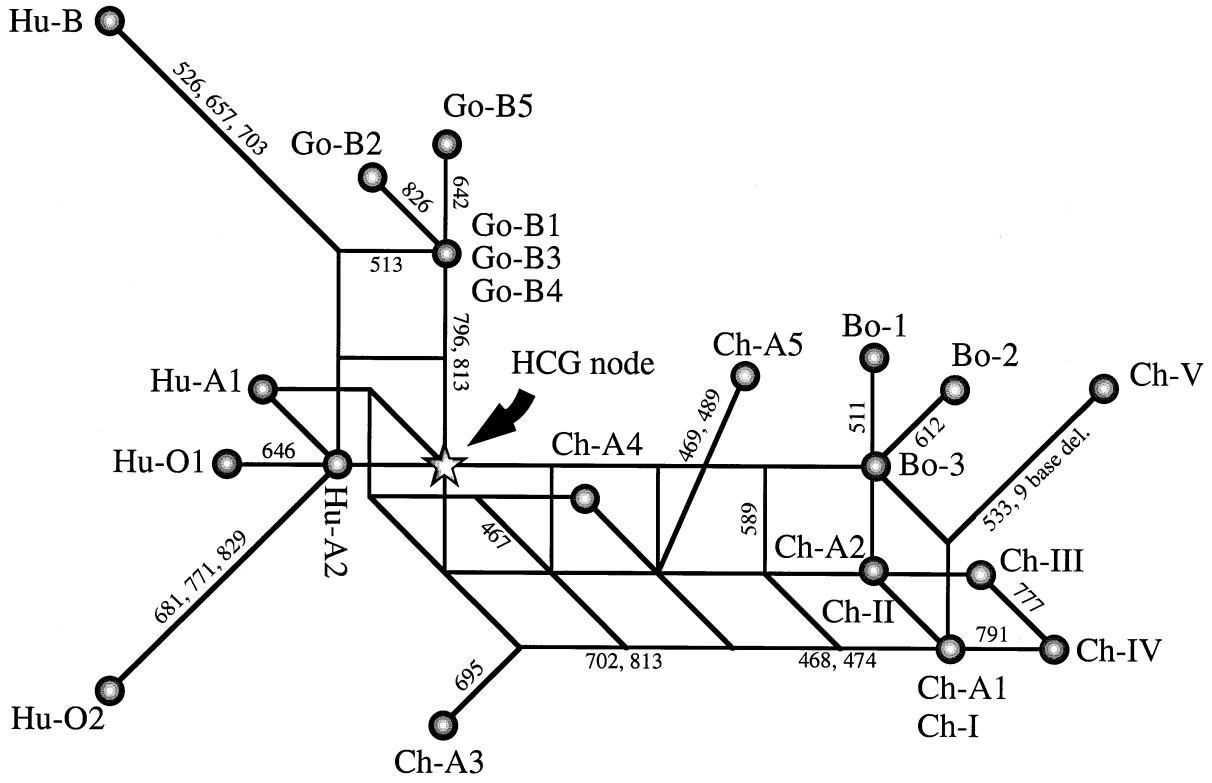

Fig. 2. The phylogenetic network of ABO blood group gene exon 7 sequences for common chimpanzee, bonobo, gorilla, and human. Numbers are nucleotide positions responsible for corresponding edges, and edge lengths are proportional to number of nucleotide differences. Abbreviations of alleles are the same as in Table 1. A star indicates human–chimpanzees–gorilla (HCG) common ancestral node.

there are two kinds of variations in the first part of exon 7 (positions 375 to 489). Positions 533 and 589 characterize allele Ch-V. The 9-base deletion is another unique feature for allele Ch-V. Those create another variation in the middle part of exon 7 (positions 513 to 695). The last part of exon 7 has more complicated polymorphism, because position 777 is incompatible with positions 702, 813, and 791. This might be a consequence of recombination, and if this is the case, at least one recombination has occurred between positions 777 and 791.

There is no separate branch for the bonobo specific cluster, but the three bonobo sequences are closely related.

3.3. Nucleotide diversities in chimpanzee ABO blood group genes

We computed nucleotide diversities for the common chimpanzee and bonobo ABO blood group exon 7 sequences. Table 2 shows the genotype at the ABO blood group locus for each individual. Chimpanzee CH-90 and bonobo BO-8 are heterozygous, but only one allele could be identified. Nucleotide diversities of common chimpanzee and bonobo were 0.384% and 0.204%, respectively (see Table 3). Li and Sadler (1991) estimated nucleotide diversity in humans to be 0.110% by using 49 loci. Nucleotide diversities among alleles of chimpanzee ABO blood group genes seem to be higher than that of alleles of most human genes. Kaessmann et al. (1999) showed that the nucleotide diversity among alleles of chimpanzee for chromosome X non-coding is

Table 2
The list of chimpanzees and their genotypes for ABO blood group gene exon 7

Individual name	Genotype
Common chimpanzee (*Pan troglodytes*)	
CH-46	Ch-III/Ch-III
CH-75	Ch-I/Ch-I
CH-76	Ch-I/Ch-II
CH-80	Ch-II/Ch-V
CH-83	Ch-II/Ch-II
CH-86	Ch-II/Ch-II
CH-90	Ch-IV/–[a]
CH-206	Ch-V/Ch-V
CH-220	Ch-V/Ch-V
CH-235	Ch-III/Ch-III
Bonobo (*Pan paniscus*)	
BO-3	Bo-1/Bo-1
BO-4	Bo-2/Bo-3
BO-5	Bo-2/Bo-3
BO-8	Bo-3/–[a]

[a] CH-90 and BO-8 are heterozygous, but another allele could not be determined.

Table 3
Nucleotide diversities in chimpanzee ABO blood group genes

Sample size	Number of sites compared (bp)	Nucleotide diversity (%)
Common chimpanzee (*Pan troglodytes*)		
19	468	0.384
Bonobo (*Pan paniscus*)		
7	468	0.204

higher than that of most human genes, and our result on the ABO gene agrees with their study. Kitano et al. (2000) estimated nucleotide diversity values of 0.219% and 0.208% for intron 6 sequences of the common chimpanzee and bonobo ABO blood group gene. This result suggested that the nucleotide diversity of exon 7 is higher than that of intron 6 for common chimpanzee ABO blood group genes.

Saitou and Yamamoto (1997) estimated allelic differences of the coding region of human ABO blood group gene to be 0.8–1.3% per nucleotide site. Those values are much larger than those of two chimpanzee species. The higher level of nucleotide diversity and the literature suggesting association between allelic variation at the ABO blood group locus and a variety of infectious diseases raises the possibility that the higher nucleotide diversity in humans is driven by position selection (Mourant et al., 1978). In chimpanzee, this kind of selection may be weak or neutral.

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