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Intragenic haplotypes and molecular evolution of the human α2-HS glycoprotein (fetuin) gene

M. Osawa [a,*], I. Yuasa [b], J. Henke [c], M. Kaneko [a], K. Umetsu [a]

[a] *Department of Forensic Medicine, Yamagata University School of Medicine, 2-2-2 Iida-nishi, Yamagata 990-9585, Japan*
[b] *Department of Legal Medicine, Tottori University School of Medicine, Yonago 683-8503, Japan*
[c] *Institut für Blutgruppenforschung, 50501 Köln, Germany*

Abstract

α2-HS glycoprotein (AHSG/fetuin) is a human plasma protein that is polymorphic with two common alleles and many variants. To investigate molecular evolution at this locus, intragenic haplotypes was analyzed by a contiguous genomic sequencing, and their frequencies were determined for 309 subjects. Judging from the aligned nucleotide sequences in the human and chimpanzee genes, it was concluded that the type 1 allele is older and has evolved into four major suballeles. The type 2 allele was generated from one major branch of the type 1 allele. The AHSG gene had a low mutation rate and nonhomogeneous distribution of the substitutions.

Keywords: α2-HS glycoprotein; Molecular evolution

1. Introduction

α2-HS glycoprotein (AHSG/fetuin) is a human 46 kDa plasma protein with extensive polymorphism by isoelectric focusing. The two common alleles, *AHSG*1* and **2*, are found in all population groups, which have been used as genetic markers in forensic hemogenetics [1]. In a previous study, to find nucleotide differences in the two common alleles, we examined the sequence of cDNA obtained by the RT-PCR method, and double amino acid replacements, Thr230Met and Thr238Ser, were detected in *AHSG*2* [2]. In this study, we examined intragenic haplotypes through analysis of the whole genomic

* Corresponding author. Fax: +81-23-6285273.
E-mail address: mosawa@med.id.yamagata-u.ac.jp (M. Osawa).

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sequence of the AHSG gene, including the common alleles and two variants of AHSG, and we demonstrate the gene diversity and linkage from an evolutionary aspect.

2. Materials and methods

Overlapping segments of the AHSG gene were amplified using 14 pairs of oligonucleotide primers, which spanned a total of 10,309 bases [2,3]. Then, the PCR product was labeled with a sequencing kit, followed by sequencing using an autoanalyzer. This contiguous scanning was applied to 20 samples of homozygotes at known substitutions. To estimate the frequency of six determined suballeles in the common alleles, seven specific substitutions were examined in the case of 111 and 198 German and Japanese individuals, respectively, by the RFLP method. Multiple sequence alignment and a phylogenetic network were constructed using CLUSTAL W.

3. Results and discussion

Samples containing homozygous changes at four known variation sites were chosen. When the entire sequence of the AHSG gene spanning 10.3 kb was determined, 27 sites of nucleotide variation were found. From the identified intragenic haplotypes, we verified that the type 1 and 2 alleles could be divided into four and two distinct suballeles, respectively, and these were designated *AHSG*1A* to **1D*, and **2A* and **2B*. The allele frequencies were estimated by detecting the seven specific nucleotides in the six haplotypes (Table 1). *AHSG*1A* was predominant and the frequencies were similar in the German and Japanese groups. *AHSG*1B* and **1D*, however, were not encountered in the Japanese population. In addition, the nucleotide diversity of the AHSG locus was calculated to be 0.04% for both populations, indicating that there is no apparent difference in allele distribution between these two populations.

As shown in Fig. 1, the phylogenetic network of the human AHSG gene was constructed based on the nucleotide substitution events and the DNA sequence of the chimpanzee AHSG. Through evolution from the chimpanzee, the AHSG alleles formed three major branches, *AHSG*1A*, **1B/C* and **1D*, and the type 2 allele continuously diverged from one branch of **1B/C*. Then, we also examined type 3 and 5 variants. It was revealed that both contained a single nucleotide substitution in the coding region. In the case of *AHSG*3*, a C to T transition was evident in the *AHSG*1D* suballele, which should be accompanied by an amino acid change from Arg to Cys at a.a. position 299. The *AHSG*5* allele had a G to A transition in the *AHSG*1A* suballele, which should result in a change from Asp to Asn at

Table 1
Frequencies of AHSG alleles in German and Japanese populations

	**1A*	**1B*	**1C*	**1D*	**2A*	**2B*	**3*	**5*
Germans ($n=111$)	0.437	0.131	0.027	0.072	0.045	0.270	0.018	ND
Japanese ($n=198$)	0.543	ND	0.182	ND	0.091	0.179	ND	0.005

ND indicates not detected.

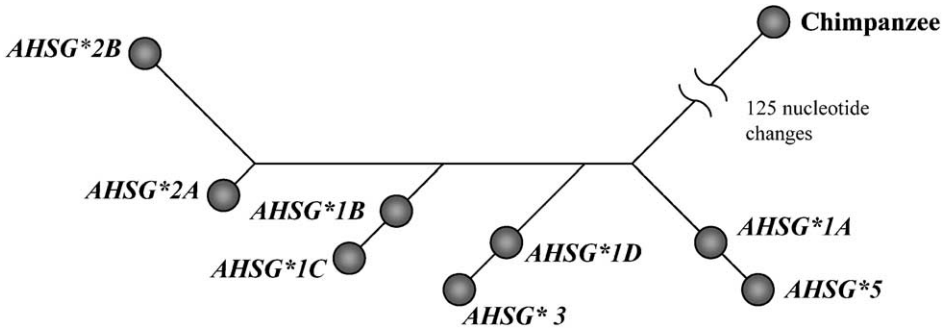

Fig. 1. The estimated phylogenetic network for the human and chimpanzee AHSG genes. Filled circles indicate the alleles shown in Table 1. The edge length corresponds to the number of nucleotide differences.

a.a. position 258. This indicated that both were generated relatively recently through a single nucleotide substitution in the coding region of the original allele, which was consistent with the fact that rare allelic variants have a highly restricted distribution.

The AHSG locus is polymorphic with two common alleles and approximately 30 unique variants that serve as markers of certain ethnic groups [1]. Analysis of a contiguous sequence of 10.3 kb including non-coding regions revealed that the two common alleles were apparently heterogeneous and consisted of two or four distinct suballeles with the linkage and evolution of the locus. Judging from the conserved Thr residues at a.a. positions 230 and 238 of the deduced amino acid sequence of the chimpanzee AHSG, the type 1 allele appears to be the ancestral gene in humans, and it has evolved in three major directions with divergence to the major type 2 allele. In addition, it has been proposed that the phenotype involves differences in structural features such as height and bone mineral density due to an influence on development [4]. While the more recently generated type 2 allele has been widespread, it is unknown if this is the consequence of any selection.

We estimated the rate of nucleotide substitutions (λ) as well (not shown). Our estimates were lower than other reported genes, indicating that AHSG is a protein with a conserved sequence. Furthermore, all four non-synonymous substitutions at a.a. positions 230, 238, 258 and 299 are concentrated in the carboxyl terminal domain. This non-homogeneous distribution of sites of variation suggests that a mutational hotspot is present within the AHSG gene.

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