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SHORT COMMUNICATION

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Molecular epidemiology of C9 deficiency heterozygotes with an Arg95Stop mutation of the C9 gene in Japan

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Abstract Deficiency of the ninth component of human complement (C9) is the most common complement deficiency in Japan, with an incidence of approximately one homozygote in 1000, but is very rare in other countries. Genetic analyses of Japanese C9 deficiency have shown that a C-to-T transition leading to TGA stop codon for Arg95 in exon 4 of the *C9* gene (Arg95Stop) is common in Japanese C9 deficiency. To determine the prevalence of heterozygous carriers of the Arg95Stop mutation in a Japanese population, we collected DNA samples from 300 individuals in two of the four main islands of Japan. Heterozygote detection was performed with an allele-specific polymerase chain reaction (PCR) system designed to detect exclusively only one of the normal and mutant alleles, followed by confirmation with PCR/single-strand conformation polymorphism (SSCP) analysis and direct sequencing. Twenty individuals were heterozygous for the Arg95Stop mutation. None was homozygous. The prevalence of carriers of the Arg95Stop mutation was 6.7% (20/300). An estimated frequency (0.12%) of complete C9 deficiency due to homozygous Arg95Stop mutation was consistent with frequencies determined by serological studies.

Key words C9 deficiency · *C9* gene · Nonsense mutation · Heterozygote detection · Prevalence · Japan

Introduction

Congenital deficiency of the ninth component of human complement (C9) is inherited as an autosomal recessive trait and shows extreme ethnic predominance (Winkelstein et al. 1995). In Japan, it is one of the most frequent genetic disorders, with its incidence being approximately one in 1000 (Hayama et al. 1989; Fukumori et al. 1989), whereas only a few patients with C9 deficiency have been identified in European countries. Although most individuals with C9 deficiency appear to be healthy, our previous study (Nagata et al. 1989) demonstrated that the risk of development of meningococcal meningitis in C9-deficient individuals was much higher than that in normal individuals, based on the incidence of C9 deficiency among blood donors in Fukuoka and the number of C9-deficient individuals in that area who developed meningococcal meningitis.

Genetic analyses of three unrelated C9-deficient individuals in north west Europe have revealed five different point mutations: four nonsense mutations at codons 33, 95, 133, and 406 in exons 2, 4, 4, and 9, respectively, and a missense mutation at codon 98 in exon 4 (Witzel-Schlömp et al. 1997, 1998). We have shown that four Japanese C9-deficient patients with meningococcal meningitis showed a homozygous nonsense mutation at codon 95 in exon 4 of the *C9* gene (Arg95Stop) (Kira et al. 1998). Horiuchi et al. (1998) have reported that the Arg95Stop mutation was identified in 18 (90%) of 20 alleles from ten unrelated C9-deficient individuals, mainly healthy blood donors. Thus, two independent studies in Japan suggest that the nonsense mutation may be responsible for most Japanese C9 deficiency. To determine the prevalence of the Arg95Stop mutation in Japan, we screened for Arg95Stop heterozygotes in 300 individuals in Kyushu and Honshu, two of the four main islands of Japan.

Subjects and methods

In this hospital-based study, we obtained DNA samples from 300 Japanese individuals, 150 from Kyushu University (Fukuoka, Kyushu Island) and 150 from Tottori University (Yonago, Honshu Island). Patients with neisserial infections were not included. Other personal information, in-

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cluding name, age, sex, and the presence of other diseases was not analyzed by intent.

To detect the Arg95Stop mutation in exon 4 of the *C9* gene, allele-specific polymerase chain reaction (PCR) (Wu et al. 1989) was carried out with genomic DNA, as previously described (Kira et al. 1998). Briefly, the common primer is complementary to the coding strand of exon 3. The common primer and normal primer were used for the amplification of the normal *C9* gene (N primer set). The common primer and the mutant primer were used as for the amplification of the *C9* deficiency mutant gene (M primer set). The DNA samples from the 300 individuals were amplified with each primer set using Ready-To-Go PCR Beads (Pharmacia Biotech, Uppsala, Sweden).

PCR/single-strand corformation polymorphism (SSCP) analysis was carried out in 20 individuals who showed amplified PCR products with the N and M primer sets. Exon 4-specific PCR amplification (Witzel-Schlömp et al. 1997) was performed using Ready-To-Go PCR Beads (Pharmacia Biotech). After the PCR products were digested with Pst I (TaKaRashuzo, Otsu, Japan), SSCP analysis was performed on GeneGel Excel 12.5/24 (Pharmacia Biotech) with 20 mA at 20°C, according to the manufacturer's instructions. DNA samples from previously identified homozygotes and heterozygotes for the mutation and normal individuals were used as authentic markers. Direct sequencing of exon 4-specific PCR products (Kira et al. 1998) was performed in 5 individuals randomly chosen from among the 20 individuals who showed amplified PCR products with the N and M primer sets.

Results and discussion

Allele-specific PCR showed that ten individuals from Fukuoka and ten from Yonago were heterozygous for the Arg95Stop mutation, since 461-bp fragments appeared in both the N and M primer sets (Fig. 1). The 461-bp fragment was amplified only with the N primer set but not with the M primer set in the 280 other individuals. Nobody was homozygous for the nonsense mutation. Thus, DNA analysis of 300 individuals revealed 20 heterozygotes for the Arg95Stop mutation. The carrier frequency was calculated to be 6.7%. The estimated frequency of the C9 deficiency with the homozygous Arg95Stop mutation in our hospital-based DNA study was 0.12%, which was similar to the frequencies of the C9 deficiency determined by serum studies, including both hospital-based (Hayama et al. 1989) and blood donor-based (Fukumori et al. 1989) studies. There were no significant differences in the incidence of C9 deficiency among the eight areas of the four main islands in Japan (Hayama et al. 1989). We identified an equal frequency of Arg95Stop heterozygotes in two areas in two of the four main islands. Therefore, the same mutation may be responsible for most of the C9-deficient individuals in Japan. Hondo (main islands)-Japanese are derived mainly from migrants who came from the northeast Asian continent after the Aeneolithic Yayoi period (300 BC-300 AD)

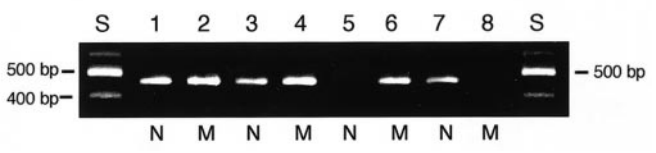

Fig. 1 Allele-specific polymerase chain reaction (PCR) of *C9* gene for detection of normal and mutant alleles (a C-to-T transition at codon 95) in exon 4. The 461-bp fragments generated by PCR using the normal primer set (*N*) and mutant primer set (*M*) are displayed. *Lanes 1 and 2*, Detected heterozygous carrier; *lanes 3 and 4*, father of C9-deficient patient (heterozygote); *lanes 5 and 6*, C9-deficient patient (homozygote); *lanes 7 and 8*, normal individual. The normal alleles are evident in the heterozygotes and the normal individual. The mutant alleles are observed in heterozygotes and a C9-deficient patient. *S*, Size marker: 100-bp ladder

(Omoto and Saitou 1997). Determination of the prevalence of the Arg95Stop mutation in other Asian populations may help to elucidate the importance of evolutionary mechanisms such as founder effect, heterozygote advantage, random drift, and population bottlenecks during the spread of this mutation.

All 20 heterozygous subjects detected by allele-specific PCR showed a heterozygous pattern on exon 4-specific PCR/SSCP analysis (data not shown). Direct sequencing revealed the Arg95Stop mutation in all five individuals randomly chosen from the 20 heterozygotes (data not shown). The allele-specific PCR system correctly allowed for the determination of the genotypes with no false positives. This method may be a powerful and easy approach for the genetic diagnosis of Japanese C9 deficiency and its carrier screening without requiring the additional steps of probe hybridization, ligation, or restriction enzyme cleavage.

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