Neurogenetics (2005) 6: 229–232
DOI 10.1007/s10048-005-0016-y

SHORT COMMUNICATION

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Association of sporadic Creutzfeldt–Jakob disease with homozygous genotypes at *PRNP* codons 129 and 219 in the Korean population

Received: 15 June 2005 / Accepted: 17 August 2005 / Published online: 11 October 2005

Abstract Human prion protein gene (*PRNP*) is considered an important gene in determining the incidence of human transmissible spongiform encephalopathies or prion diseases. Polymorphisms of *PRNP* at codon 129 in Europeans and codon 219 in Japanese may play an important role in the susceptibility to sporadic Creutzfeldt–Jakob disease (CJD); data regarding codon 129 in the Japanese population have led to divergent interpretations. In order to determine which, if any, of the *PRNP* genotypes in Korean people are associated with sporadic CJD, we examined the genotype and allelic distributions of human *PRNP* polymorphisms in 150 patients with sporadic CJD. All Korean sporadic CJD patients were Met/Met at codon 129, Glu/Glu at codon 219 and undeleted at the octarepeat region of *PRNP*. Our study showed significant differences in genotype frequency of *PRNP* at codon 129 (χ^2=8.8998, P=0.0117) or 219 (χ^2=12.6945, P=0.0004) between sporadic CJD and normal controls. Furthermore, the genotype frequency of the heterozygotes for codons 129 and/or 219 showed a significant difference between the normal population and sporadic CJD patients (χ^2=21.0780, P<0.0001).

Keywords Prion protein gene · Polymorphism · Creutzfeldt–Jakob disease · Population genetics

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Introduction

The human prion diseases are neurodegenerative disorders that are characterized by the accumulation of an abnormal protease-resistant isoform of the prion protein, PrP^{Sc} [1]. Human prion protein contains 253 amino acids encoded by prion protein gene (*PRNP*), which is located on chromosome 20 in humans. *PRNP* plays an important role in conferring susceptibility or resistance to prion disease. A number of point and insertion mutations of *PRNP* have been linked to familial Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease (GSS), and fatal familial insomnia (FFI) [2–5]. Moreover, polymorphisms of *PRNP* appear to be able to influence expression of prion disease in sporadic and iatrogenic CJD [6–8]. Homozygosity of methionine (Met) and valine (Val) at codon 129 of *PRNP* may cause a predisposition to sporadic and iatrogenic CJD in Europeans [6–9]. Results from a study in Japanese patients with sporadic CJD did not confirm the findings from the European studies [10]. All cases of variant CJD are homozygous for Met at codon 129 [11]. The increased frequency of homozygosity at codon 129 among 21 sporadic CJD patients (95.5%) compared to 106 normal individuals (49%) in UK suggested the possibility that this codon may contribute to the development of sporadic CJD [7]. However, in the Japanese population, there were differences from the above data regarding the frequency of homozygotes, which showed 92% homozygotes among 164 of the general population but 82% homozygotes among 21 sporadic CJD cases [10]. In our previous study, there was no difference in the frequency of Met homozygotes (94.33 vs 93.0%) at codon 129 of *PRNP* between healthy Korean and Japanese populations (χ^2=0.5785, P=0.4469). Glutamic acid (Glu)/Lysine (Lys) heterozygous polymorphism at codon 219 has been reported to occur in Asian populations but not in Caucasians [12–14]. Recently, it has been shown that the genotype of codon 219 influences the clinicopathologic features of GSS with a codon 102 mutation [12], and the heterozygotes at codon 219 prevent the induction of sporadic CJD [8]. Polymorphisms at codon 171 and differences in the octarepeat region have been

found in Caucasians, but these are not known to influence the occurrence of prion disease [15].

In the present study, the purpose was to investigate the frequencies of various *PRNP* polymorphism genotypes in 150 Korean sporadic CJD patients and to determine the correlation between specific genotype and the incidence of sporadic CJD in the Korean population.

Materials and methods

CJD patient population

We used previously established criteria [16] for diagnosis of sporadic CJD in Korea. The sporadic CJD cases in Korea have been reported previously [17–19]. Of the 150 suspected CJD cases, 17 were classified as definite CJD and 133 were classified as probable CJD.

Blood samples

Blood samples were collected from all sporadic CJD patients between May 1996 and April 2005. The study was approved by the Ethical Committee of Chunchon Sacred Heart Hospital. All blood samples were frozen at −70°C prior to analysis.

Polymerase chain reaction (PCR)

Genomic DNA was extracted from 200 μl of blood using the QIAamp DNA blood mini kit (QIAGEN) following the supplier's instructions. PCR was performed with T-1 (GATGCTGGTTCTCTTTGTGG) and T-2 (CCCACT ATCAGGAAGATGAG) primers. These primers were designed to amplify a 738-bp product in the complete open reading frame (ORF; 759 bp) of *PRNP*. The PCR reagents contained 50 pmol of each primer, 5 μl of 10× *Taq* DNA polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP mixtures, and 2.5 units of *Taq* DNA polymerase (Promega). The PCR conditions were 94°C for 5 min to denature, and then 35 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min, and then 1 cycle of 72°C for 10 min to extend the reaction. The PerkinElmer Cetus DNA thermal cycler (PerkinElmer) was used.

Nucleotide sequencing analysis

The purification of PCR products for sequencing was done using a QIAquick gel extraction kit (QIAGEN). The PCR products were directly sequenced on an ABI 377 automatic sequencer using a *Taq* dideoxy terminator cycle sequencing kit (ABI) and the same primers as above. Nucleic acid sequences were assembled and edited using a combination of the ABI 377 DNA Sequencer Data Analysis Program and Sequence Navigator Software.

Statistical analysis

Statistical analysis was performed using the SAS 8.1 Software (SAS Institute Inc.). χ^2 tests were used to analyze differences in genotype frequencies between the normal population and sporadic CJD cases.

Results

To examine the correlation between Met/Met homozygosity at codon 129 and susceptibility of sporadic CJD in Koreans, we tested the genotype of 150 sporadic CJD cases. All 150 cases were homozygous for Met at codon

Table 1 Genotype and allele frequencies of the polymorphisms at codons 129 and 219 and octapeptide repeat regions in samples of Korean sporadic CJD patients compared to the frequencies in healthy controls

Polymorphism	Samples	Total, *n*	Genotype frequency, *n* (%)			Allele frequency	
			Met/Met	Met/Val	Val/Val	Met	Val
Codon 129	Controls	529	499 (94.33)	29 (5.48)	1 (0.19)	0.971	0.029
	Sporadic CJD	150	150 (100)	0 (0)	0 (0)	1	0
Polymorphism	Samples	Total, *n*	Genotype frequency, *n* (%)			Allele frequency	
			Glu/Glu	Glu/Lys	Lys/Lys	Glu	Lys
Codon 219	Controls	529	487 (92.06)	42 (7.94)	0 (0)	0.960	0.040
	Sporadic CJD	150	150 (100)	0 (0)	0 (0)	1	0
Polymorphism	Samples	Total, *n*	Genotype frequency, *n* (%)			Allele frequency	
			5/5	5/4[a]	4/4	5	4
Octapeptide repeat region	Controls	529	527 (99.62)	2 (0.38)	0 (0)	0.998	0.002
	Sporadic CJD	150	150 (100)	0 (0)	0 (0)	1	0

[a]5 indicates no deletion of octarepeat (24 bp), and 4 indicates a deletion of octarepeat

Table 2 Genotype distributions for codons 129 and 219, and octarepeat region of *PRNP* in Korean sporadic CJD patients

Genotype	Normal controls (n=529)	Sporadic CJD patients (n=150)
$^{M}129^{M}/^{Q}219^{Q}/^{+}Octa^{+a}$	459 (86.7%)	150 (100%)
$^{M}129^{M}/^{Q}219^{K}/^{+}Octa^{+}$	38 (7.2%)	0 (0%)
$^{M}129^{V}/^{Q}219^{Q}/^{+}Octa^{+}$	25 (4.7%)	0 (0%)
$^{M}129^{V}/^{Q}219^{K}/^{+}Octa^{+}$	4 (0.8%)	0 (0%)
$^{M}129^{M}/^{Q}219^{Q}/^{+}Octa^{-}$	2 (0.4%)	0 (0%)
$^{V}129^{V}/^{Q}219^{Q}/^{+}Octa^{+}$	1 (0.2%)	0 (0%)

[a]+Octa indicates no deletion of octarepeat (24 bp), and −Octa indicates a deletion of octarepeat

129 (Table 1). Although frequency of Met/Met homozygosity at codon 129 in Koreans is similar to the Japanese results for a normal population, the 100% Met/Met homozygosity in Korean sporadic CJD cases was statistically different from the value (76% Met/Met homozygosity) reported for sporadic CJD cases in the Japanese population [10]. These results suggest that Met/Met homozygosity at codon 129 may be a risk factor for sporadic CJD (χ^2=8.8998, P=0.0117).

We also examined Korean sporadic CJD cases to determine whether the Glu/Glu homozygosity correlated with sporadic CJD. Of the 150 sporadic CJD cases, all were homozygous for Glu at codon 219 (Table 1). Korean sporadic CJD cases have the same allele frequency (1:0 Glu/Lys) as the Japanese sporadic CJD cases at codon 219. This result supports the Japanese data that Glu/Lys heterozygosity polymorphism at codon 219 may protect against sporadic CJD (χ^2=12.6945, P=0.0004).

In addition, we looked for the presence of known *PRNP* polymorphisms (codons 117, 124, 161, and 171) as well as the deletion of a single octarepeat; none of these was found in Korean sporadic CJD patients. All other nucleotides in CJD patients were the same as reported for the normal population.

In relation to the three polymorphisms found in healthy Koreans, the genotype frequency of $^{M}129^{M}/^{Q}219^{Q}/^{+}Octa^{+}$ was found in 100% of Korean sporadic CJD patients (Table 2). Statistical comparison of the heterozygotes at codons 129 and/or 219 of *PRNP* showed a significant difference between sporadic CJD patients and healthy Koreans (χ^2=21.0780, P<0.0001). This suggests that heterozygotes of *PRNP* polymorphisms at codons 129 and/or 219 confer decreased susceptibility to sporadic CJD.

Discussion

We have shown that all the 150 Korean sporadic CJD patients had the same genotype ($^{M}129^{M}/^{Q}219^{Q}/^{+}Octa^{+}$). This finding suggests that heterozygosity is somewhat protective regarding the occurrence of sporadic CJD. To our knowledge, our data are the first study of strong correlation between codon 129 polymorphism of *PRNP* and sporadic CJD in Asian population.

We previously reported that the frequencies of *PRNP* genotypes at codon 129 in the normal Korean population (94.23% M/M, 5.54% M/V, and 0.23% V/V) do not differ significantly from those previously reported for the Japanese (92% M/M, 8% M/V, and 0% V/V) [13]. However, they are different from those observed for the British population (37% M/M, 51% M/V, and 12% V/V) [6]. The higher incidence of Met/Met homozygosity at codon 129 in sporadic CJD patients compared to the normal Korean population (100 vs 94%) is similar to the tendency in the British population (83 vs 37%) rather than in the Japanese population (82 vs 92%) [6, 10]. Iatrogenic CJD was significantly linked to Val homozygosity, and all cases of variant CJD have been homozygous for Met at codon 129 [6, 11].

The genotype and allelic frequencies of *PRNP* at codon 219 in the normal Korean population were not similar to those in the Japanese [12, 13]. In vitro studies using a culture cell system revealed that *PRNP* containing the K219 allele resists conversion to PrP^{Sc} [15]. However, neither heterozygosity nor the Lys/Lys genotype at codon 219, combined with other mutations of *PRNP*, was found in the Korean population. Interestingly, only the Glu/Glu allele has been found at codon 219 in 150 Korean patients with sporadic CJD; thus, the Glu/Lys heterozygote present in 8% of the normal Korean population may provide protection from sporadic CJD. Our data support the Japanese finding that heterozygosity at codon 219 inhibits the development of sporadic CJD.

In our study of healthy Koreans, we found a single octapeptide deletion in *PRNP* in two individuals [13]. This result shows that this polymorphism is found in Asians as well as in Europeans. Interestingly, the change found in the two Koreans was R2 deletion, not R2–R3 or R3–R4 deletion, which are usually found in Europeans. The deletions in *PRNP* were not associated with sporadic CJD. In the current study, we did not find any Korean sporadic CJD patients with any octapeptide deletion. This result may be due to the small sample size of only 150 sporadic CJD patients compared to the sample of 529 healthy Koreans. We could not assess the importance of the absence of this deletion because of the low frequency of this polymorphism in healthy individuals.

In conclusion, our data suggest that there is an influence of genotype content of two *PRNP* polymorphisms on the occurrence of sporadic CJD in the Korean population, and that these data add to the other studies that attempt to assess the role that these polymorphisms play in CJD incidence.

Acknowledgements We thank Dr. Yong-Hee Kim for help with the statistical analysis. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (A020007).

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