

PRNP 1368 polymorphism is not associated with sporadic Creutzfeldt-Jakob disease in the Korean population

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Keywords:

Creutzfeldt-Jakob disease, Korean, population genetics, prion protein gene, single nucleotide polymorphism

Received 10 March 2008

Accepted 7 May 2008

Background: Human prion protein gene (*PRNP*) is considered a critical and fundamental gene in determining the incidence of human prion diseases. Codons 129 and 219 play an important role in the susceptibility to sporadic Creutzfeldt-Jakob disease (CJD). An association between sporadic CJD and the polymorphism (*PRNP* 1368) in an upstream of *PRNP* exon 1 has been reported in the British and German populations, but study in the Dutch population has failed to confirm an association. **Purpose:** To investigate whether the *PRNP* 1368 polymorphism is associated with sporadic CJD in the Korean population. **Methods:** We compared the genotype and allele frequencies of *PRNP* 1368 polymorphism in 171 sporadic CJD patients with those in 212 healthy Koreans. **Result and conclusion:** A significant difference of genotype and allele frequencies at *PRNP* 1368 was found between the normal Korean population and various European populations. In contrast to the results in the British and German populations, our study does not show a significant difference in genotype ($P = 0.2763$) and allele ($P = 0.3750$) frequencies of *PRNP* 1368 between sporadic CJD and normal controls.

Introduction

The human prion diseases 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*), located on chromosome 20p12 in humans. *PRNP* plays an important role in conferring susceptibility to prion diseases. Various mutations in the coding region of *PRNP* have been linked to familial Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia [2–5]. Polymorphisms of *PRNP* at codon 129 and 219 appear to influence expression of prion disease in sporadic CJD [6–8]. Homozygosity of methionine (Met) and valine (Val) at codon 129 of *PRNP* may cause a predisposition to sporadic CJD in Europeans and Koreans [6–9]. Results from a study in Japanese patients with sporadic CJD did not confirm the findings from these studies, however, the power of the Japanese data is limited because of small sample size and rare frequency of Val homozygotes [10]. All cases of variant CJD are homozygous for Met at codon 129 [11]. Glutamic acid (Glu)/Lysine (Lys) heterozygous polymorphism at codon 219 has been

reported to occur in Asian populations, but not in Caucasians [12–14]. It has been shown that the heterozygotes at codon 219 prevent the induction of sporadic CJD in Japanese and Korean populations [8,15]. Recently, there has been growing interest in polymorphisms outside the coding region of *PRNP*, as there is evidence that levels of *PRNP* expression influence incubation time and the susceptibility to prion diseases [16]. Several single nucleotide polymorphisms (SNPs) were identified in intronic and upstream regions of human *PRNP*. The C/G polymorphism at position 101 amongst the three SNPs in the regulatory region of *PRNP* was associated with sporadic CJD in the British population [17]. In contrast to this study, this polymorphism in Dutch and German populations was not associated with sporadic CJD [18,19]. The polymorphism (*PRNP* 1368; ref SNP ID: rs1029273) upstream of *PRNP* exon 1 was found to be associated with sporadic CJD in British and German populations [19,20]. However, this finding could not be confirmed in the Dutch population [21]. Although the *PRNP* 1368 polymorphism has been studied in several European countries [19–21], there have been no reports concerning this polymorphism from other parts of the world. To investigate whether *PRNP* 1368 polymorphism modulates susceptibility to sporadic CJD in the Korean population, we examined the genomic DNA of 171 Korean sporadic CJD patients and 212 healthy controls matched for age.

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Materials and methods

CJD patients and controls

We used previously established criteria [22] for diagnosis of sporadic CJD in Korea. Neuropathologically confirmed patients and/or patients with immunochemical detection of PrP^{Sc} in the brain were classified as definite CJD. Patients were classified as probable CJD if they exhibited rapidly progressive dementia, periodical sharp wave complexes on EEG, 14-3-3 protein in cerebrospinal fluid, a duration of dementia <2 years and two of the following: myoclonus, visual or cerebellar symptoms or both, pyramidal or extrapyramidal signs or both, or akinetic mutism. The sporadic CJD cases in Korea have been reported previously [23]. Of the 307 suspected CJD cases, 20 were classified as definite CJD and 151 were classified as probable; it was these two groups that were analyzed. We did genetic analysis on all CJD cases by sequencing the entire *PRNP* open reading frames. Patients with mutations in the *PRNP* were excluded from analysis. The control subjects were 212 unrelated individuals (98 male and 114 female; mean age 72.10 ± 8.78) matched for ethnic background to sporadic CJD patients (Table 1). All control subjects were volunteers recruited from routine health checkups at the Chunchon Sacred Heart Hospital.

Blood samples

Blood samples were collected from all sporadic CJD patients between May 1996 and April 2006. 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

Genomic DNA was extracted from 200 µl blood using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA, USA) following the supplier's instructions. PCR was performed with J-1 (GAGAAAACCTTGCGT-

CAGCA) and J-2 (AAGGTGCAGAAAAGATGGC) primers. These primers were designed to amplify a 586 bp product in an upstream region of *PRNP* exon 1. The PCR reagents contained 50 pmole of each primer, 5 µl of 10 × *Taq* DNA polymerase buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP mixtures, and 2.5 units of *Taq* DNA polymerase (Promega, Madison, WI, USA). The PCR conditions were 94°C for 2 min to denature and 35 cycles at 94°C for 45 s, 56°C for 45 s, and 72°C for 1 min 30 s, and then 1 cycle at 72°C for 10 min to extend the reaction. The Perkin-Elmer Cetus DNA thermal cycler (Perkin-Elmer, Norwalk, CT, USA) was used.

Restriction fragment length polymorphism

Restriction cleavage sites were searched using Webcutter, version 2.0 (Carolina Biological Supply Co., Burlington, NC, USA). A 20 µl aliquot of purified PCR mixture was digested at 37°C for 1 h with 5 units of *Pvu II* (Invitrogen, Carlsbad, CA, USA). Restriction products were separated on a 1.5% agarose gel and visualized with ethidium bromide staining under ultraviolet light.

Nucleotide sequencing analysis

The purification of PCR products for sequencing was carried out using a QIAquick gel extraction kit (Qiagen). The PCR products were directly sequenced on an ABI 377 automatic sequencer (ABI, Foster City, CA, USA) using a *Taq* dideoxy terminator cycle sequencing kit (ABI) and the same primers as indicated earlier in the standard conditions.

Statistical analysis

A Chi-squared test was used to determine whether *PRNP* 1368 was in Hardy-Weinberg equilibrium (HWE) in the Korean population. Differences in genotype, allele, and haplotype frequencies between the normal population and sporadic CJD cases were tested using Fisher's exact test within SAS 8.1 software (SAS Institute Inc., Cary, NC, USA). Haplotypes and their frequencies were inferred using the algorithm developed by Stephens *et al.* [24]. A *P*-value of less than 0.05 was considered statistically significant. We also examined Lewontin's *D'* (*|D'|*) and a linkage disequilibrium coefficient *r*² between three SNPs of *PRNP*.

Results

The genotype frequencies at *PRNP* 1368 were in HWE in Korean control group (*P* = 0.782) and sporadic CJD group (*P* = 0.150) (data not shown). We

Table 1 Characteristics of sporadic Creutzfeldt-Jakob disease (CJD) patients and controls

	CJD patients	Healthy controls
Number of subjects	171	212
Gender		
Male	88 (51.46%)	98 (46.23%)
Female	83 (48.54%)	114 (53.77%)
Mean age at disease onset (years ± SD)	63.13 ± 9.44	–
Mean age at blood collection (years ± SD)	–	72.10 ± 8.78

examined genotype and allele frequencies of *PRNP* 1368 polymorphism in a Korean normal population. Of the 212 normal controls, 79 (37.3%) were homozygous for C, 34 (16.0%) were homozygous for T, and 99 (46.7%) were heterozygous at *PRNP* 1368, with allele frequency of 0.61:0.39 C:T (Table 2). There were significant differences in genotype ($P < 0.0001$) and allele ($P < 0.0001$) frequencies at *PRNP* 1368 between the Korean and British populations. The frequency of the C allele in the normal Korean population is substantially greater than French, British, and Dutch populations (Table 2). To examine the correlation between *PRNP* 1368 polymorphism and susceptibility of sporadic CJD in Koreans, we analyzed the genotype and allele frequencies in 171 sporadic CJD patients. No significant difference was found between sporadic CJD and normal Korean controls in genotype ($P = 0.276$) or allele frequency ($P = 0.375$) (Table 2). This result suggests that the *PRNP* 1368 polymorphism does not increase susceptibility to sporadic CJD. Analysis of the haplotype frequency was performed in Korean controls and sporadic CJD patients. Six haplotypes were constructed in Koreans by three polymorphisms. Significant differences in ht 3 and ht 4 frequencies were detected between sporadic CJD and controls, dependent on the *PRNP* codon 129 or 219 polymorphism (Table 3). To investigate whether there was strong linkage disequilibrium amongst the three *PRNP* polymorphisms, linkage disequilibrium ($|D'|$), and r^2 were calculated in normal Korean controls. An SNP at *PRNP* 1368 was in moderate linkage disequilibrium with two SNPs at codons 129 and 219 of *PRNP* (Table 4). These results indicate that differences in *PRNP* 1368 do not affect susceptibility to sporadic CJD in the Korean population.

Discussion

Recently, studies on a possible correlation between the *PRNP* 1368 polymorphism and sporadic CJD in several

Table 3 Haplotype frequency of three *PRNP* polymorphisms in a normal population and in sporadic Creutzfeldt-Jakob disease (CJD) patients

Haplotype	<i>PRNP</i> 1368	Codon 129	Codon 219	Frequency		<i>P</i> -value
				Controls	CJD patients	
ht1	C	A	G	248 (56.6)	218 (63.7)	–
ht2	T	A	G	148 (35.0)	124 (36.3)	0.760
ht3	T	A	A	11 (2.8)	0	0.001
ht4	T	G	G	6 (1.4)	0	0.033
ht5	C	A	A	4 (1.0)	0	0.127
ht6	C	G	G	4 (1.0)	0	0.127

Table 4 Pairwise linkage disequilibrium coefficients (r^2) between three single nucleotide polymorphisms (SNPs) of *PRNP* in Korean normal population

Polymorphisms	D'		
	<i>PRNP</i> 1368	Codon 129	Codon 219
<i>PRNP</i> 1368	–	0.35	0.57
Codon 129	0	–	0.01
Codon 219	0.02	0	–

European countries have yielded contradictory results. In the present study, we failed to detect a significant association between *PRNP* 1368 polymorphism and the risk for sporadic CJD in the Korean population, which is relatively homogenous with regard to racial characteristics and ethnic background.

The genotype and allele frequencies of the *PRNP* 1368 polymorphism in the normal Korean population differs significantly from that previously reported for the British, French, and Dutch ($P < 0.001$) (Table 2). This polymorphism in large samplings of the normal British population was reported in two papers [20,25]. As the same group performed these studies, it is possible that the controls used in the two papers overlapped to some extent.

Table 2 Genotype and allele frequencies of the *PRNP* 1368 polymorphism in a normal population and in sporadic Creutzfeldt-Jakob disease (CJD) patients from Britain, France, Holland, and Korea

Subject group	Populations	Total, <i>n</i>	Genotype frequency (%)			<i>P</i> -value	Allele frequency		
			CC	CT	TT		C	T	<i>P</i> -value
Controls	British	269 ^a	46 (17.1)	114 (42.4)	109 (40.5)	–	0.38	0.62	–
		566 ^b	101 (17.9)	277 (48.9)	188 (33.2)	0.1080	0.42	0.58	0.1228
	French ^a	205	24 (11.7)	108 (52.7)	73 (35.6)	0.0596	0.38	0.62	0.9463
	Dutch ^c	248	48 (19.4)	107 (43.2)	93 (37.5)	0.7126	0.41	0.59	0.4081
CJD patients	Korean	212	79 (37.3)	99 (46.7)	34 (16.0)	<0.0001	0.61	0.39	<0.0001
	British ^a	93	36 (38.7)	35 (37.6)	22 (23.7)	<0.0001 ^d	0.575	0.425	<0.0001 ^d
	Dutch ^c	46	10 (21.7)	20 (43.5)	16 (34.8)	0.9093 ^d	0.435	0.565	0.6481 ^d
	Korean	171	65 (38.0)	88 (51.5)	18 (10.5)	0.2763 ^d	0.637	0.363	0.3750 ^d

^aMead *et al.* [20]; ^bRohrer *et al.* [25]; ^cCroes *et al.* [21]; ^dcomparisons are between results for control and for CJD patients of the same nationality.

Our negative results in the *PRNP* 1368 polymorphism are in agreement with the Dutch finding but not the British and German reports [19–21]. These conflicting results may be because of the limited sample size analyzed or to the difference in the genotype distribution of *PRNP* 1368 polymorphism between Asian and European ethnic groups (Table 2).

The familial forms of prion diseases are caused by mutations in the coding region of *PRNP*. These mutations are believed to be linked to differences in the prion protein structure and its stability. However, most CJD cases are not associated with mutations in the coding region of *PRNP*. Therefore, alternative mechanisms could be involved in susceptibility to prion diseases. Polymorphisms in the *PRNP* promoter region are associated with increased susceptibility to prion diseases in both cattle and mice [26–31]. These *PRNP* promoter polymorphisms influence the *PRNP* gene expression level, and these might be responsible for differences in incubation period [30,32]. Overexpression of *PRNP* gene in transgenic mice showed a decrease in incubation time, whereas *PRNP* knockout mice were resistant to prion disease after infection [16,27].

In previous studies, in addition to the SNPs in the coding region of *PRNP*, other SNPs were identified and associated with higher risk of developing sporadic CJD [19,20]. In particular, it has been shown that polymorphisms at *PRNP* 1368 and *PRNP* 34296 amongst these SNPs are associated with susceptibility to sporadic CJD, independent of the *PRNP* codon 129 polymorphism [19]. Thus, further investigations in the Korean population will be necessary to assess association between sporadic CJD and *PRNP* 34296 polymorphism, a SNP located approximately 8 kb downstream of the *PRNP* 3' coding region.

The present work showed neither significant association of *PRNP* 1368 polymorphism with sporadic CJD nor strong linkage disequilibrium between SNPs at codons 129/219 and *PRNP* 1368 polymorphism in the Korean population (Table 4); these findings differ from results obtained in previous studies based on British and German populations. This result suggests that *PRNP* 1368 polymorphism is not probably a functional SNP.

In our previous studies, we found that polymorphisms within the coding region of *PRNP* are not associated with sporadic Alzheimer's disease or vascular dementia (VaD) [33,34]. The downstream prion-like protein (doppel) encoded by prion-like protein gene (*PRND*) shares structural homology with the prion protein. We also observed that a polymorphism at 3' untranslated region +28 of the *PRND* is associated with sporadic CJD, whereas several polymorphisms within the coding region did not correlate with devel-

opment of sporadic CJD [35,36]. In future, we will investigate whether the polymorphisms in non-coding regions of *PRNP* and *PRND* are associated with sporadic AD or VaD in the Korean population.

In conclusion, *PRNP* 1368 polymorphism did not significantly contribute to the development of sporadic CJD in the Korean population. This finding was similar to the results of a sampling of Dutch subjects, but was different from results from British and German populations, which indicated that *PRNP* 1368 polymorphism is strongly associated with sporadic CJD. Our result is the first genetic association study of the *PRNP* 1368 polymorphism with sporadic CJD in an Asian population.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-311-E00034) and (KRF-2006-312-C00368).

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