

No association of prion protein gene polymorphisms with Alzheimer's disease in Korean population

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Abbreviations: AD, Alzheimer's disease; CJD, Creutzfeldt-Jakob disease; *PRNP*, human prion protein gene

Abstract

The polymorphism at codon 129 (M129V) of the human prion protein gene (*PRNP*) is a known risk factor for Creutzfeldt-Jakob disease (CJD) in Caucasians. There are few reports of this polymorphism's effect on memory and on the risk of Alzheimer's disease (AD). The M129V genotype distributions among Asians are very different from Caucasians. Another polymorphism, codon 219 (E219K) is not found in Caucasians. We investigated two polymorphisms of *PRNP*, M129V (rs1799990) and E219K (rs1800014) in 297 Korean AD patients and 217 healthy subjects. The analysis of the genotype and allele distributions showed no significant difference between the AD patients and the controls in both polymorphisms ($P=0.19$ genotype, $P=0.51$ allele for M129V; $P=0.64$ genotype, $P=0.50$ allele for E219K). Also, the *PRNP* polymorphisms were not significantly associated with AD when the populations were stratified for the presence or absence of apolipoprotein E- $\epsilon 4$ (ApoE- $\epsilon 4$) allele. These results suggest that the *PRNP* genetic variants are not associated with the risk for AD in Korean population.

Keywords: Alzheimer disease; apolipoproteins E; prion diseases; Creutzfeldt-Jakob syndrome; polymorphism, single nucleotide; PRNP protein, human

Introduction

Alzheimer's disease (AD) and Creutzfeldt-Jakob disease (CJD) have a common pathogenic mechanism in that the protein deposits in the brain due to the conversion of a soluble, normal protein into an insoluble, aggregated form leading to fatal neurodegeneration. In AD, beta amyloid peptide (A β), aggregates to form senile plaques. Prion diseases including CJD, Gerstmann-Sträussler-Scheinker disease, kuru and fatal familial insomnia in humans are characterized by the conformational change of the cellular prion protein (PrP^C) into a pathological and infectious isoform (PrP^{Sc}) (reviewed in Prusiner, 1994; Aguzzi and Haass, 2003). A β in AD and PrP^{Sc} in CJD can coexist. Prion protein was localized to senile plaques in the aged and AD brain and promoted plaque formation in 3 month-old bigenic mice carrying mutant human APP and prion protein genes (Kovacs *et al.*, 2002; Schwarze-Eicker *et al.*, 2005). Also, both diseases show a consistent pattern of cortical degeneration (reviewed in Forman *et al.*, 2004; Armstrong *et al.*, 2005).

The human prion protein gene (*PRNP*) on chromosome 20 is reported to have more than 10 polymorphisms that lead to amino acid substitutions. Among them the single nucleotide polymorphisms (SNPs) at codons 129 and 219 seem to be pathologically significant. The polymorphism at codon 129 encoding either methionine (M) or valine (V) affects genetic susceptibility to human prion diseases and appears to critically influence the propagation of PrP^{Sc}. The survival period was shorter for homozygotes (MM and VV) than heterozygote (MV) ($P < 0.0001$) (Pocchiari *et al.*, 2004) and MV heterozygote individuals had some protection against kuru epidemic among the Fore tribe of Papua New Guinea (Mead *et al.*, 2003). In case of polymorphism at codon 219 of the *PRNP* gene in which lysine is substituted for glutamic acid (E219K), the K allele was found to be protective against sporadic CJD in Asia and Pacific (Soldevila *et al.*, 2003).

Berr *et al.* (1998) observed the correlation bet-

ween the codon 129 polymorphisms and cognitive performance. Risk of cognitive impairment was increased significantly in VV individuals compared with either MV or MM subjects. Down Syndrome (DS) patients carrying at least one V allele showed faster decline in intellectual ability and the earlier onset of dementia than MM subjects (Del Bo *et al.*, 2003). AD patients with VV genotype showed acceleration of cognitive decline (Del Bo *et al.*, 2005). There have been reports showing significant association between early onset Alzheimer's disease (EOAD) and homozygosity of the codon 129 polymorphism. The VV genotype was highest in Dutch EOAD patients and MM genotype in German AD patient with onset age of 70 or younger (Dermaut *et al.*, 2003; Riemenschneider *et al.*, 2004). Also, in Polish population, the MM and VV genotypes were higher in AD patients (Golanska *et al.*, 2004). On the other hand, a study with Spanish sporadic AD patients showed no association between the homozygosity and AD even after stratifying by ApoE genotype, age at onset, and gender (Combarros *et al.*, 2000). The two polymorphisms, M129V and E219K, were not associated with AD (Ohkubo *et al.*, 2003) in Japanese population. Because of the potential importance of the *PRNP* polymorphisms in association with AD and the very different allele frequencies of Koreans from Caucasians, we investigated the *PRNP* codon 129 and 219 polymorphisms of Korean AD patients and an age-matched control group.

Materials and Methods

Subjects and blood samples

Blood samples were collected from 297 AD patients and 217 cognitively healthy control subjects. All AD patients were self-referring to the Samsung Medical Center Memory Disorder Clinic and met NINCDS-ADRDA and neuropsychological battery (Welsh *et al.*, 1994) for probable AD. Age-matched controls were randomly selected among healthy volunteers who visited Health Promotion Center at Samsung Medical Center for health checkup. The study was approved by the Ethics Committee and informed consents were obtained from the volunteers. Blood samples of each subject were taken by venous puncture after obtaining informed consent.

Genotype analysis

Genomic DNA was extracted from 200 μ l blood using the QIAamp DNA blood mini kit (Qiagen, USA) following the supplier's instructions. PCR

was performed with PCR-forward (GATGCTGG-TTCTCTTTGTGG) and PCR-Reverse (CCCACTA-TCAGGAAGATGAG) primers. The PCR reagents contained 50 pmol of each primer, 5 μ l of 10 \times *Taq* DNA polymerase buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP mixture, and 2.5 U of *Taq* DNA polymerase (Promega, USA). The PCR conditions were 94°C for 5 min to denature; 30 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min; and 1 cycle of 72°C for 10 min to extend the reaction using Perkin-Elmer Cetus DNA thermal cycler (Perkin-Elmer). The PCR products were purified using a PCR purification kit (Qiagen). DNA sequencing was carried out on an ABI 377 automatic sequencer using a *Taq* dideoxy terminator cycle sequencing kit (ABI). The PCR products were sequenced with PCR-forward and PCR-Reverse primers and also with two nested primers; Seq-forward (AAGCCTGGAGGATGGAACAC), Seq-reverse (ACACATCTGCTCAACCACGC). Nucleic acid sequences were assembled and edited using a combination of the ABI 377 DNA Sequencer Data Analysis program and Sequence Navigator software. For restriction fragment-length polymorphism (RFLP), 20 μ l aliquot of purified PCR mixture was digested at 37°C for 1 h with 5 units of *Nsp*I (MBI). Restriction products were separated on a 1.5% agarose gel and visualized with ethidium bromide staining under UV light. The APOE genotyping was done with INNO-LiPA ApoE Kit (Innogenetics N.V., Belgium) following manufacturer's instructions.

Statistical analysis

SAS 8.0 software (SAS Institute, Inc.) was used for statistical analysis. Differences in genotype frequencies between AD patients and the control subjects were analyzed by χ^2 tests. The genotypes were in Hardy-Weinberg equilibrium.

Results

For AD patients, the mean age was 72.6 \pm 9.2 years (men 28.3%) and for the control group, 66.6 \pm 6.7 yr (men 35.5%). The distributions of genotypes and allele frequencies of *PRNP* polymorphisms of the AD patients and the control group are shown in Table 1. As previously reported (Jeoung *et al.*, 2004), the 129V allele frequency (0.0331) and that of 219K (0.0467) were very low in Korean population. Neither the genotype frequencies nor the allele frequencies of codon 129 showed association with AD ($P = 0.19$, genotype; $P = 0.51$, allele). There was no significant difference in codon 219 between AD and the controls

Table 1. PRNP codon 129 and codon 219 genotype and allele frequencies in AD patients and control subjects.

			Total (n = 514) n (%)	AD (n = 297) n (%)	Control (n = 217) n (%)	P-value
PRNP_129	Genotype	M/M	481 (93.58)	275 (92.59)	206 (94.93)	0.1926
		M/V	32 (6.23)	22 (7.41)	10 (4.61)	
		V/V	1 (0.19)	0 (0.00)	1 (0.46)	
PRNP_129	Allele	M	994 (96.69)	572 (96.30)	422 (97.24)	0.5127
		V	34 (3.31)	22 (3.70)	12 (2.76)	
PRNP_219	Genotype	E/E	469 (91.92)	273 (91.92)	196 (90.32)	0.6366
		E/K	42 (7.74)	23 (7.74)	19 (8.76)	
		K/K	3 (0.58)	1 (0.34)	2 (0.92)	
PRNP_219	Allele	E	980 (95.33)	569 (95.79)	411 (94.70)	0.5034
		K	48 (4.67)	25 (4.21)	23 (5.30)	

Table 2. PRNP genotype, allele frequencies in AD patients and control subjects with and without ApoE-ε4.

		ApoE-ε4 (-) (n = 306)				ApoE-ε4 (+) (n = 181)			
		Total n (%)	AD n (%)	Control n (%)	P-value	Total n (%)	AD n (%)	Control n (%)	P-value
<i>PRNP_129</i>									
Genotype	M/M	292 (95.42)	133 (94.33)	159 (96.36)	0.3291	164 (90.61)	132 (91.03)	32 (88.89)	0.7498
	M/V	13 (4.25)	8 (5.67)	5 (3.03)		17 (9.34)	13 (8.97)	4 (11.11)	
	V/V	1 (0.33)	0 (0.00)	1 (0.61)		0 (0.00)	0 (0.00)	0 (0.00)	
Allele	M	597 (97.55)	274 (97.16)	323 (97.88)	0.7577	345 (95.30)	277 (95.52)	68 (94.44)	0.7551
	V	15 (2.45)	8 (2.84)	7 (2.12)		17 (4.70)	13 (4.48)	4 (5.56)	
<i>PRNP_219</i>									
Genotype	E/E	277 (90.52)	127 (90.07)	150 (90.91)	0.9213	167 (92.27)	136 (93.79)	31 (86.11)	0.0765
	E/K	27 (8.82)	13 (9.22)	14 (8.48)		13 (7.18)	9 (6.21)	4 (11.11)	
	K/K	2 (0.61)	1 (0.71)	1 (0.61)		1 (0.55)	0 (0.00)	1 (2.78)	
Allele	E	581 (94.93)	267 (94.68)	314 (95.15)	0.9364	347 (95.86)	281 (96.90)	66 (91.67)	0.0890
	K	31 (5.07)	15 (5.32)	16 (4.85)		15 (4.14)	9 (3.10)	6 (8.33)	

($P = 0.64$, genotype; $P = 0.50$, allele), either. The stratification of AD patients to early-onset (< 65 years) and late-onset (≥ 65 years) did not reveal any association of the two SNPs with AD (data not shown).

The samples were stratified by the presence and the absence of ApoE-ε4 allele and the genotype and allele frequencies of the two PRNP SNPs are shown in Table 2. We found no significant difference in the frequency of PRNP polymorphisms between ApoE-ε4 allele carriers and non-carriers

of AD patients ($P > 0.05$).

Discussion

There seem to be great differences among various populations in the codon 129 allele and genotype distributions. The M allele frequency is highest in Central and East Asia ($> 90\%$), high in the Pacific (approximately 81%), in Europe (57-75%) and low (approximately 30%) in Native Americans (Palmer,

1991; Soldevila *et al.*, 2003; Jeong *et al.*, 2004; Yu *et al.*, 2004; Lucotte and Mercier, 2005). Similarly, the MM genotype is highly abundant (> 93%) in East Asia. With regard to the extremely high prevalence of MM genotype in Koreans, much attention has been paid to its association with AD because the MM and/or VV homozygotes is known to be associated with either CJD or AD in certain Caucasians. In German population, MM and VV genotypes conferred increasing risk for AD with decreasing age at onset whereas no association was obtained in patients with onset at older than 70 yr. More MM genotype in AD patients is observed especially among non-ApoE- ϵ 4 allele carriers (Riemenschneider *et al.*, 2004). In Polish population, the MM and VV homozygotes were higher in AD patients than in the control subjects (Golanska *et al.*, 2004). However, we found no association between M129V polymorphism and AD in Korean population. Thus, our study contrasted with the results obtained from the German and Polish populations but is consistent with that obtained from Japanese that showed no association of the M129V polymorphism with AD regardless of the age at onset or ApoE- ϵ 4 genotypes. Also, no significant difference was observed in Spanish population and in Italian population (Combarros *et al.*, 2000; Del Bo *et al.*, 2005). With regard to such discrepancy among ethnic groups, meta-analysis of six published reports showed that Caucasian subjects homozygous at codon 129 had a moderate but significant risk [OR = 1.3, 95%, CI: 1.0-1.6, $P = 0.05$] of developing AD compared to heterozygous individuals (Del Bo *et al.*, 2005). Since ethnic background strongly influences on the M129V polymorphism and the genetic susceptibility to AD, further evaluation of the data from East Asians and across ethnic groups will solve the controversial issues of a genetic role of *PRNP* in AD and cognitive decline in the elderly.

The frequencies of the EE genotype and of the E allele at codon 219 were significantly higher in the Korean population than the Japanese population (Jeong *et al.*, 2004). The 219 polymorphism was not associated with AD in Korean population regardless of the age of onset or ApoE- ϵ 4 status as seen in Japanese population (Ohkubu *et al.*, 2003). This polymorphism is not found in Caucasians (Petraroli and Pocchiari, 1996). Thus, these data suggest that the genetic risk for AD by the codon 219 polymorphism seems to be minimal although it may lower and counteract the susceptibility to CJD by the codon 129 polymorphism (Soldevila *et al.*, 2003).

The present study, the first genetic study of the

PRNP gene in association with diseases in Korean population, does not support the hypothesis that the *PRNP* SNP might play a role in the genetic susceptibility to AD. The negative outcome might be attributable to the fact that both VV genotype (one in 514) and V allele are very rare (3.3%) in Korean population compared to European populations (> 30%). Even though the physiological function of normal prion protein and the mechanism of brain damage by its infectious form remain to be identified, PrP might be still involved in A β aggregation and hence AD pathology.

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