

## SHORT REPORT

# Polymorphism at 3' UTR + 28 of the prion-like protein gene is associated with sporadic Creutzfeldt–Jakob disease

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The downstream prion-like protein (doppel or Dpl) shares significant biochemical and structural homology with the cellular prion protein, PrP<sup>C</sup>, which is considered as a responsible protein for the transmissible spongiform encephalopathies (TSEs) or prion diseases. Recently, polymorphisms in open reading frame (ORF) of the prion-like protein gene (*PRND*) have been analysed in relation to the occurrence of prion diseases and other neurodegenerative disorders. We examined the role of a single-nucleotide polymorphism (SNP) at 3' untranslated region (UTR) + 28 of *PRND*. We analysed this polymorphism in 110 Korean patients with sporadic Creutzfeldt–Jakob disease (CJD) and 102 healthy control subjects. Significant differences in genotype ( $P=0.005$ ) and allele ( $P=0.032$ ) frequencies at 3' UTR + 28 were observed between sporadic CJD and normal controls. This result suggests that the *PRND* polymorphism at 3' UTR + 28 might be associated with the occurrence of sporadic CJD.

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## Introduction

Human prion protein contains 253 amino acids encoded by the prion protein gene (*PRNP*), which is located on chromosome 20 in humans.<sup>1</sup> *PRNP* plays an important role in conferring susceptibility 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).<sup>2–4</sup> Moreover, polymorphisms of *PRNP*

appear to be able to influence expression of prion disease in sporadic and iatrogenic CJD.<sup>5–7</sup>

The downstream prion-like protein (doppel or Dpl) shares significant biochemical and structural homology with the cellular prion protein, PrP<sup>C</sup>. The PrP-like protein gene (*PRND*) is located at approximately 27 kb downstream of the human *PRNP*.<sup>8</sup> In the *PRND*, the codons 25, 56, and 174 and the 3' untranslated region (UTR) + 28 site have been shown to be polymorphic.<sup>9,10</sup> The *PRND* polymorphism at 3' UTR + 28 is a noncoding thymidine (T)–cytidine (C) change. Recently, polymorphisms in the open reading frame (ORF) of human *PRND* have been analysed in relation to the occurrence of prion diseases and other neurodegenerative disorders.<sup>9–14</sup> However, an association between *PRND* polymorphism at 3' UTR + 28 and sporadic

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CJD as well as genotype and allele distributions of this polymorphism in the normal population have not been reported thus far.

Therefore, we have examined *PRND* polymorphism at 3' UTR +28 in 102 healthy Koreans and 110 sporadic CJD patients to investigate whether the genotype and allele frequencies of this *PRND* polymorphism is associated with sporadic CJD in a Korean population.

## Materials and methods

### CJD patient population

We used previously established criteria<sup>15</sup> to enhance the level of accuracy of diagnosis of sporadic CJD in Korea. Neuropathologically confirmed patients and/or patients with immunochemical detection of PrP<sup>Sc</sup> in the brain were classified as definite CJD. Patients were classified as probable CJD if they exhibited a rapidly progressive dementia, periodical sharp wave complexes (PSWC) on electroencephalography (EEG), 14-3-3 protein in CSF, a duration of dementia <2 years and two of the following: myoclonus, visual and cerebellar symptoms or both, pyramidal or extrapyramidal signs or both, or akinetic mutism. The sporadic CJD cases, which were diagnosed by immunohistochemistry and DNA sequencing, have been reported previously in Korea.<sup>16</sup> Of the 110 suspected CJD cases, 17 cases were classified as definite CJD and 93 cases were classified as probable CJD. Blood samples were collected from 102 healthy volunteers and 110 sporadic CJD patients in Korea. The study was approved by the Ethical Committee of Chunchon Sacred Heart Hospital.

### Laboratory analyses

Genomic DNA was extracted from 200  $\mu$ l of blood using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA, USA) following the supplier's instructions. For polymerase chain reaction (PCR) amplification of *PRND* polymorphism at 3' UTR +28, we designed the Hdpl-1 (5'-GTTTCTCTGGCAGGTCTG-3') and Hdpl-2 (5'-GAGAAGAGCTGGGTCAC TT-3') primers producing a 623 bp fragment. The PCR reagents contained 50 pmol of each primer, 5  $\mu$ l of 10  $\times$  *Taq* DNA polymerase buffer, 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP mixture, and 2.5 units of *Taq* DNA polymerase (Promega, Madison, WI, USA). The PCR conditions were as follows: an initial denaturation step of 95°C for 2 min, 30 cycles of 94°C

for 40 s, 56°C for 45 s, and 72°C for 90 s, and final extension at 72°C for 10 min. The purification of PCR products for sequencing was performed using a QIAquick gel extraction kit (Qiagen, Valencia, CA, USA). The DNA sequencing was performed on an ABI 377 automatic sequencer using a *Taq* dideoxy terminator cycle sequencing kit (ABI, Foster City, CA, USA). Nucleic acid sequences were assembled and edited using a combination of the ABI 377 DNA Sequencer Data Analysis Program and Sequence Navigator Software.

### Statistical analyses

$\chi^2$  tests were used to determine whether *PRND* 3' UTR +28 were in Hardy-Weinberg equilibrium (HWE) in the Korean population. Differences in genotype and allelic frequencies between healthy Koreans and patients with sporadic CJD were compared by  $\chi^2$  tests using the SAS 8.1 Software (SAS Institute Inc., Cary, NC, USA). We also examined Lewontin's *D'* ( $|D'|$ ) and linkage disequilibrium coefficient  $r^2$  between three single-nucleotide polymorphisms (SNPs) of *PRND* and *PRNP*.<sup>17,18</sup>

## Results

The genotype frequencies at *PRND* 3' UTR +28 were in HWE in Korean control group ( $P=0.373$ ) (Table 1). We analysed genotype and allele distributions of 3' UTR +28 polymorphism in a Korean normal population. Of the 102 normal samples, 24 (23.5%) were homozygous for T, 20 (19.6%) were homozygous for C, and 58 (56.9%) were heterozygous at 3' UTR +28, with allele frequency of 0.52:0.48 T:C (Table 1). To investigate whether there is an association between this polymorphism and sporadic CJD, we analysed the genotype and allele frequencies of Korean sporadic CJD cases. Of the 110 sporadic CJD cases, 48 (43.6%) were homozygous for T, 21 (19.1%) were homozygous for C, and 41 (37.3%) were heterozygous at 3' UTR +28, with allele frequency of 0.623:0.377 T:C (Table 1). Significant differences were found between sporadic CJD and normal controls in genotype distribution ( $P=0.005$ ) and in allele frequency ( $P=0.032$ ) at 3' UTR +28. Furthermore, as shown in Table 2, neither age nor gender had any influences on the *PRND* polymorphism at 3' UTR +28. We have also examined whether there are significant differences in genotype frequency of this polymorphism between definite cases or probable cases and controls.

**Table 1** Genotype and allele frequencies of the polymorphism at 3' UTR +28 of the *PRND* in samples of the normal Korean population and sporadic CJD patients

Subject groups	Total, n	Genotype frequency, n (%)			Allele frequency, n (%)		P-value for HWE
		T/T	C/T	C/C	T	C	
Control	102	24 (23.5)	58 (56.9)	20 (19.6)	106 (52.0)	98 (48.0)	0.373
Sporadic CJD	110	48 (43.6)	41 (37.3)	21 (19.1)	137 (62.3)	83 (37.7)	0.095
		$(\chi^2 = 10.6569, df = 2, P = 0.005)$			$(\chi^2 = 4.6006, df = 1, P = 0.032)$		

**Table 2** Genotypes at 3' UTR +28 of the *PRND*, age, and sex in Korean normal population and definite/probable cases with sporadic CJD

	Total, <i>n</i>	Genotype frequency, <i>n</i> (%)			<i>P</i> -value <sup>a</sup>
		T/T	C/T	C/C	
Normal controls	102	24 (23.5)	58 (56.9)	20 (19.6)	—
Age (years)	57.7 ± 8.6 <sup>b</sup>	59.5 ± 9.5	57.1 ± 8.7	58.3 ± 8.2	
Gender (M/F)	47/55	11/13	27/31	9/11	
Sporadic CJD patients	110	48 (43.6)	41 (37.3)	21 (19.1)	0.005
Definite CJD cases	17	9 (52.9)	5 (29.4)	3 (17.6)	0.036
Age (years)	57.2 ± 9.6	58.0 ± 10.2	54.8 ± 6.0	59.0 ± 1.7	
Gender (M/F)	10/7	4/3	3/2	3/1	
Probable CJD cases	93	39 (41.9)	36 (38.7)	18 (19.4)	0.015
Age (years)	63.8 ± 11.9	63.9 ± 9.6	64.5 ± 11.5	62.2 ± 16.8	
Gender (M/F)	43/50	18/21	15/21	10/8	

M/F = Male/Female.

<sup>a</sup>Based on comparison of genotype frequencies between controls and sporadic CJD patients.<sup>b</sup>Data are expressed as mean ± SD.**Table 3** Pairwise linkage disequilibrium coefficients between three SNPs of *PRND* and *PRNP* in Korean control samples

Polymorphisms	$ D' ^a$		
	<i>PRNP</i> codon 129	<i>PRNP</i> codon 219	<i>PRND</i> 3' UTR +28
$r^2^b$ PRNP codon 129	—	1	0.1
PRNP codon 219	0	—	0.46
PRND 3' UTR +28	0	0.01	—

<sup>a</sup>The Lewontin's standardized coefficient.<sup>b</sup>The correlation coefficient.

There was significant difference in genotype frequencies between normal controls and definite sporadic CJD ( $P=0.036$ ) as well as a significant difference between controls and probable sporadic CJD ( $P=0.015$ ) (Table 2). *PRNP* polymorphisms at codon 129 and 219 are thought to play an important role in susceptibility to sporadic CJD.<sup>6,7</sup> These polymorphisms in Korean are also associated with sporadic CJD (data not shown). To examine whether strong linkage disequilibrium is observed between the SNP of *PRND* at 3' UTR + 28 and the two SNPs of *PRNP*, linkage disequilibrium coefficients ( $|D'|$ ) and  $r^2$  between three SNPs were calculated in normal Korean controls (Table 3). Absolute linkage disequilibrium ( $|D'|=1$  and  $r^2=1$ ), complete linkage disequilibrium ( $|D'|=1$  and  $r^2 \neq 1$ ), and strong linkage disequilibrium ( $|D'|>0.9$ ) were not observed in three SNPs. An SNP at *PRND* 3' UTR + 28 was in no linkage disequilibrium with two SNPs at *PRNP* codons 129 and 219.

## Discussion

Polymorphisms in the *PRND* have been reported in several European countries, whereas analysis of *PRND* sequence in

Asian countries has not been examined previously. In this study, we studied polymorphism at 3' UTR + 28 of *PRND* in a sampling of the Korean population. We have found evidence that a specific genotype (TT) or allele (T) at 3' UTR + 28 of the *PRND* is a risk factor for sporadic CJD.

In a previous study, it has been shown that the genotype frequencies of the ORF of *PRNP* at codon 219 as well as codon 129 are associated with susceptibility to sporadic CJD.<sup>6,7</sup> The doppel protein has 25% homology to the C-terminal part of the prion protein and the overexpression of doppel is associated with cerebellar neurodegeneration of PrP-knockout mice.<sup>8</sup> These studies strongly suggest that polymorphisms of the *PRND* could play a role in prion diseases.

The major *PRND* polymorphism reported in several European countries was substitution of threonine (Thr) with methionine (Met) at codon 174. In these studies, codon 174 polymorphism in British and French populations was not associated with sporadic CJD or variant CJD,<sup>9,10</sup> nor was this polymorphism in Spanish people associated with sporadic Alzheimer's disease (AD).<sup>12</sup> In contrast, in German and Dutch populations codon 174 polymorphism was associated with sporadic CJD and/or AD.<sup>11,13</sup> In the German control group, genotype frequencies were not in HWE proportions ( $P=0.002$ ). This prevents the exact interpretation of the study since HWE may be deviated by bias in sample selection or genotyping errors.<sup>11</sup> The polymorphisms at codons 26 and 56 of *PRND* were not associated with susceptibility to sporadic CJD.<sup>9,10</sup> The *PRND* polymorphism at 3' UTR + 28 has been reported in two studies.<sup>9,19</sup> The allele frequency for *PRND* polymorphism at 3' UTR + 28 in the normal Korean population (52% T, 48% C) is not significantly different from that previously reported for healthy French controls (51% T, 49% C) ( $\chi^2=0.0579$ ,  $df=2$ ,  $P=0.810$ ).<sup>19</sup> The genotype frequency of this *PRND* polymorphism is not related to

those of polymorphisms at codons 129 and 219 of PRNP, as the genotype frequencies for the codons 129 and 219 in Korean are quite different from those in French. It has been known that SNPs in 3' UTR of genes can affect gene expression.<sup>20</sup> It is possible that the 3' UTR +28 polymorphism is involved in either doppel gene expression or gene stability and that this may play a role in susceptibility to sporadic CJD. Recently, it was found that Dpl levels in brain tissue of sporadic CJD patients were similar to the levels in normal controls.<sup>21</sup> This result suggests that expression levels of Dpl may not be influenced in prion disease.<sup>21</sup> Unexpectedly, genotype frequency of T/C heterozygotes in Korean sporadic CJD patients (37.3%) was lower than that in normal controls (56.9%). There may be various explanations for this result. One explanation is that this polymorphism may be in linkage disequilibrium with a nearby locus involved in sporadic CJD. Undetected polymorphisms in strong linkage disequilibrium with this polymorphism may cause a spurious association. Finally, similar to our results, an association between SNP at 3' UTR and the incidence of other diseases has been reported.<sup>22,23</sup> In the current study, comparing the total percentage of TT vs the combined TC/CC for CJD patients yielded 43.6% TT and 56.4% TC/CC compared to 23.5% TT and 76.5% TC/CC for controls; this was significantly different ( $\chi^2 = 9.5403$ ,  $df = 1$ ,  $P = 0.002$ ).

Our results are the first genetic association study of the PRND noncoding region with sporadic CJD. Recently, we reported that the distributions of codons 129 and 219 genotypes of PRNP in a Korean population differ significantly from those reported for other ethnic groups.<sup>24</sup> Thus, further investigations in different ethnic groups including Europeans will be necessary to assess association between sporadic CJD and the PRND 3' UTR +28 polymorphism. Furthermore, since it is unknown whether this polymorphism affects mRNA stability or gene expression of PRND, further experiments should be conducted to clarify the role of this polymorphism in PRND function.

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