Lack of association between \textit{PRNP} 1368 polymorphism and Alzheimer’s disease or vascular dementia

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Running title: PRNP polymorphism and dementia
Abstract

Background: Polymorphisms of the prion protein gene (PRNP) at codons 129 and 219 play an important role in the susceptibility to Creutzfeldt-Jakob disease (CJD), and might be associated with other neurodegenerative disorders. Several recent reports indicate that polymorphisms outside the coding region of PRNP modulate the expression of prion protein and are associated with sporadic CJD, although another studies failed to show an association. An association between the polymorphism (PRNP 1368) in an upstream of PRNP exon 1 and either Alzheimer’s disease (AD) or vascular dementia (VaD) has not been reported.

Methods: To investigate whether the PRNP 1368 polymorphism is associated with the occurrence of AD or VaD in the Korean population, we compared the genotype, allele, and haplotype frequencies of the PRNP 1368 polymorphism in 152 AD patients and 192 VaD patients with frequencies in 268 healthy Koreans.

Results and Conclusion: Significant differences in genotype, allele and haplotype frequencies of PRNP 1368 polymorphism were not observed between AD and normal controls. There were no significant differences in the genotype and allele frequencies of the PRNP 1368 polymorphism between Korean VaD patients and normal controls. However, in the haplotype analysis, haplotype Ht5 was significantly over-represented in Korean VaD patients. This was the first genetic association study of a polymorphism outside the coding region of PRNP in relation to AD and VaD.
Keywords Prion protein gene · Alzheimer’s disease · vascular dementia · single nucleotide polymorphism · population genetics · Korean
Background

Alzheimer’s disease (AD), the most common cause of dementia in the aged population, is characterized neuropathologically by the presence of neurofibrillary tangles and amyloid plaques in the brain and clinically by gradual loss of memory. Several genes associated with AD have been identified, including amyloid precursor protein gene (APP), presenilin-1 gene (PS1), presenilin-2 gene (PS2), and the apolipoprotein E gene (ApoE) [1]. AD and prion diseases, such as Creutzfeldt-Jakob disease (CJD), share a number of clinical, pathogenetic and pathological features. A structural hallmark of AD is amyloid-β peptide (Aβ) aggregates in extracellular amyloid deposits defined as senile plaques, while in CJD there is an accumulation of abnormal protease-resistant isoform (PrP<sup>res</sup>) in neurons and extracellular amyloid-like aggregates. Aβ-positive senile plaques commonly contain PrP deposits in AD [2-4] and incidentally are positive for PrP in prion diseases such as CJD [5].

Vascular dementia (VaD) is the second most common cause of dementia after AD. VaD in Korea was more prevalent than AD in the 1980s [6]. VaD is a clinical syndrome causing cognitive decline due to cerebrovascular lesions. Risk factors for VaD are age, sex, race, hypertension, smoking, diabetes mellitus, and hypercholesterolemia. However, there is no conclusive evidence for the association of genetic polymorphisms with VaD. VaD patients show pathophysiologic similarities to sporadic CJD, including a role for oxidative stress and the occurrence of dementia.

Prion protein contains 253 amino acids encoded by prion protein gene (PRNP), located on chromosome 20p12.3 in humans. PRNP plays an important role in conferring susceptibility or resistance to prion disease. A number of mutations in the open reading frame (ORF) are linked to the familial form of prion diseases [7,8]. Polymorphisms at
codons 129 or 219 of *PRNP* are susceptibility factors to sporadic CJD [9-11]. In several European populations, an association between the *PRNP* codon 129 polymorphism and AD was reported [12-15]. In contrast to these studies, other studies failed to detect a significant association between this polymorphism and AD [16-18], and in Asian populations, no association between the *PRNP* codons 129/219 polymorphisms and AD was reported [19]. Recently, there has been growing interest in polymorphisms outside the ORF of *PRNP*. Several polymorphisms were identified in intronic and upstream regions of human *PRNP*. The single nucleotide polymorphism (SNP) at position -101 (*PRNP* 12533) within the regulatory region of *PRNP* was associated with sporadic CJD in the British population [20]. This is not consistent with the data in Dutch and German populations [21,22]. The polymorphism (*PRNP* 1368) in an upstream of *PRNP* exon 1 was found to be associated with sporadic CJD in British and German populations [22,23], but this association was not seen in Dutch and Korean populations [24,25]. In a British population, there was no association of *PRNP* 1368 polymorphism with frontotemporal lobar degeneration (FTLD) [26]. Although *PRNP* 1368 polymorphism has been studied in sporadic CJD and FTLD patients, the association study between the *PRNP* 1368 polymorphism and either AD or VaD has not been reported so far.

In the present study, the purpose was to investigate the genotype and allele frequency of a polymorphism outside the coding region of *PRNP* in Korean AD and VaD patients and to determine the correlation between this polymorphism and the incidence of AD and VaD in the Korean population.
**Methods**

Analysis included 152 Korean patients with AD (51 male and 101 female; mean age at disease onset 73.48 ± 8.00 years), which were diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association criteria (NINCDS-ADRDA) [27]. One hundred ninety two Korean patients with VaD (100 male and 92 female; mean age: 71.95 ± 8.92 years) were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [28] and National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l’Enseignement en Neurosciences (NINDS-AIREN) criteria [29]. The control subjects were 268 unrelated individuals (118 male and 150 female; mean age 71.17 ± 8.68 years) matched for age and ethnic background to AD patients and VaD patients (Table 1). All control subjects were volunteers recruited from routine health checkups at the Chunchon Sacred Heart Hospital. None of them presented symptoms of dementia or any movement disorders. Absence of dementia was determined by considering past history and Korean Mini-Mental State Examination (K-MMSE) criterion (score of >24).

Blood samples were collected from 268 healthy Korean volunteers, 152 AD patients and 192 VaD patients between May 2000 and June 2008. The study was approved by the Ethical Committee of Chunchon Sacred Heart Hospital and an informed consent was given by all subjects or their caregivers. All blood samples were frozen at −70°C prior to analysis.

Genomic DNA was extracted from 200 μl blood using the QIAamp DNA blood mini kit.
(Qiagen, USA) following the supplier’s instructions. Polymerase chain reaction (PCR) was performed with J-1 (GAGAAAACCTTGCAGTCAGCA) and J-2 (AAGGTGCAGAAAAGATGGGC) 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, USA). The PCR conditions were 94°C for 2 min to denature, and 35 cycles of 94°C for 45 sec, 56°C for 45 sec, and 72°C for 1 min 30 sec, and then 1 cycle of 72°C for 10 min to extend the reaction. The Perkin-Elmer Cetus DNA thermal cycler (Pekin-Elmer, USA) was used.

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

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

A χ² test was used to determine whether the PRNP 1368 polymorphism was in Hardy-Weinberg equilibrium (HWE) in the Korean population. Odds ratios (OR) with 95% confidence interval (CI) and P-values were calculated by the logistic regression models.
Fisher’s exact test was used to analyze differences in haplotype frequency between the normal population and patients with AD and VaD.
Results

The genotype frequencies at PRNP 1368 were in HWE in Korean control group ($P = 0.742$) and AD group ($P = 0.226$), not in VaD group ($P = 0.025$) (data not shown). To examine a correlation between the PRNP 1368 polymorphism and susceptibility of AD in Koreans, we examined the genotype and allele frequencies of this polymorphism in 152 Korean AD patients and in 268 healthy controls. No significant difference between Korean AD patients and controls was found in genotype or allele frequency of the PRNP 1368 polymorphism (Table 2). This result suggests that the PRNP 1368 polymorphism does not increase susceptibility to AD. When our data set was stratified by gender, there was no significant association between this polymorphism and gender (data not shown).

We also investigated the genotype and allele frequencies of PRNP 1368 in 192 Korean VaD patients to determine whether this polymorphism correlated with VaD. There were no significant differences in genotype and allele frequencies between VaD patients and controls (Table 3). In addition, analysis of the haplotype frequency was performed in AD patients, VaD patients and controls. Six haplotypes of the 3 PRNP polymorphisms were constructed in Koreans. One (ht 5) of these six haplotypes was significantly over-represented in Korean VaD patients (Table 4). These results suggest that the PRNP 1368 polymorphism is not associated with the occurrence of VaD in the Korean population.
Discussion

VaD is a clinical syndrome causing cognitive deterioration due to cerebrovascular lesions. However, the mechanisms for cognitive impairment in VaD patients are not completely understood. In recent studies, the evidence for associations between polymorphisms of many genes and VaD has been reported [30]. AD is one of the most serious health problems in the industrialized world. The accumulation of amyloid β peptide (Aβ) in the brain is a key event in the pathogenesis of AD. Autosomal dominant mutations in APP, PSEN 1, and PSEN 2 have been shown to cause AD, while a variant of APOE significantly increases the risk for AD [1].

Although the exact function of the prion protein is not fully understood, it might be involved in the development and intensity of oxidative stress and, thereby, contribute to neurodegeneration. Thus, polymorphisms in the cording region of PRNP might influence other neurodegenerative disorders in addition to prion diseases. Many studies on a correlation between the PRNP codon 129 and AD in various populations have yielded contradictory results [12-19]. This controversial result may be due to the different sample size of the population analyzed, to a difference in frequency of PRNP genotypes between different ethnic groups [31], or even to a difference in age of onset. In our previous studies, we failed to detect a significant association between PRNP polymorphism at codons 129/219 and the risk for AD or VaD in the Korean population [32,33]. Recently, there has been growing concern about several polymorphisms outside the cording region of PRNP. Polymorphisms in the PRNP promoter region may be associated with increased susceptibility of prion diseases in cattle and mice [34-36]. These PRNP promoter polymorphisms influence the PRNP gene expression level [37].
An association between sporadic CJD and polymorphism at the PRNP 1368 has been reported in British and German populations [22-23], but the studies in Dutch and Korean populations have failed to confirm an association [24,25]. However, this polymorphism and its association with AD or VaD have not been studied so far. In this study, we failed to detect a significant association between the PRNP 1368 polymorphism and the occurrence of AD and VaD in the Korean population (Tables 2 and 3).

The downstream prion-like protein (doppel) encoded by prion-like protein gene (RPND) shares biochemical and structural homology with the prion protein. Our previous studies assessing the correlation of the coding region and 3’ UTR region of PRND with sporadic CJD have been reported [38,39]. In one of these studies, polymorphism at 3’ UTR +28 of the PRND was associated with sporadic CJD [38]. Further research on the role of PRND gene in AD or VaD in the Korean population is needed.
Conclusion

PRNP 1368 polymorphism was not significantly associated with incidence of sporadic AD and VaD in Koreans. Our report is the first association study of a polymorphism outside the coding region of PRNP with AD and VaD.
Competing interests

The author(s) declare that they have no competing interests.

Authors’ contribution

BHJ and YSK designed the study. BHJ, KHL, and YJL performed the genotyping. BHJ, YJK, YHK, YSC, and YSK analyzed the data. BHJ and YJK performed statistical analysis. BHJ, YJK, RIC, and YSK wrote the paper. All authors read and approved the final manuscript.

Acknowledgments

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Table 1 Characteristics of AD and VaD patients and controls

<table>
<thead>
<tr>
<th></th>
<th>AD patients</th>
<th>VaD patients</th>
<th>Healthy controls</th>
</tr>
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<tbody>
<tr>
<td>Number of subjects</td>
<td>152</td>
<td>192</td>
<td>268</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51 (33.55%)</td>
<td>100 (52.1%)</td>
<td>118 (44.0%)</td>
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<tr>
<td>Female</td>
<td>101 (66.45%)</td>
<td>92 (47.9%)</td>
<td>150 (56.0%)</td>
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<tr>
<td>Mean age at disease onset</td>
<td>73.48 ± 8.00</td>
<td>71.95 ± 8.92</td>
<td>-</td>
</tr>
<tr>
<td>(years ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age at blood collection</td>
<td>-</td>
<td>-</td>
<td>71.17 ± 8.68</td>
</tr>
<tr>
<td>(years ± SD)</td>
<td></td>
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Table 2 Genotype and allele frequencies of the PRNP 1368 polymorphism in the normal population and in AD patients

<table>
<thead>
<tr>
<th>Genotype frequency</th>
<th>AD (n = 152)</th>
<th>Control (n = 268)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>CC</td>
<td>56 (36.8)</td>
<td>103 (38.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>67 (44.1)</td>
<td>124 (46.3)</td>
<td>0.994</td>
<td>0.640 – 1.544</td>
<td>0.978</td>
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<tr>
<td>TT</td>
<td>29 (20.1)</td>
<td>41 (15.3)</td>
<td>1.301</td>
<td>0.731 – 2.315</td>
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<table>
<thead>
<tr>
<th>Allele frequency</th>
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</tr>
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<tbody>
<tr>
<td>C</td>
<td>179 (58.9)</td>
<td>330 (61.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>125 (41.1)</td>
<td>206 (38.4)</td>
<td>1.119</td>
<td>0.839 – 1.491</td>
<td>0.444</td>
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Table 3: Genotype and allele frequencies of the PRNP 1368 polymorphism in the normal population and in VaD patients

<table>
<thead>
<tr>
<th></th>
<th>VaD (n = 192)</th>
<th>Control (n = 268)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td><strong>Genotype frequency</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>84 (43.8)</td>
<td>103 (38.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>CT</td>
<td>74 (38.5)</td>
<td>124 (46.3)</td>
<td>0.732</td>
<td>0.487 – 1.100</td>
<td>0.133</td>
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<tr>
<td>TT</td>
<td>34 (17.7)</td>
<td>41 (15.3)</td>
<td>1.017</td>
<td>0.594 – 1.742</td>
<td>0.952</td>
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<tr>
<td><strong>Allele frequency</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>242 (63.0)</td>
<td>330 (61.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>142 (37.0)</td>
<td>206 (38.4)</td>
<td>0.940</td>
<td>0.717 – 1.232</td>
<td>0.654</td>
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Table 4 Haplotype frequency of three PRNP polymorphisms in the normal population, AD patients, and VaD patients

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>1368 Codon 129</th>
<th>Codon 219</th>
<th>Frequency</th>
<th>P value</th>
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<td>CTL</td>
<td>AD vs CTL</td>
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</tr>
<tr>
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<td>A</td>
<td>G</td>
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<td>A</td>
<td>0.0263</td>
</tr>
<tr>
<td>ht4</td>
<td>T</td>
<td>G</td>
<td>G</td>
<td>0.0197</td>
</tr>
<tr>
<td>ht5</td>
<td>C</td>
<td>A</td>
<td>A</td>
<td>0.0164</td>
</tr>
<tr>
<td>ht6</td>
<td>C</td>
<td>G</td>
<td>G</td>
<td>0.0066</td>
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