



Association between CAG repeat length in the *PPP2R2B* gene and Alzheimer disease in the Japanese population

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ABSTRACT

We analyzed the association between *PPP2R2B* gene CAG repeat length and Alzheimer disease (AD) susceptibility in the Japanese population. Blood samples were collected from 218 late-onset AD patients and 86 controls. DNA fragments containing the target CAG repeat region were amplified using polymerase chain reaction (PCR). PCR products were sequenced using ABI PRISM 310 genetic analyzer. The mean CAG repeat length did not differ significantly between the control and AD groups. In contrast, the frequency of CAG repeats shorter than 15 was significantly higher in AD group, specifically in the AD with APOE4 subgroup, than in the control group. The results suggest that CAG repeat lengths in the *PPP2R2B* gene may be potential genetic markers for AD susceptibility in the Japanese population.

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Alzheimer disease (AD) is the most common cause of dementia in the elderly, and is characterized by progressive cognitive decline and cerebral atrophy. The primary pathological feature of AD is the presence of neurofibrillary tangles and senile plaques in the brain [26]. The presence of the $\epsilon 4$ allele of the apolipoprotein E (APOE) gene (*APOE4*) confers a heightened risk of late-onset AD in multiple genetic backgrounds [4]. Although trinucleotide repeats are common features of the human genome, the trinucleotide repeat number varies among individuals and the lengths of these repeats is associated with many genetic diseases, including Huntington disease (HD) and Dentatorubral-pallidolusian atrophy (DRPLA) [25]. A majority of spinocerebellar ataxias (SCAs) are caused by the expansion of trinucleotide repeats. SCAs are a group of autosomal dominant progressive neurodegenerative disorders that are characterized by overlapping and variable phenotypes [20]. Spinocerebellar ataxia type 12 (SCA12) is caused by CAG repeat expansion in the non-coding region of the *PPP2R2B* gene [11]. Clinical symptoms of SCA12 include dementia, upper limb tremor, and extra pyramidal symptoms. Brain magnetic resonance images of the affected individuals revealed cerebral and cerebellar atrophy [11,23].

The *PPP2R2B* gene, which encodes a brain-specific regulatory B subunit of the serine/threonine protein phosphatase 2A (PP2A), is located on chromosome 5q31–33 and is widely expressed in brain neurons [21]. PP2A has been implicated in cell cycle and proliferation and development and regulation of multiple signal

transduction pathways [30]. In addition, PP2A dephosphorylates the hyperphosphorylated tau protein [7]. It is suggested that PP2A-mediated dephosphorylation of tau is facilitated by the B regulatory subunit of PP2A [6]. Tau, an axonal microtubule-associated protein, promotes microtubule assembly and stabilization [17], and tau phosphorylation has been implicated, to varying degrees, in AD pathogenesis [12]. Because of the overlap between the SCA12 phenotype and certain aspects of AD, including the functional role of PP2A, it is important to determine the association between the *PPP2R2B* gene and AD. Recently, Chen et al. reported that the presence of short alleles of the CAG repeat in the *PPP2R2B* gene is associated with increased AD susceptibility in the Han Chinese [3]. However, the existence of such an association among other population group is uncertain. In the present study, we investigated the association between *PPP2R2B* gene CAG repeat lengths and AD susceptibility in the Japanese population.

Patients with late-onset AD were diagnosed with definite or probable AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke Alzheimer's Disease and Related Disorders Association [22]. The control group consisted of non-demented elderly subjects from the general population. After written informed consent was obtained, peripheral blood was collected from 218 late-onset AD patients (mean age: 79.0 years; women: 65.6%) and 86 control subjects (mean age: 74.7 years; women: 52.3%). The protocol for specimen collection was approved by the Genome Ethical Committee of Osaka University Graduate School of Medicine.

DNA was extracted from peripheral blood nuclear cells using the phenol–chloroform method or the QIAamp DNA Blood Kit (Qiagen). CAG repeats in the *PPP2R2B* gene were identified

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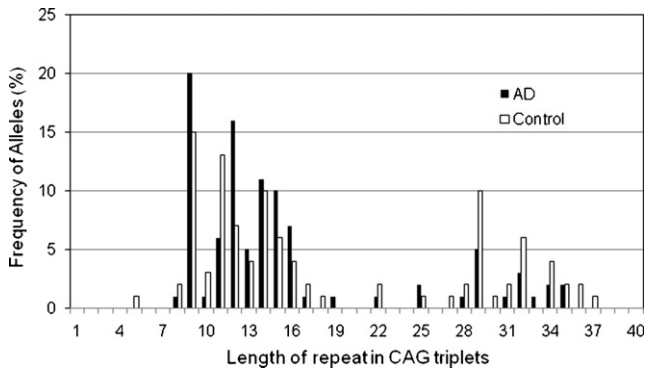


Fig. 1. Distribution of allele frequencies against the CAG repeat numbers in the *PPP2R2B* gene of control subjects and AD patients.

by polymerase chain reaction (PCR) amplification using 6FAM dye-labeled forward (5'-TGCTGGGAAAGAGTCGTG-3') and reverse (5'-GCCCGCGCACTCACCTC-3') primers. The PCR was performed with 36 cycles consisting of two cycles of 30 s at 95 °C and 30 s at 70 °C, two cycles of 30 s at 95 °C and 30 s at 65 °C, two cycles of 30 s at 95 °C and 30 s at 60 °C, and 30 cycles of 30 s at 95 °C, 30 s at 56 °C, and 30 s at 72 °C preceded by 10 min at 95 °C and followed by 10 min at 72 °C. PCR products were electrophoresed in a capillary in an automated ABI PRISM 310 genetic analyzer (Applied Biosystems). Analysis was performed with GenScan analysis software (Applied Biosystems) [11]. The *APOE* genotype was determined using a PCR-RFLP method [15].

Statistical analysis was performed using JMP (version 7.0, SAS Institute, Cary, NC). The 2-sided Mann–Whitney's *U*-test was used to evaluate the difference in CAG repeat distribution between the AD and control groups. The difference in the CAG repeat allele frequencies between the groups was further tested by the Chi-square test. Each value represents mean (standard error). A *p*-value of <0.05 was considered statistically significant.

The frequency distribution of CAG repeat alleles in the *PPP2R2B* genes was analyzed in 218 LOAD patients and 86 controls. In Fig. 1, the CAG repeat number (*X*-axis) is plotted against the frequency of distributions (%) (*Y*-axis). The repeat range was 5–37 and 8–35 in the control and AD groups, respectively. Pathological expansion of CAG repeats was not detected in the AD and control groups. The most common lengths were 9 (15.3%) triplets in the control group. Similarly, in the AD group, the most common lengths were 9 (20.0%) triplets. The mean CAG repeat lengths in the AD and control groups (14.2 and 16.6, respectively) were not statistically different (*p* = 0.158). In addition, when we divided the AD group into *APOE4* and non-*APOE4* subgroups, we found that the mean CAG repeat lengths of both subgroups (13.9 and 14.5, respectively) were not significantly different from that of the control group (Table 1).

Table 1
Comparison of CAG repeat numbers in control subjects and AD patients.

Group	Control			AD		
	Total	<i>APOE4</i> (+)	<i>APOE4</i> (–)	Total	<i>APOE4</i> (+)	<i>APOE4</i> (–)
Number	86	12	74	218	106	112
Allele range	5–37	9–34	5–37	8–35	8–35	8–35
Allele with maximum frequency						
Allele	9	9	9	9	9	9
Frequency (%)	15.3	14.2	16.7	20.0	20.1	17.5
Mean (SE)	16.6 (0.8)	14.4 (1.8)	16.9 (0.8)	14.2 (0.5)	13.9 (0.6)	14.5 (0.7)
<i>p</i> value		0.942	0.114	0.158	0.110	0.362

The differences between the CAG repeat numbers in the control and AD groups were assayed using Mann–Whitney's *U*-test. SE: standard error of the mean.

Table 2
Short (≤ 15) and long (> 15) alleles: CAG repeat number in *PPP2R2B*; the short and long allele repeat numbers in the AD and control groups were compared.

Group	Allele number			<i>p</i> value	OR
	Total	Short (≤ 15)	Long (> 15)		
Control	172	110 (64%)	62 (36%)		
Control with <i>APOE4</i>	24	16 (67%)	8 (33%)	0.267	
Control without <i>APOE4</i>	148	94 (64%)	54 (36%)	0.022*	1.58
AD	436	320 (73%)	116 (27%)	0.021*	1.55
AD with <i>APOE4</i>	212	163 (77%)	49 (23%)	0.005*	1.87
AD without <i>APOE4</i>	224	157 (70%)	67 (30%)	0.197	

Differences in the allele repeat numbers in the AD and control groups were determined using Chi-square test.

* *p* < 0.05, statistically significant.

OR, odds ratio.

Because the mean CAG repeat length among all subjects was 15, we dichotomized the alleles into short (≤ 15) and long (> 15) categories. Statistical analysis revealed that the frequency of CAG repeats shorter than 15 was significantly higher in the AD group than in the control group (*p* = 0.021, odds ratio = 1.55) (Table 2). Compared to the controls, the AD subgroups, *APOE4* and non-*APOE4*, each had a significantly higher frequency of CAG repeats shorter than 15 (*p* = 0.005, odds ratio = 1.87). However, there was no significant difference in the allele frequency distribution between the non-*APOE4* AD group and the control group (*p* = 0.197) (Table 2). Additionally, a comparison of the allele frequency distributions of the control subgroups, *APOE4* and non-*APOE4* with that of the AD revealed that the frequency of CAG repeats shorter than 15 was significantly higher in the AD groups than in the control without *APOE4* groups (*p* = 0.022, odds ratio = 1.58) (Table 2).

SCA12 is a relatively rare late-onset neurodegenerative disorder characterized by diffuse cerebral and cerebellar atrophy [11]. The phenotype typically involves action tremor of upper extremities and various symptoms, including dementia. SCA12 is caused by CAG repeat expansion in the non-coding region of the *PPP2R2B* gene [10,11]. Pathogenic CAG repeat expansions have been detected in SCA12 patients in the range of 55–69 to 66–78, but normal individuals from different ethnic populations have exhibited ranges from 7–28 to 9–45 [2,3,5,11,27–29]. A correlation between the SCA12 phenotype and certain aspects of AD has been suggested. However, the lone study that analyzed the association between CAG repeat expansions in the *PPP2R2B* gene and AD susceptibility reported that the frequency of the Han Chinese individuals carrying the short 5-, 6-, and 7-triplet alleles was notably higher in AD patients [3].

In the present study, we investigated the length of *PPP2R2B* gene CAG repeats in AD patients and control subjects in the Japanese population. The mean CAG repeat lengths in the AD and control groups were not statistically different. In contrast, we found that the frequency of CAG repeats shorter than 15 was significantly higher in the AD group, specifically the AD with *APOE4* subgroup

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