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ORIGINAL ARTICLE

The production ratios of AICDε51 and Aβ42 by intramembrane proteolysis of βAPP do not always change in parallel

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Abstract

Background: During intramembrane proteolysis of β-amyloid protein precursor (βAPP) by presenilin (PS)/γ-secretase, ε-cleavages at the membrane-cytoplasmic border precede γ-cleavages at the middle of the transmembrane domain. Generation ratios of Aβ42, a critical molecule for Alzheimer's disease (AD) pathogenesis, and the major Aβ40 species might be associated with ε48 and ε49 cleavages, respectively. Medicines to down-regulate Aβ42 production have been investigated by many pharmaceutical companies. Therefore, the ε-cleavages, rather than the γ-cleavage, might be more effective upstream targets for decreasing the relative generation of Aβ42. Thus, one might evaluate compounds by analyzing the generation ratio of the βAPP intracellular domain (AICD) species (ε-cleavage-derived), instead of that of Aβ42.

Methods: Cell-free γ-secretase assays were carried out to observe *de novo* AICD production. Immunoprecipitation/MALDI-TOF MS analysis was carried out to detect the N-termini of AICD species. Aβ and AICD species were measured by ELISA and immunoblotting techniques.

Results: Effects on the ε-cleavage by AD-associated pathological mutations around the ε-cleavage sites (i.e., βAPP V642I, L648P and K649N) were analyzed. The V642I and L648P mutations caused an increase in the relative ratio of ε48 cleavage, as expected from previous reports. Cells expressing the K649N mutant, however, underwent a major ε-cleavage at the ε51 site. These results suggest that ε51, as well as ε48 cleavage, is associated with Aβ42 production. Only AICDε51, though, and not Aβ42 production, dramatically changed with modifications to the cell-free assay conditions. Interestingly, the increase in the relative ratio of the ε51 cleavage by the K649N mutation was not cancelled by these changes.

Conclusion: Our current data show that the generation ratio of AICDε51 and Aβ42 do not always change in parallel. Thus, to identify compounds that decrease the relative ratio of Aβ42 generation, measurement of the relative level of Aβ42-related AICD species (i.e., AICDε48 and AICDε51) might not be useful. Further studies to reveal how the ε-cleavage precision is decided are necessary before it will be possible to develop drugs targeting ε-cleavage as a means for decreasing Aβ42 production.

Key words: *AICDε51, Alzheimer's disease, presenilin/γ-secretase, βAPP, γ-cleavage, ε-cleavage.*

INTRODUCTION

The transmembrane domain of β-amyloid protein precursor (βAPP) is proteolysed by presenilin (PS)/γ-secretase.[1] Analysis of the resultant products has shown that the proteolysis proceeds by at least two distinct cleavages. The 'ε-cleavage' liberates its intracellular domain (i.e., βAPP intracellular domain (AICD)) into the cytoplasm, whereas the 'γ-cleavage' releases Alzheimer's disease (AD)-associated amyloid β-protein (Aβ).[2–6]

There are some variations in both the γ- and ε-cleavages of βAPP.[6–8] The major N-termini of AICD species consist of leucine-49, valine-50 and leucine-52 (Aβ-numbering), whereas the major C-termini of Aβ species are comprised of valine-40 and alanine-42. (Fig. 1a).[6] Among these, highly aggregatable Aβ42 is the major component of senile plaques in AD brains.[9]

Are there any relationships between the ε- and γ-cleavages? How do these cleavages occur? Ihara *et al.* have tried to address these questions and recently showed that ε-cleavage precedes γ-cleavage in *in vitro* γ-secretase assays.[10] βAPP-CTF stubs, βAPP membrane-tethered remnants after β-cleavage, first undergo ε-cleavage.[10] The ε-cleavage liberates AICD from the membrane and produces a membrane-bound 48/49 amino-acid-long Aβ species that undergoes further C-terminal truncation by PS/γ-secretase.[11] Stepwise cleavages remove every three amino-acid residues from the C-terminus of the long Aβ species, which finally secretes Aβ40/42.[12–14] For example, mutant PS causes increases in both ε48 and γ42 cleavages.[8] Thus, the γ-cleavage seems to occur in an ε-cleavage-dependent manner.[10] Furthermore, these results show that the production process for pathological Aβ42 is distinct from that of Aβ40.[15] That is, the major ε49 cleavage causes the production of Aβ40, whereas a minor ε48 cleavage causes the production of pathological Aβ42.[14]

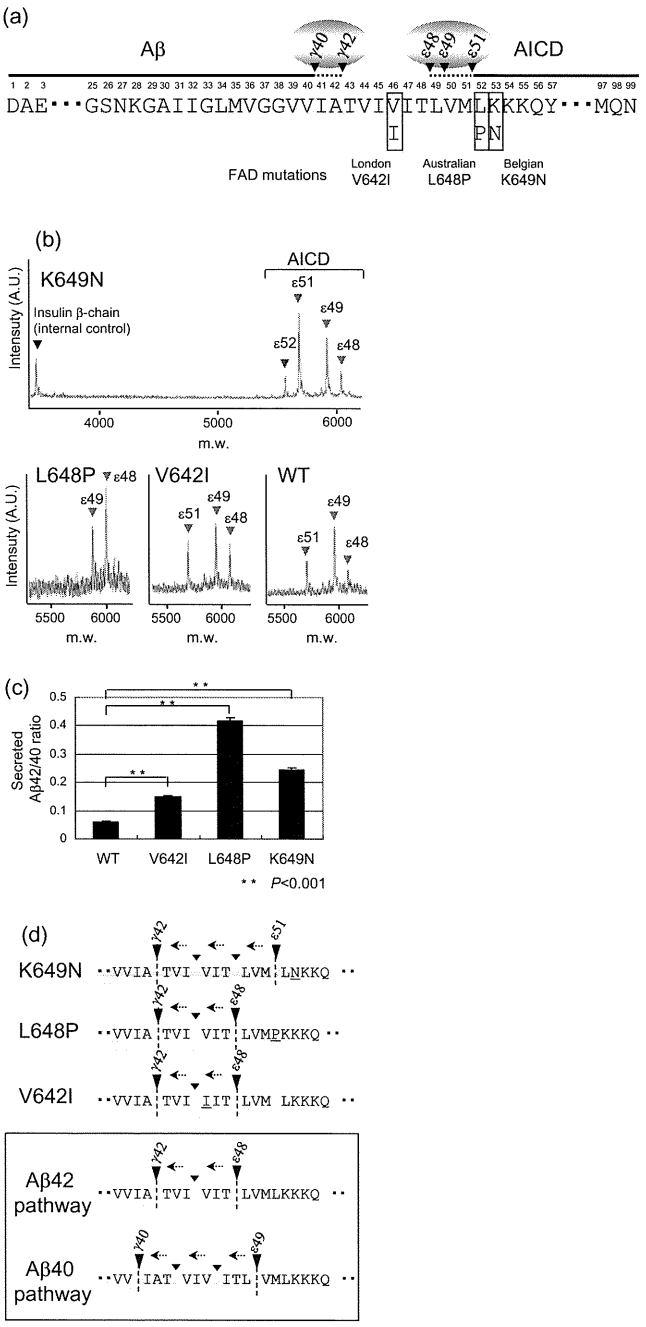

Figure 1 Effect of familial Alzheimer's disease (AD)-associated β-amyloid protein precursor (βAPP) mutations around the ε-cleavage site. (a) Schematic diagram of intramembrane cleavage sites of βAPP and the familial AD mutations used in the present study. The amino acid sequence around the juxta membrane region of human βAPP is described (amyloid β-protein (Aβ) numbering). Filled inverted triangles indicate the cleavage sites. Substituted amino acids of the familial AD mutations are shown in open rectangles. The site of each mutant is also shown using APP695 numbering. (b) Mass spectra of *de novo* βAPP intracellular domain (AICD) species in the cell-free assay. Crude membrane fractions obtained from wild-type (wt) βAPP and the indicated βAPP mutant cells were used. (c) Relative secreted Aβ42 to Aβ40 ratio in the conditioned media of wt βAPP and the indicated βAPP mutant cells. The asterisks indicate statistical significance (*$P < 0.05$, **$P < 0.001$, one-way analysis of variance (ANOVA) and Tukey–Kramer method). Error bars indicate standard error of the mean (SEM). (d) Hypothesis for explaining increased γ42 cleavage in each mutant βAPP (upper panels) and differential production of Aβ40 and Aβ42 (lower panels). FAD, familial Alzheimer's disease; m.w., molecular weight.

Modulation of γ-secretase function to specifically inhibit Aβ42 production is one of the promising strategies for developing drugs to modify the disease course of AD[16]. Given the possible correlation between the ε- and γ-cleavages, we think that targeting the upstream ε-cleavages will be a novel and more efficient method for developing Aβ42-lowering drugs. To test if precision of the ε-cleavage can be used as a novel target for drug development, we investigated the ε-cleavage pathway, particularly ε51 cleavage, which has previously not been well-characterized.[7]

MATERIALS AND METHODS

Cell culture and cDNA constructs

cDNAs of βAPP V642I, L648P and K649N mutants were generated by PCR-based mutagenesis using a Quickchange mutagensis kit (Stratagene, La Jolla, CA, USA) or KOD plus (Toyobo, Osaka, Japan) with wt βAPP695 cDNA as a template. K293 cells were transfected and cultured as previously described.[17]

Membrane preparation

The crude membrane fraction was prepared as previously described with a slight modification.[7,18] In the present study, the homogenization buffer contained 0.25 mol/L sucrose and 50 mmol/L HEPES (pH 7.4) containing a protease inhibitor cocktail (Roche Basel, Switzerland). To prepare the alkaline pretreated membrane, the membrane fraction was suspended in a 50 mmol/L bicarbonate buffer (pH 11.0) and incubated at 4°C for 1 h. The suspension was then centrifuged at 100 000 × *g* for 1 h followed by washing once with a 50 mmol/L Mes buffer (pH 6.0).

Cell-free γ-secretase assay

The cell-free γ-secretase assay was carried out as previously described with a modification.[7,18] The reaction buffer in the present study contained a 150 mmol/L citrate buffer (pH 6.0), 50 mmol/L MES (pH 6.0), 167 mmol/L NaCl and a protease inhibitor mixture comprised a 5x complete protease inhibitor cocktail (Roche), 0.5 mmol/L DIFP (WAKO, Osaka, Japan), 1 μg/ml TLCK (Sigma-Aldrich, St. Louis, MO, USA), 10 μg/ml antipain (Peptide Institute, Osaka, Japan), 10 μg/ml leupeptin (Peptide Institute), 5 mmol/L 1,5 phenanthroline (Sigma-Aldrich), 10 μmol/L amastatin (Peptide Institute), 10 μmol/L bestatin (WAKO), 1 μmol thiorphan (Sigma-Aldrich), 10 μmol/L phosphoramidon (Peptide Institute) and 1 μmol/L pepstatin A (Peptide Institute). To prepare the pH 7.4 buffer, 50 mmol/L HEPES (pH 7.4) was used instead of the citrate and MES buffers.

Immunoprecipitation/MALDI MS analysis

Immunoprecipitation/MALDI MS (IP-MS) analysis followed by cell-free incubation was carried out as previously described.[7,18,19] The heights of the MS peaks and molecular weights were calibrated using angiotensin and bovine insulin β-chain as standards (Sigma-Aldrich).

ELISA analysis for Aβ

Aβ40 and Aβ42 levels in conditioned media were quantified by ELISA (WAKO).

Immunoblotting of Aβ

SDS-solubilized proteins were separated by SDS-PAGE using an 8 mol/L urea gel[17,18,19] and transferred to a nitrocellulose membrane. Immunoblotting of Aβ species using 82E1 (IBL) was carried out as previously described.[20]

RESULTS

The βAPP K649N Belgian mutant increased both the relative ratio of AICDε51 and Aβ42 production in a cell-free γ-secretase assay

To test if the ε51 cleavage precedes the γ42 cleavage, we analyzed the effects of three βAPP mutants (V642I,[21] L648P,[22] and K649N[23]) around the ε-site. The L648P and K649N mutants (βAPP695 numbering) are located downstream of the ε51 site, and the V642I mutant is located upstream of the ε48 site (Fig. 1a). Each of the three mutants is familial AD-associated and therefore increases the relative ratio of Aβ42 production. We raised K293 cells stably expressing each of the mutants, prepared the crude membrane fractions[24] and carried out the cell-free γ-secretase assays.[7,18]

As shown in Figure 1b, the K649N βAPP mutant caused a marked increase in the relative ratio of AICDε51 production (see also Table 1). However, the other two mutants caused completely different effects on the cleavage. The L648P mutant produced a barely detectable level of AICDε51, whereas in the V642I mutant cells, the ratio of AICDε51 production was comparable to that of wild-type (wt) expressing cells. It is of note that, instead of increased AICDε51 pro-

Table 1 Molecular species of β-amyloid protein precursor intracellular domain generated in the cell-free assay

AICD species		m/z Calculated (M + H)	Observed (M + H) Mean	SD
AICDε51 (52–99)	Wild-type	5677.79	5678.38	0.64
	V642I	5677.79	5678.30	0.70
	K649N	5663.74	5663.96	0.23
AICDε49 (50–99)	Wild-type	5907.9	5908.35	0.29
	V642I	5907.9	5908.49	0.21
	L648P	5891.87	5892.48	0.20
	K649N	5893.84	5894.10	0.27
AICDε48 (49–99)	Wild-type	6020.98	6021.36	0.40
	V642I	6020.98	6021.59	0.42
	L648P	6004.96	6005.59	0.33
	K649N	6006.93	6007.51	0.17
AICDε52 (53–99)	K649N	5550.65	5551.01	0.27

M + H, protonated molecular mass.

duction, these V642I and L648P mutants substitutively increased the relative ratio of AICDε48 production. Next we measured Aβ species secretion by the stable cells in conditioned media using ELISA (Fig. 1c). As expected, we observed a significant increase in the ratio of Aβ42 to total Aβ secretion in the conditioned medium of the mutant cells. This data shows that the K649N mutant increased the ratio of Aβ42 production through upregulation of the ε51 cleavage, whereas the V642I and L648P mutants increased Aβ42 production through the ε48 cleavage. Based on these results, we suggest that not only the ε48, but also the ε51 cleavage precedes Aβ42 production, possibly by sequential three amino-acid C-terminal truncation[14] (Fig. 1d).

Incubation in higher pH does not cancel the K649N βAPP mutant effects

We previously found that the precision of ε-cleavage changes depending on the buffer pH.[7,18] The relative ratio of AICDε51 production is the most sensitive to such changes. Therefore, we next determined whether the relative ratio of AICDε51 and/or Aβ42 production by the K649N mutant is affected by changing the buffer pH during the cell-free assay. As expected, incubation in the higher pH (pH 7.4 *vs* pH 6.0) buffer decreased the relative ratio of AICDε51 generation in both the K649N mutant and wt βAPP membrane fraction. However, the pH effect was not so strong as to cancel the AICDε51 upregulation effect by the K649N mutant (Fig. 2a). We further analyzed the pH effects on the increase in the relative ratio of Aβ42 production by the mutant (Fig. 2b). Surprisingly, the assay pH elevation did not cause any changes in the relative ratio of Aβ42 generation. Therefore, unlike the effects of the K649N mutant on the ε51- and γ42-cleavages, the elevation of the buffer pH causes a decrease in the relative ratio of AICDε51 production, but does not cause any changes in Aβ42 production. The data suggest that two distinct mechanisms might contribute to the determination of the relative ratio of AICDε51 production.

Alkali pretreatment of the crude membrane fraction cancels the effect of higher pH cell-free incubation on ε-cleavage

Because the ε51 cleavage occurs at the membrane-cytosol interface, we considered that membrane-bound substances might induce the pH-dependent effects on AICDε51 production. Many substances detach from the membrane on treatment with alkali solution.[25] To test this theory, we washed the wt βAPP membrane fraction in a pH 11 solution (see 'Materials and Methods'), then we carried out the cell-free assay at pH 6.0. The relative ratio of AICDε51 production markedly decreased (Fig. 3a), whereas that of the Aβ42 did not (Fig. 3b). The phenomena are reminiscent of the effects of raising the pH of the incubation buffer (see Fig. 2). Thus, we further considered that the decrease in the AICDε51 production resulting from the use of a higher incubation buffer pH might also be a result of detachment of substances from the membrane. When the membrane fraction was incubated in a pH 7.4 buffer after alkali treatment, we could no

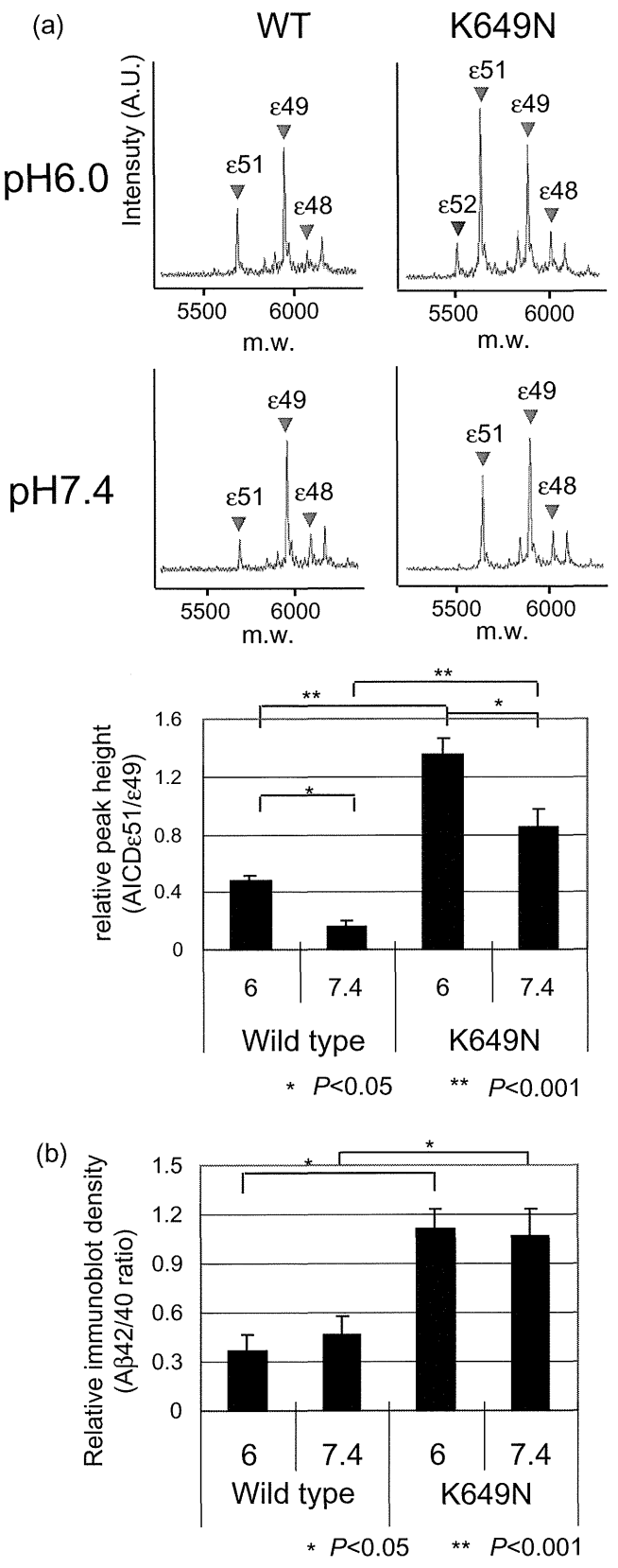

Figure 2 Effect of cell-free incubation pH levels on the precision of ε/γ-cleavages. (a) Mass spectra of β-amyloid protein precursor intracellular domain (AICD) generated in the cell-free assay carried out at the indicated pH (upper and middle panels). Peak heights of AICDε49 and ε51 were measured and the ratios of AICDε49 to ε51 were calculated (lower panel). The asterisks indicate statistical significance (*$P < 0.05$, **$P < 0.001$, one-way ANOVA and Tukey–Kramer method). Error bars indicate SEM. (b) Levels of amyloid β-protein (Aβ) generated at the indicated pH. Levels of Aβ40 and 42 were measured by western blotting and the Aβ42 to 40 ratios calculated. The asterisks indicate statistical significance. Error bars show SEM. m.w., molecular weight.

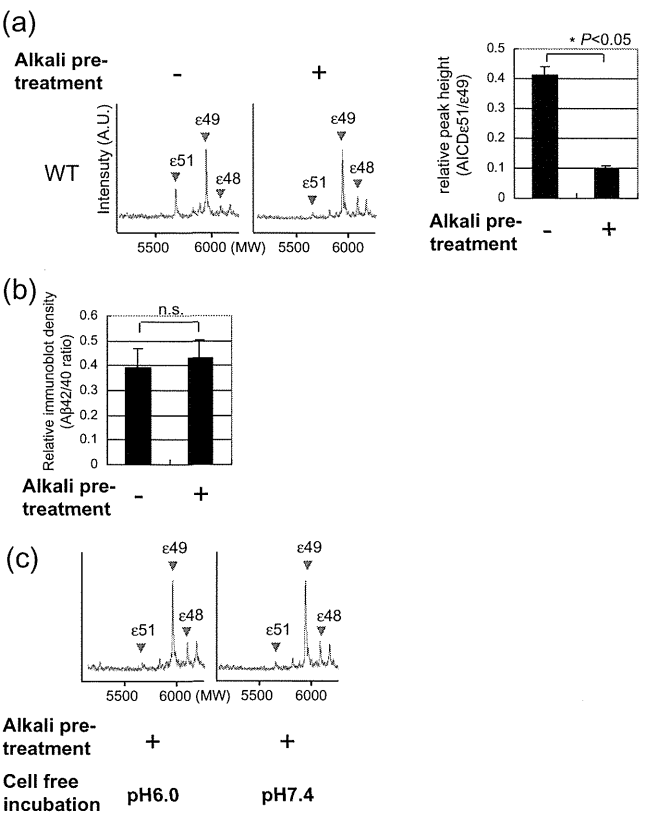

Figure 3 Effect of alkali pretreatment on the precision of ε/γ-cleavages of wild-type (wt) β-amyloid protein precursor. (a) Mass spectra of β-amyloid protein precursor intracellular domain (AICD) generated in the cell-free assay with and without alkali pretreatment. Peak heights of AICDε49 and ε51 were measured and the AICDε49 to ε51 ratios calculated. The asterisk indicates statistical significance (*$P < 0.05$, paired *t*-test). Error bars indicate SEM. (b) Levels of β-amyloid (Aβ) generated in the cell-free assay after alkali pretreatment. Levels of Aβ40 and 42 were measured by western blotting with and without alkali pretreatment and the Aβ42 to 40 ratios calculated. (c) Mass spectra of AICD generated in the cell-free assay at the indicated pH after alkali pretreatment. MW, molecular weight; WT, wild type.

longer observe the pH-dependent incubation buffer effects on the AICDε51 ratio (Fig. 3c). Collectively, although incubation at lower pH buffer increased in the AICDε51 ratio (Fig. 2a), the effects were cancelled by the alkali pretreatment (Fig. 3a). These results suggest that substances removed by the alkali treatment might induce the changes in the relative ratio of AICDε51 production.

Alkali pretreatment of the crude membrane fraction did not cancel the effects of the K649N mutant on the ε-cleavage

As shown in Figure 1, the K649N βAPP mutation causes upregulation of both the AICDε51 and Aβ42 ratio, whereas alkali pretreatment causes downregulation of only the AICDε51 ratio (Fig. 3). These data show that changes in the AICDε51 ratio caused by the mutation and by the treatment occur by two distinct processes. A further experiment was carried out to confirm whether the K649N mutation cause a change in the relative ratio of AICDε51 production through the effect of the alkali treatment (Fig. 4a). After treatment of the K649N mutant membrane fraction in the alkali solution, the cell-free assay was carried out at pH 6.0. As shown in Figure 4a, even after the alkali treatment, the K649N mutant membrane produced a relatively higher level of AICDε51 than that of the wt fraction (Fig. 3a). Furthermore, the elevated Aβ42 ratio was not changed by the pretreatment (Fig. 4b).

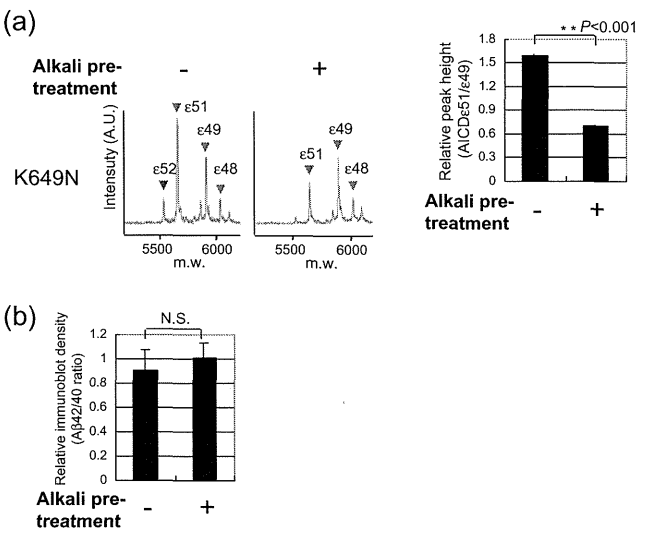

Figure 4 Effect of alkali pretreatment on the precision of ε/γ-cleavages of βAPP K649N Belgian mutant. (a) Mass spectra of β-amyloid protein precursor intracellular domain (AICD) generated in the cell-free assay with and without alkali pretreatment. (b) Levels of of β-amyloid generated in the cell-free assay following alkali pretreatment. N.S, not significant.

DISCUSSION

In the present study, we determined that there are at least two factors that change the precision of ε-cleavage: (i) a process induced by a pathological βAPP mutation; and (ii) another process induced by possibly unidentified substances removed from the membrane fraction by alkali pretreatment. In the case of βAPP mutations, the relative ratio of ε51 and ε48 production increases in parallel with the ratio of AD-associated Aβ42.

It has been reported that ε-cleavage precedes γ-cleavage and γ-cleavage seems to occur in an ε-cleavage-dependent manner.[10] Considering these reports and our own preliminary results, it seemed possible that measurement of the relative ratio of AICDε48/AICDε51 production might help develop Aβ42-lowering anti-AD drugs. Further study showed, however, that the relative level of AICDε51 production is drastically affected by the removal of unidentified substances from the membrane as a result of alkali pretreatment. Interestingly, the alkali pretreatment did not cause any changes in the relative ratio of Aβ42 generation. These results show that changes in the precision of ε-cleavage do not always cause parallel alterations in the precision of γ-cleavage, even though ε-cleavage occurs upstream of the γ-cleavage. Therefore, although measuring the levels of AICD species is a potentially attractive new target for developing Aβ42 lowering compounds, challenges must still be overcome before screening methods for such compounds can be established. For example, the paradoxical mechanism discussed previously must first be understood before an assay in which the ε-cleavage precision accurately reflects the γ-cleavage precision can be developed.

How does alkali pretreatment result in a decreased ratio of AICDε51 production? One might consider the presence of unknown substances that (i) transiently associate with the PS/γ-secretase and affect its intramembrane cleavage precision, or (ii) truncate a couple of N-terminal amino-acid residues of AICD produced by the ε-cleavage. The second possibility is reminiscent of angiotensin-converting enzyme activity to truncate the C-terminus of Aβ42.[26] Of course, the

possibility that alkali pretreatment might change the character of PS/γ-secretase itself also cannot be excluded.

CONCLUSION

Our current data suggest that the precision of ε-cleavage does not always change in parallel with the precision of γ-cleavage, even though ε-cleavage occurs upstream of the γ-cleavage. Thus, to measure the levels of AICD species might be an attractive new target for developing Aβ42 lowering compounds, there still remain some challenges.

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ORIGINAL PAPER

Association Study Between the Pericentrin (*PCNT*) Gene and Schizophrenia

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Abstract Disrupted-in-schizophrenia 1 (*DISC1*), a known genetic risk factor for schizophrenia (SZ) and major depressive disorder (MDD), interacts with several proteins and some of them are reported to be genetically associated with SZ. Pericentrin (*PCNT*) also interacts with *DISC1* and recently single-nucleotide polymorphisms (SNPs) within the *PCNT* gene have been found to show significant associations with SZ and MDD. In this study, case-controlled association analysis was performed to determine if the *PCNT* gene is implicated in SZ. Nine SNPs were analyzed in 1,477 individuals (726 patients with SZ and 751 healthy controls). No significant difference was observed between the controls and the patients in allelic frequencies or genotypic distributions of eight SNPs. Although allelic distribution of rs11702684 was different between the two groups ($P = 0.042$), the difference did not reach statistical significance after permutation correction for multiple comparisons. In the haplotypic analysis, we could not find any significant association in our subjects, either. This gene may not play a major role independently in the etiology of SZ in the Japanese population.

Keywords Schizophrenia · PCNT · Kendrin · Case–control association study · DISC1

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Introduction

Schizophrenia (SZ) is a complex psychiatric disorder that afflicts approximately 1% of the population throughout the world and has high heritability (Craddock et al. 2005). The pericentrin gene (the official symbol; *PCNT* and also called kendrin) is located at 21q22.3, which is one of chromosomal lesions prevalent in SZ by cytogenetic analysis (Demirhan and Tastemir 2003). *PCNT* is a coiled-coil protein localized specifically to the centrosome throughout the cell cycle (Flory et al. 2000) and an integral component of the pericentriolar material (Li et al. 2001). This protein provides sites for microtubule nucleation in the centrosome by anchoring gamma-tubulin complex (Takahashi et al. 2002), then it plays an important role in microtubule organization, spindle organization, and chromosome segregation (Doxsey et al. 1994; Purohit et al. 1999). Disrupted-in-schizophrenia 1

(*DISC1*), a known genetic risk factor for SZ and major depressive disorder (MDD) (Cannon et al. 2005; Chen et al. 2007; Hashimoto et al. 2006; Hennah et al. 2003; Millar et al. 2000; Thomson et al. 2005), localizes to the centrosome by binding to *PCNT* (Miyoshi et al. 2004). Shimizu et al. showed that overexpression of the *DISC1*-binding regions of *PCNT* or the *DISC1* deletion mutant lacking the *PCNT*-binding region impaired the microtubule organization and they suggested that the *DISC1–PCNT* interaction played a key role in the microtubule network formation (Shimizu et al. 2008). Recently, single-nucleotide polymorphisms (SNPs) within the *PCNT* gene have been found to show allelic associations with SZ and MDD (Anitha et al. 2009; Numata et al. 2009). In addition, Mitkus et al. reported a trend for an increase mRNA levels of the *PCNT* gene in the dorsolateral prefrontal cortex of patients with SZ, compared with the control groups (Mitkus et al. 2006). In this study, case-controlled association analysis was performed in the Japanese population to determine if the *PCNT* gene is implicated in SZ.

Materials and Methods

Subjects

We used genomic DNA samples from 726 SZ patients: 406 male (mean age 48.6 ± 13.8 years), 320 female (mean age 49.2 ± 14.5 years) from the Tokushima University Hospital, affiliated psychiatric hospitals of the University of Tokushima, the Ehime University Hospital and the Osaka University Hospital in Japan. The diagnosis of SZ was made by at least two experienced psychiatrists according to DSM-IV criteria on the basis of extensive clinical interviews and review of medical records. Seven hundred fifty-one controls, 422 male (mean age 45.5 ± 11.1 years) and 329 female (mean age 45.2 ± 10.5), were selected from volunteers who were recruited from hospital staff and students and company employees documented to be free from either psychiatric problems or past mental histories. All subjects were unrelated Japanese origin and signed written informed consent to participate in the genetic association studies approved by the institutional ethics committees.

Genotyping

We initially selected eight tagging SNPs by SNPBrowser 3.5 (De La Vega et al. 2006) (Applied Biosystems, Foster, CA, USA, Pair-wise $r^2 > 85\%$, MAF > 20%, Japanese population) (rs11702684, rs2249057, rs11701058, rs2839226, rs2839231, rs3788265, rs2073376, rs1010111) (Supplementary Table 1). After that, we selected rs2073380 additionally because eight tagging SNPs did not seem to cover the third block of the *PCNT* gene from HapMap data. Genotyping was performed using commercially available TaqMan probes for the *PCNT* gene with ABI Prism 7900 HT Sequence Detection System and ABI 7500 Real Time PCR System (Applied Biosystems). Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al. 2005). Blocks were defined according to the criteria of Gabriel et al. (2002).

Statistical Analysis

Allelic and genotypic frequencies of patients and control subjects were compared using χ^2 test. The SNPAlyze 3.2Pro software (DYNACOM, Japan) was used to estimate haplotype frequencies, linkage disequilibrium (LD), permutation *P*-values (10,000 replications) and deviation from Hardy–Weinberg Equilibrium (HWE) distribution of alleles. Power calculations for our sample size performed using the G*Power program (Erdfelder et al. 1996). The criterion for significance was set at $P < 0.05$ for all tests.

Results

Genotypic and allelic frequencies of the *PCNT* gene are shown in Table 1. Genotypic distributions of these nine SNPs did not deviate significantly from HWE in either group ($P > 0.05$). No significant difference was observed in genotypic frequency between the controls and patients in eight SNPs. Although allelic distribution of rs11702684 was different between the two groups ($P = 0.042$), the difference did not reach statistical significance after permutation correction for multiple comparisons. In power calculations using the G*Power program, our sample size had >0.98 power for detecting a significant association (alpha < 0.05) when an effect size index of 0.2 was used.

Several papers reported that there were gender-specific genetic components involved in the pathology of SZ in the *DISC1* gene (Hennah et al. 2003; Chen et al. 2007) and the *DISC1*-related genes (Hennah et al. 2007; Pickard et al. 2007; Qu et al. 2008). In our study, when the data were subdivided on the basis of gender, allelic distribution of rs11702684 was different between the two groups in only male samples ($P = 0.033$). However, the difference did not survive statistical significance after permutation correction for multiple comparisons.

There were three LD blocks in the *PCNT* gene with rs2249057, rs11701058, rs2839226, and rs2839231 residing in block 1 and rs3788265 and rs2073376 residing in block 2, and rs2073380 and rs1010111 residing in block 3 (Gabriel et al. 2002, Fig. 1). These constructed marker haplotypes of blocks 1–3 were not associated with SZ (permutation $P = 0.184$, 0.137, and 0.601, respectively).

Table 1 Genotypes and allele frequencies of nine single SNPs in the PCNT gene in patients with SZ and controls

SNP	Diagnosis	Allele		*P*-value	Genotype			*P*-value	Frequency
rsl1702684		C	T		C/C	C/T	T/T		
	SC	913	515	0.042	296	321	97	0.085	0.361
	CT	892	588		265	362	113		0.397
		C	A		C/C	C/A	A/A		
rs2249057	SC	862	590	0.504	255	352	119	0.691	0.406
	CT	870	626		247	376	125		0.418
		C	T		C/C	C/T	T/T		
rs11701058	SC	669	783	0.181	153	363	210	0.297	0.461
	CT	728	772		168	392	190		0.485
		C	T		C/C	C/T	T/T		
rs2839226	SC	378	1072	0.111	47	284	394	0.19	0.261
	CT	353	1147		34	285	431		0.235
		A	G		A/A	A/G	G/G		
rs2839231	SC	408	1042	0.562	63	282	380	0.52	0.281
	CT	405	1085		53	299	393		0.272
		G	T		G/G	G/T	T/T		
rs3788265	SC	821	627	0.998	234	353	137	0.506	0.433
	CT	846	646		230	386	130		0.433
		A	G		A/A	A/G	G/G		
rs2073376	SC	445	1001	0.403	75	295	353	0.51	0.308
	CT	478	1006		77	324	341		0.322
		C	A		C/C	C/A	A/A		
rs2073380	SC	642	796	0.839	144	354	221	0.552	0.446
	CT	669	817		141	387	215		0.45
		A	G		A/A	A/G	G/G		
rs1010111	SC	1079	363	0.298	402	275	44	0.343	0.252
	CT	1141	351		428	285	33		0.235

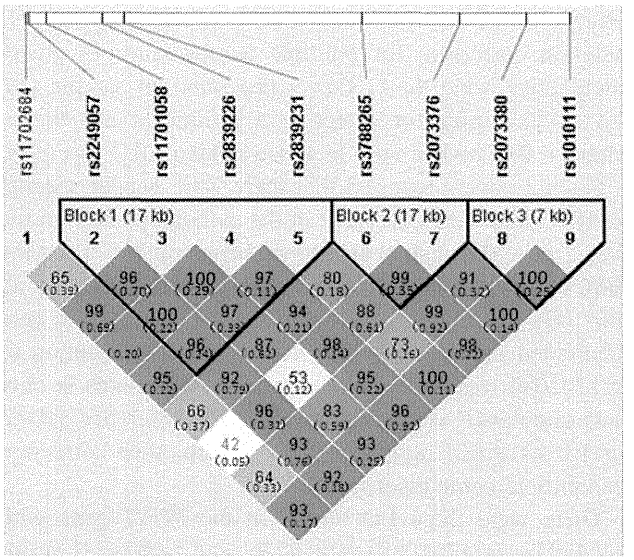

Fig. 1 LD and haplotype structure of the PCNT gene. Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al. 2005). Blocks were defined according to the criteria of Gabriel et al. (2002). Each box represents the D' (r^2) values corresponding to each pair-wise SNP

Discussion

In this study, we examined the association of nine SNPs in the *PCNT* gene and SZ. No significant difference was observed between the controls and the patients in either allelic frequencies or genotypic distributions of nine SNPs after permutation correction for multiple comparisons. In the haplotypic analysis, we could not find any significant association in our subjects. This result was concordance with another study in a Caucasian population (Tomppo et al. 2009).

During the preparation of this article, Anitha et al. reported that rs2249057 of the *PCNT* gene and haplotypes involving this SNP were significantly associated with SZ after correction for multiple comparisons in the Japanese population (Anitha et al. 2009). Although SNPs examined in our study contained rs2249057, we could not find any significant associations in our subjects. The statistical power of our study was sufficient to detect an association between the variants and SZ (SZ $n = 726$; control $n = 751$). Surprisingly, the control minor allele frequency of rs2249057 in Anitha's study (0.48) was higher than

those of our study, HapMap data, and ABI data (0.42, 0.40, and 0.41, respectively). This differing allele frequency between these two studies may be caused by samples' recruited areas. Anitha et al. used subjects from further east compared to ours. However, it is reported that there is no significant population stratification in Japanese (Arinami et al. 2005; Yamaguchi-Kabata et al. 2008).

There are several limitations in our study. First, we applied MAF > 20% when we selected the tagging SNPs and it is difficult to evaluate the association of rare variants in our study. Second, we cannot rule out a possibility that *DISC1–PCNT* interaction may be involved in the etiology of SZ. Third, our findings only represented the Japanese population and studies in other populations would still be warranted due to differing allele frequencies between populations.

Conclusions

In conclusion, we did not find any significant association between the *PCNT* gene and the SZ in the Japanese population. This gene may not play a major role independently in the etiology of SZ.

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ORIGINAL INVESTIGATION

Association study of *KIBRA* gene with memory performance in a Japanese population

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Abstract

Objectives. Papassotiropoulos et al. (Science 314: p 475) discovered that a single nucleotide polymorphism (SNP) of the *KIBRA* gene (rs17070145) was associated with delayed recall performance in Caucasians. KIBRA is highly expressed in the brain and kidneys, and is reported to be involved in synaptic plasticity. Therefore, we first tried to replicate the association between the SNP and memory performance in a Japanese subjects. *Methods.* We examined the association between the SNP and memory performance measured by the Wechsler Memory Scale-Revised (WMS-R) in 187 healthy Japanese people. *Results.* The T allele carriers had significantly better verbal memory, attention/concentration and delayed recall performance than the C/C carriers (corrected $P=0.044$, 0.047 and 0.0084, respectively). Furthermore, the C/T carriers and the T/T carriers had better delayed recall performance than the C/C carriers (post hoc $P=0.0017$ and 0.0096). *Conclusions.* This data suggest that the C/C genotype might have an impact on memory performance in Asian populations as well as in Caucasian populations. Further investigation to clarify the association of the *KIBRA* gene with memory in other ethnic groups is warranted.

Key words: *KIBRA, single nucleotide polymorphism(SNP), memory, delayed recall, WMS-R*

Introduction

The mechanism of human memory is important to know fundamental brain functions. Twin and family studies have demonstrated that most cognitive traits are moderately to highly heritable, although the particular genes that underlie the heritability have only recently been investigated (Greenwood 2003). Papassotiropoulos et al. (2006) initially presented evidence that the SNP of the *KIBRA* gene (rs17070145) might be associated with human memory performance in healthy Caucasian subjects. Rs17070145 is a common T→C substitution within the ninth intron of the *KIBRA* gene (GenBank accession number NM_015238 [GenBank]). They showed that the T allele carriers of the *KIBRA* gene had better delayed recall performance than the C/C carriers. They also showed that high levels of truncated KIBRA transcripts are expressed in memory-related brain regions in humans, additionally high levels of KIBRA are expressed in hippocampus of mice. Furthermore, hippocampal activation in the T allele carriers was higher than that in the C/C carriers during memory

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retrieval by functional magnetic resonance imaging (MRI). Thus, the *KIBRA* gene has lately attracted considerable attention on the mechanism of memory. There were six replication studies that supported a significant effect of the *KIBRA* SNP on cognition in healthy subjects (Almeida et al. 2008; Nacmia et al. 2008; Bates et al. 2009; Schaper et al. 2008; Preuschhof et al. 2009; Zhang et al. 2009) and two studies failed to confirm the association between the *KIBRA* SNP and memory performance (Need et al. 2008; Need et al. 2009).

KIBRA is a cytoplasmic protein, highly expressed in the brain and kidneys and represents a new member of the family of signal transducers. It contains two amino-terminal WW domains, a C2 like domain and a carboxyl-terminal glutamic acid-rich stretch (Kremerskothen et al. 2003). It interacts with dynein light chain 1 to activate the oestrogen receptor (Rayala et al. 2006) and discoidin domain receptor 1 to modulate collagen-induced signalling (Hilton et al. 2008). Recently, protein kinase Mζ, a brain-specific variant of PKCζ, was reported to be combined with KIBRA and supposed to modulate molecular pathways of memory formation (Yoshihama et al. 2009).

Although these results strongly suggest that the *KIBRA* SNP should contribute interindividual differences of human memory function, at least two issues remain to be clarified. First, the previous studies demonstrated different memory performance between T allele carriers and C/C carriers (Papassotiropoulos et al. 2006; Almeida et al. 2008; Nacmias et al. 2008; Need et al. 2008; Schaper et al. 2008; Bates et al. 2009; Zhang et al. 2009). The difference among three genotypes of the *KIBRA* SNP and their association with memory performance is unclear. Second, the genotype distribution of the *KIBRA* SNP is largely different among ethnics according to the National Center for Biotechnology Information database of genetic variation (dsSNP) (T allele frequencies in Caucasian: 26%, in Japanese: 81%, in Chinese: 78%, in African: 66%, respectively). It would be possible that genotype effects of the *KIBRA* SNP on memory performance in Asian populations may differ from those in Caucasian populations. To clarify these two issues, we examined a possible association between the *KIBRA* SNP and memory performance in 187 Japanese healthy volunteers.

Materials and methods

Subjects

One hundred and eighty seven healthy subjects participated in this study (92 males and 95 females, the age range; 20–65 years, mean age ± standard deviation (S.D.); 35.9 ± 11.5 years, mean Intelligent Quotient (IQ) ± S.D.; 108.8 ± 11.9). We obtained all the data including IQ and memory performance from the database and research bioresource of healthy controls with genomic DNA in Human Brain Phenotype Consortium (http://www.med.osaka-u.ac.jp/pub/psy/www/jp/labo/sp/consortioum.htm). All the subjects were biologically unrelated Japanese and met the following criteria; (1) were recruited by local advertisements for Human Brain Phenotype Consortium at a single-institution, Osaka University, (2) had no first- or second-degree relatives with psychiatric disease, (3) were excluded if they had neurological or medical conditions that could potentially affect the central nervous system, such as substance abuse or dependence, atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, cancer in an active stage, cerebrovascular disease, epilepsy or seizures, (4) were excluded if they had any psychiatric diseases and/or received psychiatric medication, (5) were excluded if the IQ was under 70. They were screened for psychiatric disease with the non-patient edition of the modified structured clinical interview for the Diagnostic and Statistical Manual-Fourth Edition Axis I disorders (SCID-I/NP) (First et al. 1997). Tables I and II (and Supplementary Table I available at: http://informahealthcare.com/doi/abs/10.3109/15622971003797258) show characteristics of subjects according to genotypes. After a description of the study, written informed consent was obtained from every subject. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and was approved by the ethics committee at Osaka University.

Genetic analysis

Venous blood was collected from the subjects and genomic DNA was extracted from whole blood according to standard procedures. A SNP (*KIBRA*: rs17070145) was genotyped by using the TaqMan 5'-exonuclease allelic discrimination assay described in the previous study (Hashimoto et al. 2006, 2007).

Cognitive tests

A full version of the Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987), which is generally used to measure memory functions, was administered to subjects. The four indices of the WMS-R (verbal memory, visual memory, attention/concentration and delayed recall) were used for the analysis. Delayed recall is an ability to recall information after 30 minutes of intervening activity. IQ data was

Table I. Demographic characteristics and memory performance of the Japanese healthy subjects between T allele carriers and C/C allele carriers.

	C/C	C/T, T/T	*P* value
Number of subjects	7	180	
Age[a]	34.7 (9.7)	36.0 (11.6)	$t(185) = -0.30$, $P = 0.77$
Gender (M/F)[b]	6/1	86/94	$X^2(1, N = 187) = 3.88$, $P = 0.049^*$
Education years[a]	15.7 (0.8)	15.5 (2.4)	$t(185) = 0.27$, $P = 0.789$
IQ[a]	103.0 (14.4)	109.1 (11.8)	$t(185) = -1.33$, $P = 0.186$
WMS-R indices[c]			
Verbal memory	97.4 (15.3)	111.4 (12.9)	$F_{1, 184} = 6.59$, $P = 0.011^*$
Visual memory	103.7 (12.3)	109.5 (9.7)	$F_{1, 184} = 1.47$, $P = 0.23$
Attention/concentration	97.6 (11.8)	108.6 (14.0)	$F_{1, 184} = 6.47$, $P = 0.012^*$
Delayed recall	96.6 (16.7)	112.3 (11.8)	$F_{1, 184} = 9.74$, $P = 0.0021^{**}$

M, male; F, female. WMS-R, Wechsler Memory Scale-Revised. Data are means ± SD.
$^*P < 0.05$. $^{**}P < 0.01$.
[a]Differences in clinical characteristics between genotypes were analyzed by using t-test for age, education years and IQ.
[b]Differences in clinical characteristics between genotypes were analyzed by using the chi-square test for gender.
[c]Differences in indices of WMS-R among genotypes were analyzed by using ANCOVA.

collected by using the full-scale version of the Wechsler Adult intelligence Scale (WAIS)-III ($n = 140$), the shortened version of the WAIS-Revised (WAIS-R) ($n = 44$) or the full-scale version of the WAIS-R ($n = 3$), as described previously (Ohi et al. 2009).

Statistical analysis

Statistical analysis was carried out using SPSS for Windows version 16.0 (SPSS Japan Inc., Tokyo, Japan). The presence of Hardy–Weinberg equibrium was examined by using χ^2-test for goodness of fit. Group comparisons of demographic data were performed by using χ^2-test for a categorical variable (gender), *t*-test or analysis of variance (ANOVA) for continuous variables, as appropriate. As there was a significant difference in gender between the T allele carriers and the C/C carriers, we conducted analysis of covariance (ANCOVA) for WMS-R indices with gender as a covariate (Table I). Group comparisons of WMS-R indices among three genotype groups were analyzed by ANOVA (Table II) and those between the C allele carriers and the T/T carriers were analyzed by *t*-test (Supplementary Table I available at: http://informahealthcare.com/doi/abs/10.3109/15622971003797258). Bonferroni correction was applied for multiple testing to assess the effects of the *KIBRA* genotype on four indices of the WMS-R. Post hoc comparisons were performed after ANOVA by using Bonferroni correction. We used G*Power 3.1 for power calculation (Faul et al. 2009). All *P* values reported are two tailed. Statistical significance was defined at $P < 0.05$.

Table II. Demographic characteristics and memory performance of the Japanese healthy subjects among C/C carriers, C/T carriers and T/T carriers.

	C/C	C/T	T/T	*P* value
Number of subjects	7	56	124	
Age[a]	34.7 (9.7)	36.9 (12.5)	35.6 (11.1)	$F_{2, 184} = 0.270$, $P = 0.76$
Gender (M/F)[b]	6/1	25/31	61/63	$X^2(2, N = 187) = 4.20$, $P = 0.12$
Education years[a]	15.7 (0.8)	15.4 (2.5)	15.5 (2.4)	$F_{2, 184} = 0.12$, $P = 0.89$
IQ[a]	103.0 (14.4)	107.8 (11.6)	109.6 (11.8)	$F_{2, 184} = 1.34$, $P = 0.26$
WMS-R indices[c]				
Verbal memory	97.4 (15.3)	111.6 (12.3)	111.3 (13.2)	$F_{2, 184} = 3.89$, $P = 0.022^*$
Visual memory	103.7 (12.3)	110.4 (7.3)	109.1 (10.6)	$F_{2, 184} = 1.53$, $P = 0.22$
Attention/concentration	97.6 (11.8)	108.0 (13.3)	108.8 (14.4)	$F_{2, 184} = 2.14$, $P = 0.12$
Delayed recall	96.6 (16.7)	110.9 (9.5)	112.9 (12.6)	$F_{2, 184} = 6.36$, $P = 0.0021^{**}$

M, male; F, female. WMS-R, Wechsler memory scale-revised. Data are means ± SD.
$^*P < 0.05$. $^{**}P < 0.01$.
[a]Differences in clinical characteristics among genotypes were analyzed by using ANOVA for age, education years, IQ and indices.
[b]Differences in clinical characteristics among genotypes were analyzed by using the chi-square test for gender.
[c]Differences in indices of WMS-R among genotypes were analyzed by using ANOVA.

Results

We examined the associations between the *KIBRA* SNP (rs17070145) and four indices of the WMS-R in 187 healthy Japanese subjects. The sample was composed of subjects carrying the C/C genotype (3.7%), the C/T genotype (39.9%) and the T/T genotype (66.3%). The genotype distributions of the SNP were in Hardy–Weinberg equilibrium ($\chi^2=0.046$, $P=0.83$).

According to the initial study (Papassotiropoulos et al. 2006), we categorized the subjects into two groups (the T allele carriers vs. the C/C carriers) (Table I). There was no significant difference in age, education years or IQ between the groups except for gender ratio ($\chi^2=3.88$, $P=0.049$). The T allele carriers had significant better memory performance in verbal memory ($F_{1,184}=6.59$, $P=0.011$), attention/concentration ($F_{1,184}=6.47$, $P=0.012$) and delayed recall ($F_{1,184}=9.74$, $P=0.0021$) than the C/C carriers, while there was no significant difference in visual memory ($F_{1,184}=1.47$, $P=0.23$) between genotype groups. The association of the SNP with verbal memory, attention/concentration and delayed recall survived after correction for multiple testing (corrected $P=0.044$, 0.047 and 0.0084, respectively). These results support that the T allele carriers could have better verbal memory, attention/concentration and delayed recall performance than the homozygous C subjects.

We next categorized the subjects into three genotype groups (the C/C genotype group vs. the C/T genotype group vs. the T/T genotype group) (Table II). There was no significant difference in age, education years, IQ or gender ratio between the groups. Significant genotype effects were found in verbal memory ($F_{2,184}=3.89$, $P=0.022$) and delayed recall ($F_{2,184}=6.36$, $P=0.0021$), while no significant difference was found in visual memory ($F_{2,184}=1.53$, $P=0.22$) and attention/concentration ($F_{2,184}=2.14$, $P=0.12$). The association of delayed recall survived after correction for multiple testing (corrected $P=0.0086$). However, the association of verbal memory was no longer positive after correction for multiple testing (corrected $P=0.088$). Post hoc analysis revealed that the T/T carriers and the C/T carriers had better delayed recall performance than the C/C carriers, respectively (post hoc $P=0.0017$, 0.0096) (Figure 1). Thus, the homozygous C allele might have recessive effects on delayed recall performance in our sample.

We finally categorized the sample into two groups (the C allele carriers vs. the T/T carriers) (Supplementary Table I available at: http://informahealthcare.com/doi/abs/10.3109/15622971003797258). There was no significant difference in age, education years, IQ or gender ratio between groups. No significant difference was observed between groups in any memory indices. These results suggest that there is no association of the *KIBRA* SNP with memory performance between the C allele carriers and the T/T carriers.

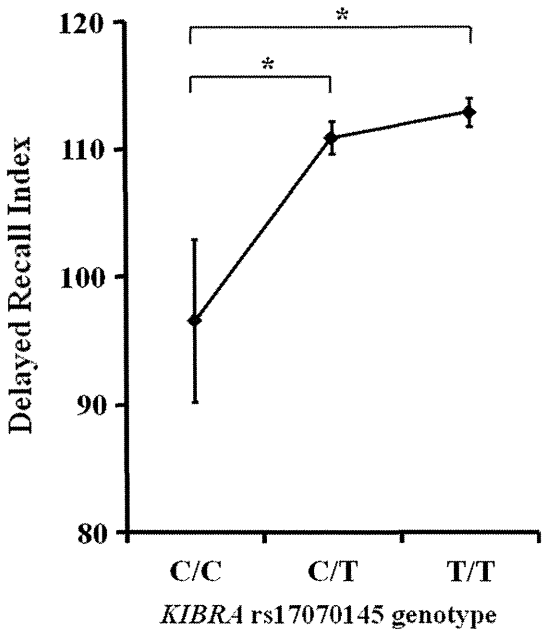

Figure 1. Delayed recall performance according to the KIBRA rs17070145 polymorphism. Mean scores of delayed recall with the C/C ($n=7$), the C/T ($n=56$) and the T/T ($n=124$) genotypes are shown (Bars represent SE). Group comparisons of delayed recall index among three genotype groups were analyzed by using ANOVA. Post hoc comparisons were performed by using Bonferroni correction. Significant differences were compared with the C/C genotype. *$P < 0.01$.

Discussion

In this study, we first examined the genotype effect of the *KIBRA* SNP among three genotype groups in healthy Japanese subjects. The C allele might have recessive effect on delayed recall performance. Moreover, it revealed that the *KIBRA* SNP had an impact on memory performance in Asian populations as well as in Caucasian populations, though the allelic distribution of the *KIBRA* rs17070145 is different between ethnic groups (T allele frequencies in Caucasian: 26%, in Japanese: 81%, in Chinese: 78%, in African: 66%, respectively).

Since the initial association study, several studies have investigated the relationship between the *KIBRA* SNP and memory performance using various methods. In the initial study, the Rey Auditory Verbal Learning Test(AVLT — a standardized test of learning a list of 30 unrelated nouns (Rosenberg et al. 1984)), Buschke's Selective Remaining Test,

and 10-min delayed recall of semantically unrelated picture stimuli were used in three healthy Caucasian cohorts. They found that the T allele (the T/T combined with the C/T) carriers had better delayed recall performance than the C/C carriers (Papassotiropoulos et al. 2006). In terms of replicating the original finding, Schaper et al. examined the association between the *KIBRA* SNP and memory performance by AVLT in a small sample (Schaper et al. 2008). They found that the T allele (the T/T combined with the C/T) carriers had significantly better total recall and delayed recognition performance than the homozygous C/C carriers. Almedia et al. found that the T allele carriers had significant better performance in immediate recall, delayed recall and recognition performance than the C/C carriers measured by the cognitive battery of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Almeida et al. 2008). Bates et al. (2009) found that the *KIBRA* SNP was related to delayed recall performance by using AVLT in subjects with asymptomatic atherosclerosis for randomized controlled trial of aspirin. Preuschhof et al. (2010) also replicated the beneficial effect of the *KIBRA* T allele on episodic memory performance and found that the *KIBRA* and the *CLSTN2* interactively modulate episodic memory performance in healthy subjects. In this way, these previous studies supported that the T allele has a more beneficial effect on episodic memory than the C allele. On the other hand, Zhang et al. (2009) suggested that the T allele carriers had less cognitive flexibility than the C/C carriers in European Americans but not in African Americans, and that current smoking status moderates this influence of the *KIBRA* SNP on cognitive performance. The other two studies did not support the association between the SNP and memory performance (Need et al. 2008, 2009). These inconsistent findings among the studies may have been led to by the possibility of publication bias, age of the subjects, differences between the memory tests which were used in the studies.

In our study, the *KIBRA* SNP had an effect on attention/concentration ($P=0.047$), similar to the previous study which suggested the association between the *KIBRA* SNP and immediate memory performance (Almeida et al. 2008); however, the *KIBRA* SNP was not associated with attention in the original study (Papassotiropoulos et al. 2006). These inconsistent findings between the studies may be led by the possibility of differences between the memory tests and ethnic groups. When we controlled the attention/concentration for verbal memory and delayed recall, genotype effects were found in delayed recall ($P=0.029$), while no significant difference was found in verbal memory ($P=0.074$).

Since the *KIBRA* gene is associated with memory in normal subjects, there are two studies to examine the association of the *KIBRA* SNP with risk for developing Alzheimer's disease (AD). One study reported that the T allele carriers of the *KIBRA* SNP had increased risk of late-onset AD (Rodriguez-Rodriguez et al. 2009); however, another study indicated that the C/C carriers had increased risk for late-onset AD (Corneveaux et al. 2010). No association between the *KIBRA* SNP and mild cognitive impairment (MCI) was found in an aged cohort without dementia (Almeida et al. 2008). Subjective memory complaints with the T allele carriers performed more poorly than those with the C/C carriers on long-term memory tests (Nacmias et al. 2008), which was opposite genotype effect found in healthy subjects including our data (Papassotiropoulos et al. 2006; Almeida et al. 2008; Schaper et al. 2008; Bates et al. 2009; Preuschhof et al. 2009). These inconsistencies among genetic association studies in patients and inconsistencies between normal control studies and studies in memory impairments could be due to the differential role of the *KIBRA* gene in a certain pathological state.

The limitation of our study is that we had a small sample size of the C/C genotype group compared with the other two genotype groups. A larger population would avoid this problem. The effect sizes f in our study were medium; 0.19 in verbal memory and 0.23 in delayed recall. It is similar to the previous studies (the effect size $d=0.2$–0.7; small to large) (Papassotiropoulos et al. 2006; Schaper et al. 2008). When we performed power calculations, the post hoc powers in our study were 0.73 in verbal memory and 0.88 in delayed recall.

Further independent study with larger sample size is warranted in the other Asian population. More investigations to clarify the association of the *KIBRA* gene with memory performance in other ethnic groups would be required. To clarify the function of the *KIBRA* gene may lead to further understandings about the brain function and the pathology of neuropsychiatric disorders with memory disturbances.

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Statement of Interest

All authors declare that they have no conflict of interest.

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Supplementary material available online

Table showing collated results

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Relationship between prepulse inhibition of acoustic startle response and schizotypy in healthy Japanese subjects

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Abstract

Prepulse inhibition (PPI) of the acoustic startle reflex (ASR) is the most common psychophysiological index of sensorimotor gating. Several studies have investigated the relationship of PPI of ASR to schizotypy in Caucasians. However, little has been reported on this relationship in Asians. We investigated a possible relationship between PPI of ASR and schizotypy in 79 healthy Japanese subjects. Schizotypy was assessed by the Schizotypal personality Questionnaire (SPQ). PPI was evaluated at signal-to-noise ratios (SnRs: difference between background noise intensity and prepulse intensity) of +12, +16, and +20 dB. The total SPQ score, cognitive/perceptual score, and interpersonal score correlated negatively with PPI at SnR of +16 and +20 dB. We conclude that PPI is associated with the trait of schizotypy in healthy Asian subjects.

Descriptors: Prepulse inhibition, Sensorimotor gating, Schizotypy, Acoustic startle response, Asians

Sensorimotor gating is thought to be a process which regulates sensory input by filtering out irrelevant or distracting stimuli, prevents sensory information overflow, and allows for selective and efficient processing of relevant information. Prepulse inhibition (PPI), which is usually defined as a reduction of the startle reflex due to weak sensory prestimulation (Braff, Stone, Callaway, Geyer, Glick, et al., 1978) of the acoustic startle reflex (ASR), is the most common psychophysiological index of sensorimotor gating.

Recently, PPI has been considered a candidate intermediate phenotype (endophenotype) of schizophrenia (Braff & Light, 2005; Turetsky, Calkins, Light, Olincy, Radant, & Swerdlow, 2007) and schizotypy (Cadenhead & Braff, 2002). Previous studies have consistently demonstrated PPI reductions in patients with schizophrenia (reviewed by Braff, Geyer, Light, Sprock, Perry, et al., 2001). Reports of PPI reductions not only in schizophrenia patients but also in unaffected relatives (Cadenhead, Swerdlow, Shafer, Diaz, & Braff, 2000; Kumari, Das, Zachariah, Ettinger, & Sharma, 2005) suggest a substantial heritability of PPI impairment (Anokhin, Heath, Myers, Ralano, & Wood, 2003). Deficient PPI is also seen in patients with schizotypal personality disorder (SPD) (Cadenhead, Geyer, & Braff, 1993; Cadenhead et al., 2000), and to a lesser extent in normal participants scoring high on psychometric measures of psychosis-proneness (Kumari, Toone, & Gray, 1997; Simons & Giardina, 1992; Swerdlow, Filion, Geyer, & Braff, 1995).

Since the profile of startle measures is thought to differ across race (Hasenkamp, Norrholm, Green, Lewison, Boshoven, et al., 2008; Swerdlow, Sprock, Light, Cadenhead, Calkins, et al., 2007; Swerdlow, Talledo, & Braff, 2005), PPI should be comprehensively explored in Asian subjects. Recent reports indicate that, as well as in Caucasians, PPI is impaired in Asian patients with schizophrenia (Kunugi, Tanaka, Hori, Hashimoto, Saitoh et al., 2007; Takahashi, Iwase, Ishii, Ohi, Fukumoto, et al., 2008). However, to our knowledge, the relationship between PPI and schizotypy has not yet been investigated in non-Caucasian subjects.

In this study, we aimed at determining a possible association between PPI and schizotypy in a sample of 79 healthy Japanese subjects. We evaluated PPI at signal-to-noise ratios (SnRs:

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difference between background noise intensity and prepulse intensity) of +12, +16, and +20 dB. To measure schizotypy, we used the three factor model (Raine, Reynolds, Lencz, Scerbo, Triphon, & Kim, 1994) of the Schizotypal Personality Questionnaire (SPQ; Raine, 1991).

Method

Participants

One hundred and nine Japanese volunteers were recruited by local advertisements in Osaka, as psychiatrically, medically, and neurologically healthy volunteers who were not receiving psychiatric medication, and had no first- or second-degree relatives with psychosis. Volunteers were screened for psychiatric disorder with the non-patient edition of the modified structured clinical interview for the Diagnostic and Statistical Manual—4th Edition, Axis I Disorders (SCID-I/NP) (First, Spitzer, Gibbon, & Williams, 1997), which was conducted by a well-trained psychologist. Two volunteers were excluded because they had psychiatric disorder (both anxiety disorder), and seven volunteers were excluded because they had first- or second-degree relatives with psychosis. As a result, 100 healthy Japanese subjects participated in this study. A portion of the subjects in the present study was from our previous sample (Takahashi et al., 2008). According to the screening interview, these subjects did not have clinically significant distress or impairment in social, occupational, or other important areas of functioning, which is necessary to be diagnosed as a personality disorder. None of the participants had any hearing impairments. Pregnant or lactating women were not included. The study procedure was conducted according to the Helsinki Declaration and approved by the Research Ethical Committee of Osaka University. All participants gave written informed consent after the study procedures were fully explained to them.

Schizotypy Questionnaire

Schizotypy was assessed using the SPQ. This is a 74-item questionnaire with a dichotomous response format (yes/no). The SPQ was developed to measure all of the nine diagnostic criteria stipulated by the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) (American Psychiatric Association, 1987) for SPD. The factor analytical study of schizotypal personality by Raine et al. (1994) showed that the nine diagnostic subscales for SPD can be reduced to three latent factors: cognitive-perceptual, interpersonal, and disorganization. The cognitive-perceptual factor reflects the positive symptoms of schizotypy, characterized by ideas of reference, odd beliefs/magical thinking, unusual perceptual experiences, and suspiciousness/paranoid ideation. The interpersonal factor reflects the negative symptoms of schizotypy, characterized by suspiciousness/paranoid ideation, constricted affect, lack of close friends, and excessive social anxiety. The disorganization factor, which represents the disorganized schizotypy, consists of symptoms such as odd speech and odd or eccentric behavior. Scores for all 74 items are summed to produce the total SPQ score and the three SPQ latent factor scores. In this study, a Japanese version of the SPQ was used, and all participants filled out the questionnaire. The questionnaire had been administered to 258 Japanese college students in a validation study (Someya, Sasaki, & Takahashi, 1994), and the validity and reliability properties of this Japanese version of the SPQ were found to be similar to those of the original version of Raine (1991).

Startle Response Measurement

The methods for the startle paradigm, eyeblink acquisition, scoring parameters, and the procedure are described in detail in one of our earlier publications (Takahashi et al., 2008). A commercial computerized human startle response monitoring system (Startle Eyeblink Reflex Analysis System Map1155SYS, Nihonsanteku Co., Osaka, Japan) was used to deliver acoustic startle stimuli, and record and score the corresponding electromyographic activity. Stimulus presentation and data acquisition were controlled through a laptop computer with Windows XP operating system installed on it. All the auditory stimuli and the background noise were produced by a custom-built tone and noise generator and delivered binaurally to the subjects through stereophonic headphones (type DR-531, Elega Acous. Co. Ltd., Tokyo, Japan) with hard plastic bells.

Startle eyeblink electromyographic responses were recorded from the left orbicularis oculi muscle with a pair of Ag/AgCl disposable electrodes (sensor area 15 mm^2) filled with wet gel. The first electrode (Blue Sensor N-00-S, Ambu, Ballerup, Denmark) was positioned approximately 1 cm directly below the pupil of the left eye and low enough to not touch the lower eyelid, while the second electrode (Blue Sensor M-00-S, Ambu) was placed laterally and slightly superior to the first one, with the centers of the electrodes separated by approximately 2 cm. The impedance between the two electrodes was measured and deemed acceptable if below 10 kΩ. The impedance was measured with an electrode impedance meter (MaP811, Nihonsanteku Co.) at a measurement frequency of 30 Hz. The ground electrode (Blue Sensor M-00-S) was placed on the left angle of the mandible.

The skin area at the electrode site was cleaned with a cotton swab saturated with rubbing alcohol, then prepared by gently rubbing a small amount of Nuprep EEG & ECG Skin Prepping Gel (Bio-Medical Instruments Inc., Warren, MI), and cleaned with a cotton swab saturated with rubbing alcohol again. Electromyography (EMG) data were measured with an EMG Telemeter (PolyTele EMG, Nihonsanteku Co.). The measurement condition was adjusted as follows: the time constant was 0.03 s, which was equivalent to the low frequency filter of 5 Hz; the high frequency filter was 300 Hz. The sensitivity of the amplifier was 1000 times. The amplification gain control for the EMG signal was kept constant for all subjects. EMG data were digitized with a 12-bit A/D converter (MaP222, Nihonsanteku Co.) and collected on the PC. The sampling frequency was 1 kHz. Sampling on each trial began 1000 ms prior to the onset of the startle eliciting stimulus and continued for 1000 ms after the onset of the startle eliciting stimulus. The resulting data were baseline corrected with a moving average. The eyeblink magnitude of every startle response was defined as the voltage of the peak activity of the EMG within a latency window of 20–85 ms following startle eliciting stimulus onset. The data were stored and exported for analyses in microvolt values.

Participants were tested in a startle paradigm, which consisted of 3 blocks with a continuously presented 70 dB sound pressure levels (SPL) background white noise. Pulse stimuli consisted of broadband white noises with an instantaneous rise/fall time lasting for 40 ms presented at 115 dB SPL. Prepulse stimuli were also broadband white noises with an instantaneous rise/fall time lasting for 20 ms presented at three different intensities (82, 86, and 90 dB SPL, equivalent to SnR of +12 dB, +16 dB, and +20 dB, respectively). The lead interval (from prepulse onset to pulse onset) was 120 ms. In block 1, the startle response for pulse alone trial (PA trial) was recorded 6 times. Block 2 consisted of PA