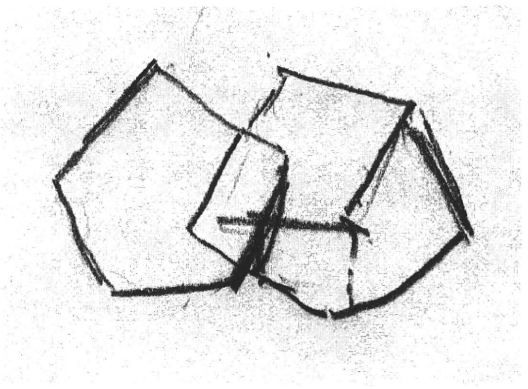

図1　五角形模写
Mini-mental state examinationにおける五角形描写で視覚認知障害が示唆された。

bodies；DLB)は，高齢期に発症する認知症として，アルツハイマー型認知症に次いで2番目に頻度の高い変性性認知症である[13]。DLBでは，必須症状である進行性の認知機能障害とともに幻視を高頻度に伴うことが特徴であり，中核症状の1つとされている。DLBの幻視の発現機序に関しては，脳機能画像や神経病理学的観点から検討が行われているが[5,19]，その生物学的機序は明らかとなっていない。今回我々は，約30年間の特発性レム睡眠行動障害(REM sleep behavior disorder；RBD)の経過の後に幻視が出現し，probable DLBと診断するに至った発症早期のDLBの1例を経験した。幻視は自宅では認められず，山岳部の別荘を訪れるたびに出現した。このことから，本症例における幻視の発現機序には，器質要因とともに心理・環境要因が関与していると考えられた。なお，匿名性に配慮し，個人が特定されないように病歴に若干の変更を加えている。

症例

〈症例〉　83歳，女性。右利き。

主訴　幻視。

既往歴　狭心症，高血圧，白内障，難聴，卵巣摘出，肺結核。

家族歴　特記すべきものなし。

生活歴　師範学校を卒業後，病院で5年間，国税局で3年間勤務し，27歳で結婚した後は専業主婦。挙子3名。結婚後はボーイスカウトの世話役や町内会長を務め，60歳から75歳まで夫の会社の経理を担当した。

病前性格　完璧主義，きちょうめん，まじめ。

現病歴：X－33年(50歳)頃から，睡眠中に夫が覚醒するほどの大声を出すことがしばしばあった。また，数か月に1回の頻度で隣に寝ている夫が患者から暴力を受けることがあり，本人は「背後から襲われたので防御した」と夢の内容を説明し，これは睡眠中の行動障害と一致していた。これらのRBDと考えられる症状は約30年間続いたが，医療機関の受診には至らなかった。

X－2年(81歳)頃から，起立時の立ちくらみ，まっすぐ歩けないなどの症状が出現し，歩行時に杖を使用するようになった。また，聞いたことを忘れる，物の置き忘れなど，健忘に気づかれていた。隣の部屋に誰かがいる気がすると夫に話すことがあった。

30年ほど前に山岳部に別荘を購入し，首都圏にある自宅との往来をしていた。近年は月2回ほど別荘に出かけ，1週間ごとに双方を行き来していた。X年3月(83歳)，別荘に滞在中に，庭の切り株をみて，「ヒトが横たわっている」と言い始めた。その後，庭の木を見て「2mの大男だ」と言い，「桜の木に8人の女がぶらさがっている」，「頭にカラスの帽子をかぶった人たちがいる」などと，徐々に幻視や錯視の訴えは増加した。さらに「カラスに化けた2mほどの大男が中心となって，多くの信者を伴って毎日2時間おきに宗教布教のため儀式を行っている」「誰かはわからないが，カラスに化けて信者を集めることでなにかを企んでおり，役場・警察に早く知らせたい」と幻視に基づく被害妄想を訴えた。しかし，夫の制止に従い，警察への通報はしていなかった。これらの幻視は別荘に滞在中のみ認められ，都市部の自宅ではみられなかった。別荘を訪れるたびに幻視が出現するため，同年7月に当院初診となった。

初診時所見　礼容は保たれ，表情の乏しさは目

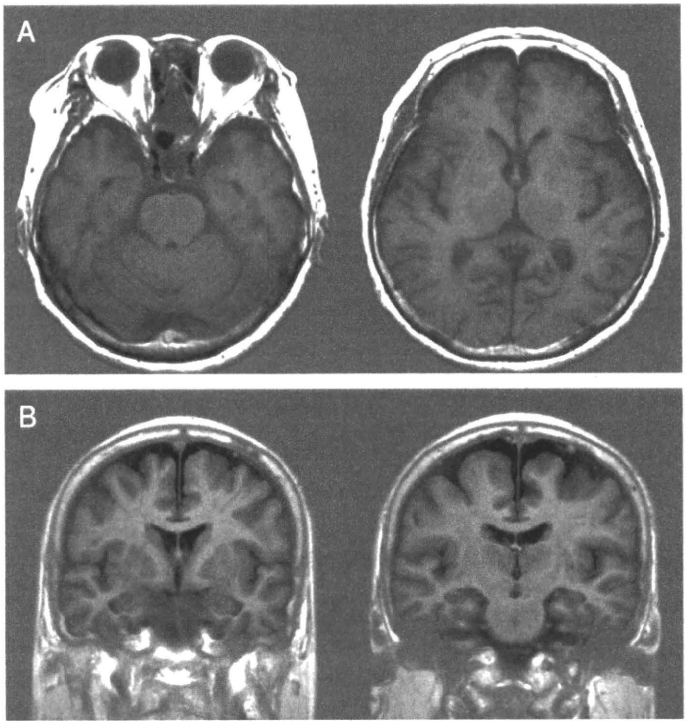

図2　頭部MRI画像
頭部MRIでは明らかな海馬領域を含む大脳萎縮は認めなかった。
T1強調画像：水平断(A)と冠状断(B)。

立たず，発語は正常であった。言語理解は良好であったが，すぐに質問内容と無関係な話題に移るなど注意力の低下が疑われた。「私はもう長くはないのかしら」と悲観的な発言がときにみられたが，食欲は良好で，不眠の訴えもなく，抑うつ状態とはみなせなかった。カラスに化けた2mの大男の外見について「長いくちばしを持ちペンギンのような白と黒の模様のコートを着て両足には白樺の木をつけ足長になっている」と幻視内容について詳細に説明した。神経学的には，不安定歩行・軽度の姿勢反射障害を認めたが，四肢の筋固縮・振戦は認められず，UPDRS(unified Parkinson's disease rating scale)-motorスコアは6点であった。自律神経症状として，便秘と起立性低血圧が認められた。

検査所見　血液・生化学検査では異常を認めなかった。神経心理検査では，改訂長谷川式認知症スケール(HDS-R)29点，mini-mental state examination(MMSE)25点であり，計算，五角形模写の項目で失点を認めた(図1)。Wechsler adult intelligence scale(WAIS-Ⅲ)では，全検査IQが89，言語性IQが93，動作性IQが87であった。群指数では，言語理解が95，知覚統合が85，作動記憶が83，処理速度が89であり，「積木模様」「数唱・語音整列」「知識」の得点が不良であった。Wechsler memory scale-revised(WMS-R)では，一般的記憶が79，言語性記憶が78，視覚性記憶が87，注意集中力が84，遅延再生が74であり，75歳以下の平均との比較では，正常下限から境界領域の記憶障害が疑われた。Bender gestalt test(BGT)では，日本語版Pascal-Suttell法の採点で111点，簡易採点法では5/16点と，それぞれDLBのcut off値である98点，5点より高値を示した[16,17]。質的には，点や線の震えとともに，いくつかの図版で描画のゆがみがあり，視覚認知障害の存在が示唆された。Clock draw-

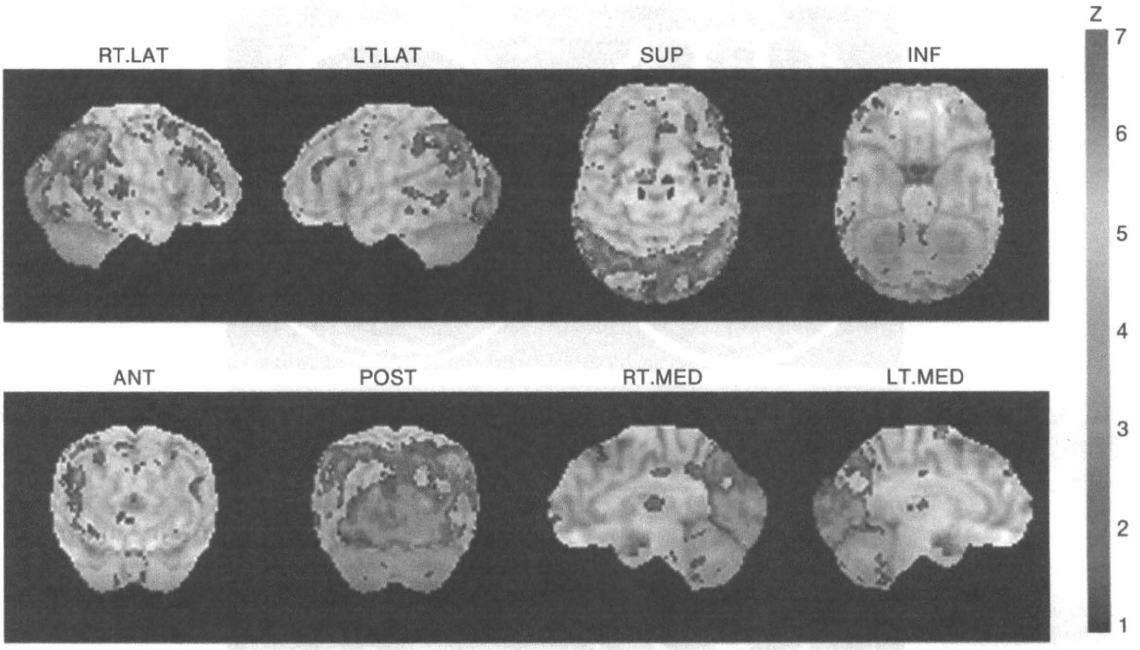

図3　頭部^{18}F-FDG PET 画像

^{18}F-FDG PET scans で 3D-SSP 解析を用いて，びまん性の頭頂後頭葉領域の糖代謝低下を認めた。
RT. LAT；right lateral view, LT. LAT；left lateral view, SUP；superior view, INF；inferior view, ANT；anterior view, POST；posterior view, RT. MED；right medial view, LT. MED；left medial view

ing test では，教示通りの時計描画が可能であった。画像検査では，頭部 MRI で左被殻にラクナを認めたが，海馬領域を含む大脳の萎縮は年齢相応であった(図2)。^{18}F-FDG PET 画像では，主に両側頭頂・後頭葉にびまん性の糖代謝低下を認めた(図3)。

臨床経過　軽度ながら進行性の認知機能障害に加え，中核症状である幻視と軽度のパーキンソニズム，支持症状である RBD を認め，神経心理学的所見や PET 所見も考慮して，probable DLB と診断した。認知機能障害と幻視に対して塩酸ドネペジルの投与を 3 mg/日より開始し，5 mg/日に増量した。また，塩酸ドネペジルの投薬とともに，神経心理学的検査と脳 PET 画像の結果を示したうえで，DLB による視覚認知障害があるために幻視や錯視が生じていることを繰り返し説明した。その結果，「別荘での出来事は気にならなくなりました」と話し，幻視に基づく妄想的な訴えはなくなった。しかし，その後，別荘を訪れると幻視は残存しており，「あれはいったい何なのでしょうか？　頭の中の問題で起こっているのかしら」と病気であることを受け入れつつも，幻視に影響を受けている様子であった。一方，塩酸ドネペジルの投与開始後，数か月に 1 回であった RBD の頻度は徐々に増加し，隔日に出現するようになった。RBD に対しクロナゼパム 0.5 mg/日の投与を開始したが，日中の傾眠を認めたため 0.4 mg/日に減量し，夜間の RBD の出現頻度は減少した。12 月になり，自宅で初めて幻視が認められ，「床の間に和服を着た男の人が座っていたが，顔は黒くて見えなかった。その頃他界したかかりつけ医だと思う」と訴えた。しかし，自宅ではその後幻視の出現は確認されていない。X+1 年 6 月現在，外来通院治療を継続中であるが，別荘でのみ幻覚は引き続き出現している。

考察

本症例では，DLB の臨床診断基準の必須症状

である進行性の認知機能障害として，記憶障害の病歴を聴取し，神経心理検査においてWMS-Rで軽度の記憶障害が確認された。また，中核症状である幻視と示唆症状であるRBDは本症例の特徴であった。不安定歩行や軽度の姿勢反射障害もみられたが，UPDRS-motorスコアは6点であり，振戦や筋固縮は認めなかった。頭部MRIで内側側頭葉の萎縮は目立たず，^{18}FDG-PETで後頭葉のびまん性糖代謝低下がみられるなど画像上の支持症状も確認されたことから，probable DLBと診断されるに至った[13]。

RBDはDLBの必須症状や中核症状に年単位で先行することが多く，特発性RBDが後に高い頻度でパーキンソン病(Parkinson's disease；PD)やDLBなどのレビー小体病を発症することが明らかとなっている[3,9]。近年，特発性RBD患者が有意な記憶障害や視覚認知障害を示し，神経心理学的特徴がレビー小体病のものと類似することが明らかとなっている[4,7]。本症例は，約30年の長期間の特発性RBDのエピソードの後に幻視が出現し，その4か月後に当院初診となっており，特発性RBDから移行した早期DLBの症例であると考えられた。特発性RBD患者の剖検例において，脳幹型レビー小体病の病理所見を認められたことが報告され[2]，本症例のRBDの長期経過はレビー小体病の臨床像の多様性を示している。本症例は，MMSEにおける五角形の模写ができず，BGTのスコアが111点と高値であり，視覚認知障害の存在が示唆された。切り株が人の顔に見えるという錯視が人物幻視を誘発し，さらにこれらに基づく妄想形成に発展したものと考えられ，視覚認知障害が幻視誘発の神経基盤となったのではないかと推測された。

芝生の中に存在する切り株，ゆらめく木々などの別荘の周囲の錯視を誘発しやすい状況が，場所依存性に幻視を誘発した一因ではないかと考えられた。DLBでは，壁にかかった服が人物に見えたり，縞模様柄を蛇と間違えたりすることがあり，対象を取り除くことによって幻視が解消されることも多い[10,14]。つまり，本症例では幻視を誘発する環境要因が多数存在する別荘においてのみ幻視が出現し，自宅では認めなかったのではないかと推察された。また，以前に別荘の近郊でカルト集団による事件があったことが患者の不安感を励起している可能性が，心理的背景として考えられた。後に，自宅でも1度だけ幻視が出現しているが，これは長年の主治医であったかかりつけ医の他界が契機となっており，やはり心理要因の関与が疑われた。すなわち，本症例の幻視の発現機序には，DLBに特徴的な視覚認知障害などの脳の器質要因のみならず，心理・環境要因も関与していることが示唆された。したがって，今後DLBの病態の進行とともに器質要因の関与が強くなると，自宅でも幻視が出現してくる可能性がある。

本症例では，塩酸ドネペジルの投与により，幻視に基づく被害妄想などの精神症状は改善したが，RBDの出現頻度の増加を認めた。特発性RBD患者やDLB患者において，塩酸ドネペジルの投与によりRBDが改善したことが報告されているが[12,18]，本症例ではRBDの出現頻度の増加を認めた。アセチルコリン分解阻害剤がREM睡眠を介した記憶に関与することが報告されており[6]，DLBの病態を考えるうえで興味深い所見であると考えられた。

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Summary

Location-dependent Visual Hallucinations in a Woman with Probable Dementia with Lewy Bodies and Idiopathic Rapid Eye Movement Sleep Behavior Disorder

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Sato Kiyoshi[1]

We report the case of an 83-year-old Japanese woman with probable dementia with Lewy bodies (DLB), who developed location-dependent visual hallucinations (VHs) after chronic idiopathic REM sleep behavior disorder (RBD). She had developed RBD at the age of 50 years, and slight memory loss, orthostatic hypotension, and unstable gait at 81 years. At the age of 83 years, she experienced VHs in the month of March, during one of her fortnightly visits to her country cottage；she had never experienced VHs at her house in the town. The hallucinations began with her identifying a stump in the garden as a person lying on the ground, and later perceiving a tree as a hefty person. She gradually developed severe VHs such as that of 8 women

hanging from a cherry tree. Finally, her condition worsened to systematic delusions ; a group of peple who transforned into crows performed a religious ceremony in her garden. At her first visit to our memory clinic in the month of July, she scored 25 points on mini-mental state examination (attention 1/5 ; construction, 0/1). On the Bender Gestalt test, she scored 111 points, which indicated visual-perceptual disability that met the threshold score for the diagnosis of probable DLB. She, however, could describe her VHs in complete detail. MRI of the head showed mild medial temporal lobe atrophy.

^{18}F-FDG PET showed bilateral diffuse parieto-occipital hypometabolism. Although donepezil 5 mg/day treatmet improved the status of her VH-based delusions, the frequency of RBD increased from once in a few months to every alternate day, thereby indicating a relationship between the dysfunction of cholinergic neurons and RBD. The occurrence of VHs might be due to certain environmental and psychological factors in addition to the organic brain dysfunction such as visual-perceptual disability.

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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.

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 ε4 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-pallidoluysian 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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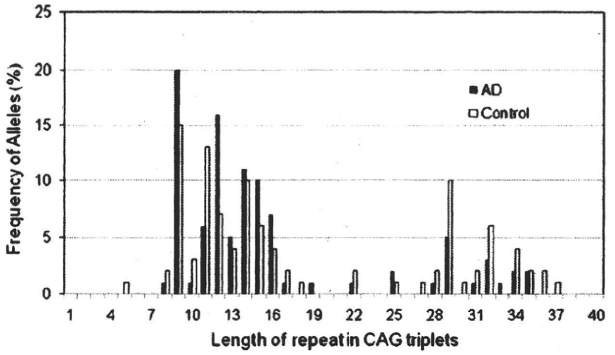

Fig. 1. Distribution of allele frequencies against the CAG repeat numbers in the *PPP2R2B* gene of control subjects and AD patients.

by polymerase chain reaction (PCR) amplification using 6FAM dye-labeled forward (5′-TGCTGGGAAAGAGTCGTG-3′) and reverse (5′-GCCCGCGCACTCACCCTC-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 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			*p* value	OR
	Total	Short (≤15)	Long (>15)		
Control	172	110 (64%)	62 (36%)		
Control with APOE4	24	16 (67%)	8 (33%)	0.267	
Control without APOE4	148	94 (64%)	54 (36%)	0.022*	1.58
AD	436	320 (73%)	116 (27%)	0.021*	1.55
AD with APOE4	212	163 (77%)	49 (23%)	0.005*	1.87
AD without APOE4	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

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

Group	Control			AD		
	Total	APOE4 (+)	APOE4 (−)	Total	APOE4 (+)	APOE4 (−)
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)
p 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.

than in the control group (Table 2). Our results suggested that AD is associated with a lower number of CAG repeats in the *PPP2R2B* gene. This was similar to the findings of a previous report by Chen et al. [3]. However, in our AD patients, we did not find short 5–7 triplet alleles which detected in AD patients in the Han Chinese population. This discrepancy may reflect a genetic differentiation between the Han Chinese and Japanese populations.

The presence of the ε4 allele of *APOE* gene confers a heightened risk of late-onset AD [4]. As compared to individuals without the ε4 alleles, the risk for AD is 2- to 3-fold and about 12-fold higher in individuals carrying one and two ε4 alleles, respectively [1,14,24]. Though several studies have attempted to elucidate the mechanism for this increased risk, how APOE4 influences AD progression has yet to be proven. In particular, we found that the frequency of short CAG repeats (≤15) was higher in the AD with APOE4 group than in the control group. Therefore, it is likely that a short number of CAG repeats of *PPP2R2B* gene play an important role for the progression of late-onset AD with APOE4.

PP2A is composed of three subunits: a catalytic subunit (C), a scaffolding subunit (A), and a regulatory subunit (B). Assembly of the complex with the regulatory B subunit is required for the specificity and regulation of PP2A [31]. In addition, PP2A is the major tau phosphatase that dephosphorylates tau at multiple sites, and its activity is decreased by 30% in the frontal or temporal cortex of AD patients compared to controls [8,18]. This down-regulation of PP2A activity in AD brains is thought to be partially responsible for abnormal tau phosphorylation. Therefore, differences in the CAG repeat lengths in the *PPP2R2B* gene may regulate PP2A activity, leading to AD progression. Through a reporter assay, the short 5–7 triplet alleles were shown to be associated with decreased PPP2R2B promoter activities [3]. However, it has not yet been demonstrated that the short CAG repeat lengths in the PPP2R2B affect PP2A function directly.

APOE plays an important role in the distribution and metabolism of cholesterol in the human body [19]. APOE4 has also been associated with tau hyperphosphorylation in several animal models [9]. In particular, high cholesterol such as in Niemann-Pick C disease might be involved in decreasing membrane fluidity [16]. Therefore, it was recently supposed that signal transduction through the interaction of APOE4 with the neuronal cell membrane might involve AD progression through various kinases and phosphatases [13].

In conclusion, our results suggest that CAG repeat lengths in the *PPP2R2B* gene may be potential genetic markers for AD susceptibility in the Japanese population. Further investigations are required to confirm the role of the *PPP2R2B* gene in AD using a larger sample size and a different population group.

Conflicts of interest

None of the authors has any conflicts of interest.

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Original Research Article

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KIBRA Genetic Polymorphism Influences Episodic Memory in Alzheimer's Disease, but Does Not Show Association with Disease in a Japanese Cohort

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Key Words
Alzheimer's disease · Episodic memory · Genetics · Neuropsychological assessment · *KIBRA* gene · Rivermead Behavioral Memory Test

Abstract

Background/Aims: A single-nucleotide polymorphism (SNP) in the *KIBRA* gene, rs17070145, was reported to be significantly associated with episodic memory in cognitively normal cohorts. This observation has expanded genetic studies on *KIBRA* to Alzheimer's disease (AD). Importantly, the association between *KIBRA* and episodic memory in AD has never been addressed. In this study, we investigated whether the *KIBRA* rs17070145 SNP influences AD episodic memory and the disease in a Japanese cohort. ***Methods:*** Blood samples from 346 AD patients and 375 normal cognitive controls were collected and genotyped for rs17070145. Episodic memory was measured in 32 AD patients, diagnosed for the first time, by use of the Rivermead Behavioral Memory Test (RBMT). ***Results:*** We found that *KIBRA* C allele carriers scored significantly lower than *KIBRA* non-C carriers on both RBMT total profile score ($p = 0.042$, effect size = 0.84) and RBMT total screening score ($p < 0.001$, effect size = 1.42). The *KIBRA* gene did not show association with AD in our Japanese cohort. ***Conclusion:*** Our results evidence a strong association between the *KIBRA* gene and episodic memory impairment in AD, but show no influence on AD in our Japanese cohort. We propose that *KIBRA* might have an effect similar to cognitive reserve.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder clinically characterized by a progressive deterioration of cognitive abilities and memory loss. For the famil-

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ial occurrence of the disease (early-onset), the existence of familial AD-responsible genes has been demonstrated, with mutations in 3 genes, *APP, PSEN1* and *PSEN2*, consistently reported. However, the genetic component that underlies sporadic AD (late-onset), which accounts for over 95% of all AD cases, is still poorly understood.

KIBRA (also known as WW and C2 domain-containing protein 1) is a protein mainly expressed in the brain and kidney [1], whose functions are still being characterized, but that, importantly, has been shown to be involved in the control of synaptic plasticity in the brain [2]. Recently, it was reported that KIBRA regulated the Salvador/Hippo/Warts network which restricted tissue size [3]. In 2005, a *KIBRA* gene single-nucleotide polymorphism (SNP), rs17070145, was reported to be significantly associated with episodic memory in 3 independent cognitively normal cohorts from Switzerland and the USA [4]. This result was later confirmed in a German sample of healthy individuals [5], a Japanese sample of healthy individuals [6] and in a cohort in which nearly 50% of individuals had a diagnosis of mild cognitive impairment [7].

These observations led to studies of the *KIBRA* rs17070145 SNP in AD, whose core feature is dysfunction of episodic memory [8]. Recently, Corneveaux et al. [9] reported an association of the *KIBRA* CC genotype (*KIBRA* CC carriers) with increased risk for late-onset AD (n = 702). Conversely, the *KIBRA* T allele (*KIBRA* CT and *KIBRA* TT carriers) was shown to be associated with an increased risk for AD in a Spanish cohort [10]. Despite the available information, *KIBRA* has not yet been established as an AD risk gene, and, importantly, no studies have ever addressed the association between *KIBRA* and episodic memory in AD.

Therefore, in this study, we investigated whether the *KIBRA* SNP rs17070145 influences AD episodic memory and AD in a Japanese cohort.

Methods

Subjects

We collected blood samples from 346 consecutive AD patients who visited Osaka University Hospital between July 27, 2001, and June 10, 2010, and from 375 cognitively normal controls, who were population-based elderly subjects (Suita City, Japan) tested by a questionnaire including the date, orientation and history. Blood samples were collected after written informed consent had been obtained from subjects and/or representatives. This study was approved by the genome ethical committee of the Osaka University Graduate School of Medicine. AD patients met the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria for probable AD [11].

We also have a research-oriented clinic for patients with cognitive impairment in the Department of Neuropsychiatry of the Osaka University Medical Hospital. It is also a clinic for early identification of dementia. In this clinic, all patients were examined comprehensively by specialists of geriatric psychiatry, and they underwent standard neuropsychological examinations including the Mini Mental State Examination (MMSE), routine laboratory tests, electroencephalography, cranial magnetic resonance imaging and radionuclear neuroimaging studies. Blood drawing for the genome study was not routine in this clinic. Eighty first-time diagnosed AD patients agreed to it, and 32 out of them agreed to an additional visit for the memory examination by use of the Rivermead Behavioral Memory Test (RBMT) between September 30, 2002, and May 23, 2007 (RBMT-AD specialized clinic subjects). RBMT-AD specialized clinic subjects were excluded from the study if they (1) had the complication of other neurological diseases, (2) had any evidence of focal brain lesions on magnetic resonance images or of cerebral arterial occlusive lesions on magnetic resonance angiography, or (3) did not have a caregiving family member familiar with their everyday life.

Rivermead Behavioral Memory Test

The RBMT, developed by Wilson et al. [12, 13], is a standardized, validated and reliable test for everyday memory, including personal events, name of persons, newspaper articles, places visited, routes followed, schedules and appointments. It is difficult to assess everyday memory with traditional memory tests [14], but the RBMT differs from conventional tests in that each of its 12 items is an analog of an everyday task, rather than a test based on experimental material, such as paired associates or list of words. The Japanese version of the RBMT was developed by Watamori et al. [15], and its reliability and validity have been previously confirmed [16–19]. Concretely, the authors reported that the RBMT can distinguish AD from both mild cognitive impairment and normal control, and strongly correlates with objective memory tests, such as the Everyday Memory Checklist caregiver rating and Clinical Dementia Rating (CDR) memory domain.

Although the RBMT has 4 parallel forms (A, B, C and D) for repeated uses, only the RBMT-A form was administered to subjects in this study. The subtests of the RBMT are (1) remembering a first name and a surname with a facial portrait, (2) remembering to ask for a personal item belonging to the subject, (3) remembering to ask about an appointment, (4) picture recognition, (5) remembering a short story (immediate), (6) remembering a short story (delayed), (7) face recognition, (8) remembering a new route (immediate), (9) remembering a new route (delayed), (10) remembering to deliver a message (immediate and delayed), (11) orientation for time, place and persons, and (12) date. In 8 of the subtests, i.e. points 1–4, 6, 7, 9 and 10 (delayed), the subjects were instructed to remember the information that they were about to be given. The subtests were then conducted 5–30 min after the information had been given. Subtests 2, 3 and 10 are tests of prospective memory. In subtest 2, the subjects were asked to hand in a personal item at the start of the session and instructed to ask for it at the conclusion of the session. The item was then placed out of sight. In subtest 3, subjects were instructed at the beginning of the test session to remember to ask for their next appointment when they heard a buzzer 20 min later. In subtest 10, they had to remember to de-

Table 1. Characterization of the *KIBRA* C carriers and non-C carriers of the RBMT-AD specialized clinic subjects

	CC/CT (n = 12)	TT (n = 20)	p
Mean age ± SD, years	68.5 ± 10.0	72.2 ± 8.1	0.267
Mean age of first abnormal memory loss episode reported by caregivers ± SD, years	63.8 ± 2.6	69.3 ± 2.0[1]	0.104
Male/female, n	5/7	8/12	1.000
APOE ε4+/−, n	10/2	12/8	0.248
CDR score 0.5/1/2, n	1/9/2	3/14/2	1.000
Mean MMSE score ± SD	17.8 ± 2.7	20.4 ± 4.6	0.093
Mean ADAS score ± SD	20.0 ± 5.8	17.6 ± 7.1[1]	0.335
Years of education			
Median	12	10	0.151
IQR	10.25–15	9–14[1]	

p values assessed by t test (continuous variables) and Fisher's exact test (categorical variables). IQR = Interquartile range (Q1–Q3).

[1] One datum was missed.

liver a message in the course of retracing a route around the room. For each subtest of the RBMT, a raw score was given. Then, two kinds of score were produced, a simple pass/fail or screening score ranging in each case from 0 to 1, and a standardized profile score ranging in each case from 0 to 2. A total screening score ranging from 0 to 12 and a total profile score ranging from 0 to 24 were used as indices of overall everyday memory status of the subjects.

Genotyping

Genotyping of *KIBRA* rs17070145 polymorphism was performed by the Taq-Man SNP assay and ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, Calif., USA) as previously described [20–23]. The apolipoprotein E *(APOE)* genotype was determined by the PCR-RFLP method [20–23].

Statistical Analysis

Baseline characteristics are presented as means ± standard deviation, medians or interquartile ranges for continuous variables, and frequencies for categorical variables. Comparisons for continuous variables and categorical variables were performed with the t test and χ^2 test or Fisher's exact test, respectively. The analysis of covariance model was used to investigate the effect of treatment on the RBMT scores with the following covariate: presence of the *KIBRA* SNP C allele (*KIBRA* CT and *KIBRA* CC), *APOE* ε4, age, the age of first abnormal memory loss episode reported by caregivers, gender, CDR stage, MMSE score, Alzheimer's Disease Assessment Scale for Japanese cognitive subscale (ADAS-Jcog) and/or years of education. The best set of covariates was selected by using Akaike's information criterion [24]. All tests were two-sided, and the statistical significance level was set at 5%. Statistical analysis was performed with SAS software version 9.02 (SAS Institute, Cary, N.C., USA), and all p values and confidence intervals (CI) presented are the original and were not corrected for multiple testing. Meta-analysis of *KIBRA* CC AD odds ratio and 95% CI was performed by the Der-Simonian-Laird method.

Results

From the RBMT-AD specialized clinic subjects, we found 1 patient with *KIBRA* CC, 11 patients with *KIBRA* CT and 20 patients with *KIBRA* TT (*KIBRA* non-C carriers). *KIBRA* CC and CT groups (*KIBRA* C carriers) were combined because there was only 1 *KIBRA* CC patient and that patient displayed memory performance similar to that of the *KIBRA* CT group (total profile score was 2, total screening score was 0). A lower frequency of the *KIBRA* C allele was observed, which was in accordance with the National Center for Biotechnology Information database of genetic variation (dbSNP) for the Asian population. Most of the patients were in an early stage of dementia (table 1). No significant differences in age, gender, *APOE* ε4, CDR, MMSE score, ADAS score and years of education were found between *KIBRA* C and *KIBRA* non-C carriers.

When analyzing the RBMT scores of the two groups, we found that C carriers scored significantly lower than non-C carriers on both the profile score ($p = 0.042$, effect size = 0.84) and screening score ($p < 0.001$, effect size = 1.42; table 2), evidencing an association of *KIBRA* rs17070145 polymorphism with episodic memory impairment in our Japanese AD cohort. We then assigned RBMT total scores as dependent variables and *KIBRA* C, age, age of first abnormal memory loss episode reported by caregivers, gender, *APOE* ε4, CDR stage, MMSE score, ADAS-Jcog score and/or years of education as independent variables and performed multiple linear regression analysis. For all the different combinations, we selected the appropriate models to which Akaike's information criteria were the smallest [24]. Model 1 was appropriate for total profile score and model 2 for total screening score. *KIBRA* C was found to be significantly associated with both total profile and screening scores after adjustment with the models shown in table 2.

We also analyzed 346 AD patients and 375 cognitively normal controls. As expected, we found significant differences in gender and *APOE* ε4 allele frequencies (table 3). *KIBRA* rs17070145 genotype and allele distribution in control and AD groups are shown in table 4. The genotype frequencies were in accordance with the Hardy-

Table 2. RBMT scores (total profile score and total screening score) between *KIBRA* C carriers and non-C carriers of the RBMT-AD specialized clinic subjects

	CC/CT	TT	p	Effect size
Total profile score (not adjusted)	2.17, 0.60–3.17	4.26, 3.01–5.51	0.042	0.84
Total profile score (model 1)	1.88, 0.42–3.34	4.22, 3.16–5.29	0.012	1.07
Total profile score (model 2)	1.79, 0.31–3.27	4.28, 3.20–5.35	0.010	1.13
Total screening score (not adjusted)	0.10, 0.00–0.36	0.93, 0.58–1.39	<0.001	1.42
Total screening score (model 1)	0.07, 0.00–0.35	0.91, 0.56–1.37	<0.001	1.54
Total screening score (model 2)	0.05, 0.00–0.31	0.93, 0.58–1.40	<0.001	1.66

Scores are expressed as mean estimates, followed by 95% CI. p values assessed by ANCOVA; model 1: adjusted for *APOE* ε4, years of education and ADAS score (this model is appropriate for total profile score); model 2: adjusted for *APOE* ε4, years of education, ADAS score and age (this model is appropriate for total screening score).

Table 3. Characterization of cognitively normal controls (NC) and AD patients

	NC (n = 375)	AD (n = 346)	p
Mean age ± SD, years	75.5 ± 4.9	75.2 ± 8.6	0.600
Male/female, n	170/205	110/236	<0.001
APOE ε4+/–, n	60/315	172/174	<0.001

p values assessed by t test (continuous variable) and Fisher's exact test (categorical variables).

Table 4. rs17070145 genotype and allele distribution in cognitively normal controls (NC) and AD patients

	CC	CT	TT	p^a	p^b	p^c
NC	13 (3.5)	128 (34.1)	234 (62.4)	0.673	0.414	0.694
AD	16 (4.6)	104 (30.1)	226 (65.3)	0.669		

Results are numbers, with percentages in parentheses.
[a] p for Hardy-Weinberg equilibrium tests (Pearson χ^2 test).
[b] p for genotype distribution (Fisher's exact test).
[c] p for allele distribution (Fisher's exact test).

Weinberg equilibrium. The *KIBRA* SNP did not show any association with AD in our Japanese cohort (table 4), even after adjustment for age, gender and *APOE* ε4 (data not shown).

Figure 1 shows KIBRA CC AD odds ratio and 95% CI in our Japanese cohort and previously reported cohorts. Our cohort's KIBRA CC AD odds ratio was 1.35 (95% CI = 0.64–2.85). Meta-analysis of them was not significant (OR = 1.10, 95% CI = 0.92–1.30).

Discussion

Despite numerous reports evidencing association of *KIBRA* with episodic memory, the relevance of *KIBRA* to AD still remains elusive. In our study, we addressed for the first time whether *KIBRA* genetic variation is associated with episodic memory impairment in AD. Our results evidence a strong association between the *KIBRA* gene and episodic memory impairment in AD and suggest a role for *KIBRA* similar to cognitive reserve, with no impact on diagnosis of AD.

There are several memory test batteries available, such as the Auditory-Verbal Learning Test (AVLT) [25], the Revised Wechsler Memory Scale Logical Memory Test [26], the Rey-Osterrieth complex figure [27] and the Takeda Three Colors Combination Test [28]. Association of *KIBRA* rs17070145 with episodic memory was shown for the first time by AVLT in 3 independent cognitively normal cohorts [3], and it has been recently confirmed in a Scottish cohort study (n = 2,091) [29]. In addition, the latter reported no association of the *KIBRA* SNP with the Revised Wechsler Memory Scale Logical Memory Test that rewards relational coding (Lothian Barth cohort, n = 542) [29], suggesting that *KIBRA* is not specific for complex episodic memory such as the Revised Wechsler Memory Scale Logical Memory Test but for simple episodic memory such as the AVLT instead. In our study, we

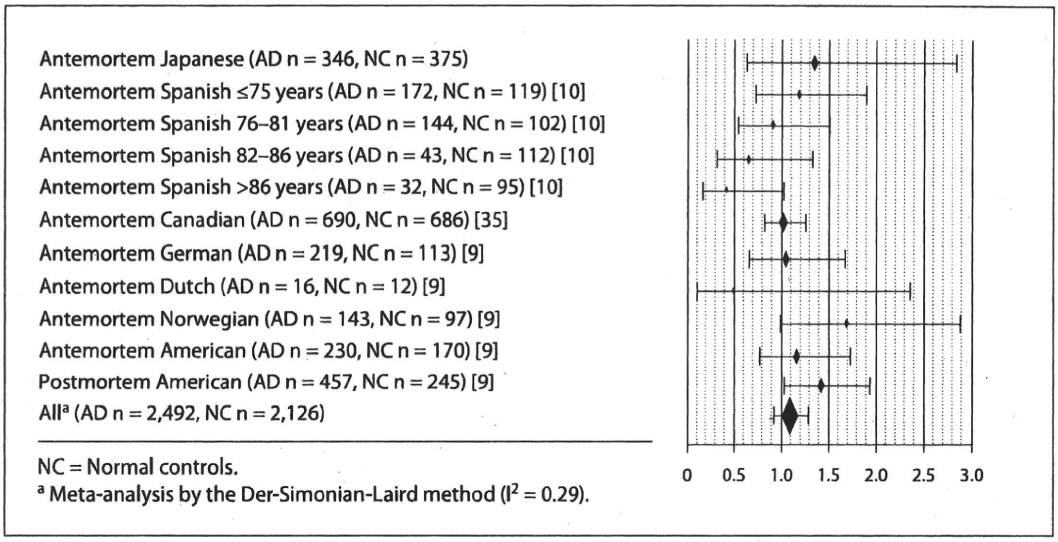

Fig. 1. *KIBRA* CC AD odds ratio and 95% CI in our Japanese cohort and previously reported cohorts.

used the RBMT, which assesses multidimensional aspects of everyday memory such as orientation, prose recall, visual recognition, prospective memory and so on. It also includes both complex episodic memory and simple episodic memory. Our results show that the *KIBRA* rs17070145 polymorphism is strongly associated with episodic memory impairment in our specialized clinic Japanese AD cohort (table 2). MMSE and ADAS-cog showed no differences between *KIBRA* C and non-C carriers (table 1). These results suggest that the *KIBRA* gene specifically seems to affect memory functions but not global cognitive status.

This association remained significant after adjustment for covariant components, which indicates that the *KIBRA* SNP might be an independent risk factor for episodic memory impairment.

Whereas an association of *KIBRA* with episodic memory has been repeatedly reported [4, 5, 7, 29], the impact of *KIBRA* on AD is still controversial (fig. 1). In a Spanish cohort, *KIBRA* CC AD odds ratio decreased continuously with age. *KIBRA* CC AD patients perhaps had earlier onsets and died soon. Hence, we tested the association between *KIBRA* and age of onset and course of the disease in RBMT-AD specialized clinic subjects. We defined the age of first abnormal memory loss episode reported by caregivers as onset age of AD. Although we found no significant differences between *KIBRA* C carriers and non-C carriers, the age of first abnormal memory loss episode reported by caregivers tended to be later in *KIBRA* non-C carriers (table 1). It is possible that the presence of the *KIBRA* T allele delays diagnosis of some AD clinical symptomatology. This effect could be similar to the well-reported effect of cognitive reserve, reflected in years of education. Highly educated individuals have better cognitive performance and, thus, tend to be judged as cognitively normal, albeit AD neuropathology is already present [30–32]. On the other hand, it appears that AD symptomatology progresses faster in people with higher education once AD is diagnosed [33]. Incidentally, in our cohort, duration from first memory loss episode to AD diagnosis was significantly shorter in the *KIBRA* TT group (2.6 ± 1.7 vs. 4.7 ± 1.2 years, p = 0.001). We propose that *KIBRA* might have an effect similar to cognitive reserve, particularly in simple word recall.

The impact of *APOE*, an established AD risk gene that accelerates AD brain pathology, on episodic memory was also examined. A recent study reported no differences, suggesting that *APOE* ε4 does not influence episodic memory (AVLT delayed recall) in cognitively normal individuals under 60 years of age [34]. In accordance, our results evidenced no significant differences in both RBMT total profile score (*APOE* ε4–: 3.40 ± 2.12 vs. *APOE* ε4+: 3.64 ± 3.16; p = 0.831) and RBMT total screening score (*APOE* ε4–: 0.80 ± 0.63 vs. *APOE* ε4+: 0.86 ± 1.17; p = 0.873) between *APOE* ε4 carriers and non-*APOE*-ε4 carriers. This lack of correlation between *APOE* and the episodic memory is intriguing and is in contrast with our findings for *KIBRA*, which seems to

have a less certain effect on AD but a more significant impact on episodic memory in young [4] and elderly subjects [4, 5, 7, 29] and even in mild AD patients, as our study shows (table 2). It is possible that *KIBRA* does not have a direct impact on AD neuropathology but could have an effect on the clinical diagnosis of AD, in a manner similar to cognitive reserve.

Compared to many reports based on Caucasian samples, our cohort evidenced lower frequencies of *KIBRA* CC. Thus, comparison of our results with those based on Caucasian samples must be carried out with caution. As our research-oriented clinic is specialized in the early identification of dementia, we should take selection bias into consideration. Further studies with larger samples, including cognitive functional and pathological data, will be carried out in the future in order to clarify the importance of the *KIBRA* SNP for episodic memory and AD pathology.

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The *chitinase 3-like 1* gene and schizophrenia: Evidence from a multi-center case–control study and meta-analysis

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ABSTRACT

The *chitinase 3-like 1* (*CHI3L1*) gene acts as a cellular survival factor in response to several environmental and psychosocial stresses. The expression level of *CHI3L1* was increased in the hippocampus and prefrontal cortex regions of patients with schizophrenia. Genetic variants of the *CHI3L1* gene have been significantly associated with schizophrenia in two distinct ethnic groups, the Chinese and Irish populations. The aims of this study are to confirm the association between the *CHI3L1* gene and schizophrenia in a Japanese population using the largest sample size to date (1463 cases and 1795 controls) and perform a meta-analysis of the combined samples (3005 cases, 3825 controls and 601 trios). We found significant associations between single nucleotide polymorphism (SNP) 4/rs4950928 ($p=0.009$), which is located in the promoter region of the *CHI3L1* gene, and haplotypes including this SNP and schizophrenia (the most significant global $p<0.001$). As the meta-analysis of the combined samples showed significant heterogeneity among studies of SNP3/rs10399805 ($p=0.026$) and SNP4 ($p<0.001$), we performed meta-analyses separately in the Japanese (2033 cases and 2365 controls) and Chinese populations (412 cases, 464 controls and 601 trios), the major groups analyzed in association studies of the *CHI3L1* gene. The meta-analysis in Japanese populations showed stronger evidence for the association of schizophrenia with SNP4 ($p=0.003$), while the meta-analysis in Chinese populations showed an association with a different variant (SNP3) ($p=0.003$). We conclude that the genetic variants in the *CHI3L1* gene have ethnic heterogeneity and confer a susceptibility to schizophrenia in Asian populations.

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1. Introduction

Schizophrenia (OMIM 181500) is a common and complex psychiatric disease. The lifetime morbidity rate is 0.5–1.0% across distinct populations. Family, twin and adoption studies of schizophrenia have indicated that there are strong genetic factors with an estimated heritability of approximately 80% (Cardno and Gottesman, 2000). Many genes have been implicated in the pathogenesis of schizophrenia (Sun et al., 2008).

The *chitinase 3-like 1* gene [*CHI3L1*, (OMIM 601525)] consists of 10 exons and spans approximately 8 kb of genomic DNA. The protein was named YKL-40 based on its three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L), and its molecular mass of 40 kDa (Johansen et al., 1992). The protein has several names, including chitinase 3-like 1, human cartilage glycoprotein-39 (HC gp39), breast regressing protein 39 (brp-39), 38-kDa heparin-binding glycoprotein (gp38k), chondrex and 40-kDa mammary gland protein (MGP-40). In this study, to avoid confounding these terms, the gene is referred to as *CHI3L1* and the protein is referred to as YKL-40.

This gene acts as a cellular survival factor in responses to a variety of adverse environments, including various types of physiologic stress, such as inflammation, hypoxia and nutrient deprivation. These stresses may induce high expression of *CHI3L1* (Junker et al., 2005; Recklies et al., 2005). YKL-40 is secreted by activated macrophages and neutrophils in different tissues during inflammation and during increased remodeling of the extracellular matrix (Kirkpatrick et al., 1995; Rehli et al., 1997; Volck et al., 1998). YKL-40 initiates mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase (PI3K) signaling cascades in fibroblasts. Signaling leads to the phosphorylation of both the extracellular signal-regulated kinase (ERK)-1/2 MAP kinase- and the protein kinase B (AKT)-mediated signaling cascades, which are associated with the control of mitogenesis (Recklies et al., 2002). The PI3K pathway and the downstream phosphorylation of AKT in particular are strongly associated with cell survival (Bakkenist and Kastan, 2004), which suggests a role for YKL-40 as an anti-apoptotic protein.

The synthesis of YKL-40 is induced by the inflammatory cytokines IL-1, IL-6 and TNF-α (Ling and Recklies, 2004; Recklies et al., 2005; Johansen et al., 2006). The genetic variants of the *CHI3L1* gene and high serum levels of YKL-40 are associated with several inflammatory diseases, including sarcoidosis, asthma and inflammatory bowel diseases (Kruit et al., 2007; Kucur et al., 2007; Ober et al., 2008). The role of YKL-40 in the nervous system is unclear. YKL-40 is elevated in the cerebrospinal fluid (CSF) of patients with spinal diseases in which the neural tissue has been damaged or stressed, including cervical myelopathy, lumbar canal stenosis and lumbar disc herniation (Tsuji et al., 2002). High levels of YKL-40 in the CSF have also been reported in patients with purulent meningitis (Ostergaard et al., 2002). The *CHI3L1* gene expression analyses demonstrated higher postmortem mRNA levels in the hippocampus and prefrontal cortex of patients with schizophrenia than in the respective tissues of controls (Chung et al., 2003; Arion et al., 2007). It has been hypothesized that YKL-40 protects cells from undergoing apoptosis and plays a role in inflammatory processes in patients with schizophrenia.

The *CHI3L1* gene is located on chromosome 1q32.1 and shows evidence of modest linkage with schizophrenia (Shaw et al., 1998; Jang et al., 2007), although recent genome-wide association studies have not identified any variant of this gene that is associated with schizophrenia (O'Donovan et al., 2008). Zhao et al. (2007) have detected genetic associations between schizophrenia and three single nucleotide polymorphisms (SNPs; rs6691378, rs10399805 and rs4950928) within the promoter region of *CHI3L1* in two independent Chinese cohorts. They found that an allele at rs4950928 impaired MYC/MAX-regulated transcriptional activation of *CHI3L1* by altering the transcription factor consensus sequences. Yang et al. (2008) subsequently indicated significant associations between schizophrenia and two SNPs in an Irish cohort. One was the same SNP (rs10399805) in the promoter that was reported in the original study and the other SNP (rs2275351) was within the gene at intron 7. These findings suggest that the *CHI3L1* gene is likely involved in predisposition to schizophrenia. However, the two studies were not replicated in two more recent studies, one conducted with Chinese trio samples and Japanese case–control samples (Yamada et al., 2008) and the other studying a small Bulgarian population (Betcheva et al., 2009). To further investigate this controversial issue, we first investigated whether the *CHI3L1* gene is associated with schizophrenia in a large Japanese population. Second, we performed meta-analyses on the overall population and separately in Japanese and Chinese populations.

2. Methods

2.1. Subjects

The subjects in our genetic association study consisted of 1463 unrelated patients with schizophrenia [54.6% males (799/664), mean age ± SD; 47.3 ± 15.0 years] and 1795 unrelated healthy controls [51.3% males (920/875), mean age ± SD; 45.5 ± 20.1 years]. The sex ratio did not differ significantly between groups ($\chi^2 = 3.7$, $p = 0.06$), while the mean age differed significantly between groups ($z = -5.1$, $p < 0.001$). These subjects were independent of those used by Yamada et al. (2008). All subjects were biologically unrelated Japanese and were recruited at three geographic regions in Japan: Osaka, Aichi and Tokushima (Yamaguchi-Kabata et al., 2008; Ohi et al., 2009). Cases were recruited from both outpatients and inpatients at university hospitals and psychiatric hospitals. Each schizophrenic research subject had been diagnosed and assessed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria, based on an unstructured clinical interview. Controls, including the hospital and institutional staff, were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University, Fujita Health University, Nagoya University and Tokushima University.

2.2. SNP selection and SNP genotyping

We designed our replication study by selecting six SNPs in the *CHI3LI* gene and the flanking regions. Five of the six SNPs were identical to the SNPs used in the original study: rs2364574 (SNP1), rs6691378 (SNP2), rs10399805 (SNP3), rs4950928 (SNP4) and rs880633 (SNP5). The designations of these SNPs in parentheses are according to Zhao et al. (2007). The remaining SNP (rs2275351) was chosen from the following study as it showed evidence for association with schizophrenia (Yang et al., 2008). Venous blood was collected from the subjects and genomic DNA was extracted from whole blood according to standard procedures. These SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, California, USA), as described previously (Hashimoto et al., 2006, 2007; Ohi et al., 2009). Detailed information on the PCR conditions is available upon request. Genotyping call rates were 99.0% (SNP1), 95.0% (SNP2), 99.2% (SNP3), 99.6% (SNP4), 99.8% (SNP5) and 97.7% (rs2275351). SNP2 was excluded from the present study because this variant was not clearly discriminated as a result of a lower call rate. No deviation from Hardy–Weinberg equilibrium (HWE) in the examined SNPs was detected in the controls ($p>0.05$), while the genotypic frequencies of two SNPs deviated from HWE in the schizophrenia patients (SNP1; $p=0.016$, rs2275351; $p<0.001$). The positions of the five SNPs analyzed in the present study are indicated in Fig. 1.

2.3. Power analysis

We performed power calculations using the Power Calculator for Two Stage Association Studies [http://www.sph.umich.edu/csg/abecasis/CaTS/; (Skol et al., 2006)]. Power estimates were based on allele frequencies in patients ranging from 0.17 (SNP4) to 0.29 (SNP3), odds ratios ranging from 1.29 (SNP3) to 1.49 (SNP4) for each associated SNP, as indicated by Zhao et al. (2007), and an alpha level of 0.05. Power was calculated under a prevalence of 0.01 using a multiplicative model, assuming varying degrees of the marker allele frequency and the odds ratio.

2.4. Meta-analysis of the CHI3L1 association studies

The studies included in the meta-analysis were selected using the Schizophrenia Research Forum (http://www.schizophreniaforum.org) and PubMed with the search terms "CHI3L1" and "Schizophrenia." The analyzed data encompass all publications up to May 2009.

2.5. Statistical analyses

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and SPSS 16.0J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls were

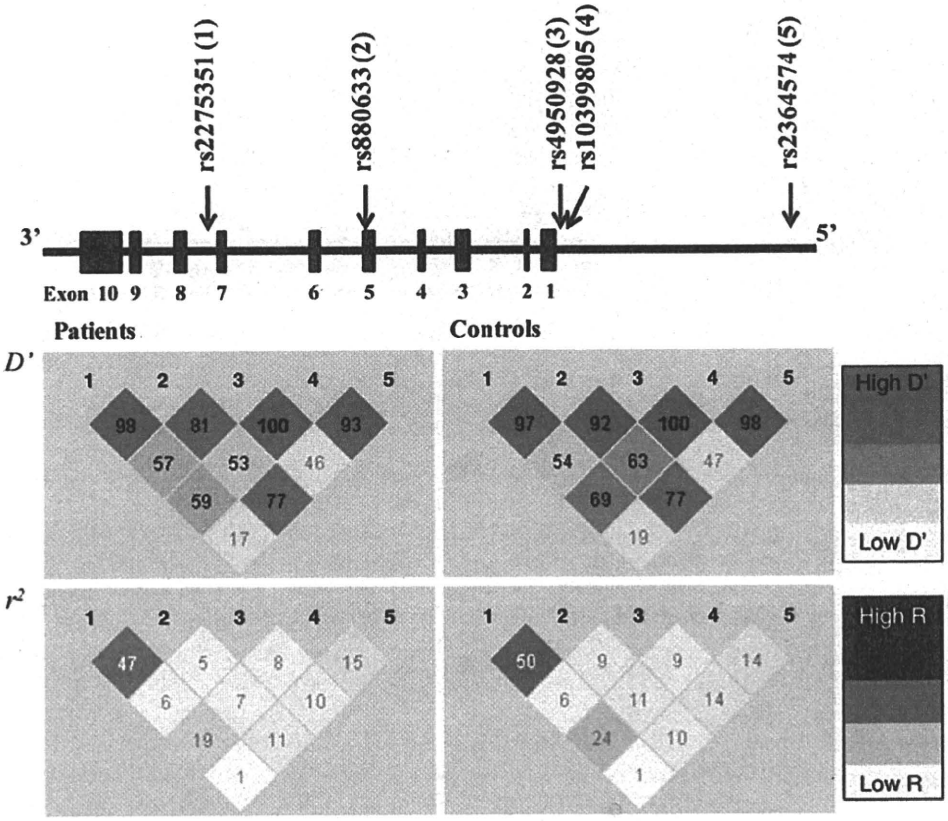

Fig. 1. Genomic structure of *CHI3L1*, including locations of the five SNPs studied, and linkage disequilibrium of these five SNPs in the patient and control groups. Based on an entry in the Entrez Gene database (National Center for Biotechnology Information), the genomic structure of *CHI3L1* is shown above. The locations of SNPs analyzed in this study are indicated by arrows. Numbers indicated in parentheses refer to numbering of the SNPs in the linkage disequilibrium (LD) diagram. The distances of exons–introns and intermarkers are drawn to scale. The LD between pairwise SNPs, using D' and r^2 values, are shown at the bottom of the map of gene structure separately for cases and controls. High levels of LD are represented by red (D') and black (r^2) coloring with increasing color intensity from 0 to 100, as shown by color bars.