

らである。近年、SD に対する症候学的研究に加え、神経画像学的研究、分子生物学的研究が精力的になされ、SD の病態解明が進んできた。本稿では診断に役立つ臨床症状や検査所見の解説に加え、最新の研究で明らかになった知見を紹介する。

## I. SD の疫学

疾患概念に対する理解がまだ十分に普及していないため、正確に診断がつけられていない可能性があり、国内に限らず海外でも疫学研究は少ない。国内では、hospital-based の研究で、高次脳機能外来を受診した連続例 330 例のうち 15 例 (4.5%) であったとの報告がなされている<sup>5)</sup>。英国における community-based の研究では、若年発症の認知症患者 108 例のうち、FTLD が 17 例、そのうち 2 例が SD であった<sup>6)</sup>。海外では日本に比べ家族発症例の FTLD が多いが、FTLD のサブタイプの中で SD の家族発症は少ないとされている<sup>7)</sup>。

## II. SD の臨床症状

SD の臨床診断は臨床症状の把握と神経画像検査で行われる。まず、最も重要な臨床症状についてまとめる。

### 1. 語義失語

SD では語義失語と呼ばれる独特の言語障害を呈する。この語義失語の概念は 1943 年に井村<sup>8)</sup>が提唱しており、わが国では欧米と比べても早い時期からその特徴をまとめていた。語義失語の中核症状は、単語に関する情報、特に意味的側面の喪失とでも呼ぶような障害である。他の言語的側面は保たれており、発語は流暢で、音の歪みもない。意味がわからない単語を含め、単語や文章を正確に復唱でき、音韻的側面は保たれる。文法的な誤りもなく、発語量も低下しない。理解面も単語の意味さえわかれば問題なく、いわゆる文レベルの理解は保たれる。書字に関しても音声言語の特徴が反映され、表音文字である仮名操作は保たれる一方、表音文字であると同時に表意文字でもある漢字の操作に障害がみられる。SD では病初期には障害が言語の側面だけにとどまるため失語の範疇でとらえることもできるが、進行するに従って後述する意味記憶障害ととらえたほうがよい状態となる。以下に SD で認められる言語症状について詳述する。

#### 1) 喚語困難・呼称障害

SD の言語症状として最も初期から目立つ症状である。自由会話の中でも適切な単語が円滑に思い出せなく

なる。「あれ」、「それ」などの指示代名詞で補うこともある。また物品をみせて、名前を答えるよう指示する呼称課題でも名前を答えられない。認知症患者のスクリーニング検査として行う mini-mental state examination (MMSE) 検査で、日常生活はほぼ自立しているにもかかわらず、物品の呼称ができず、SD が疑われることもある。また「豆腐」と答えるべき場面で「納豆」と答えるような語性錯語も認める。語性錯語は意味的に関連のある他の言葉に置き換わる意味性錯語が多いが、「とうふ」を「とうは」というように音が置き換わる音韻性錯語は目立たない。

呼称課題で、患者が答えられない単語に対しては、一般的に、語頭音をヒントとして与え、回答を促す。健忘失語の患者の場合、語頭音ヒントは有効であるが、SD 患者では正答に至らないことが多い。例えば、鉛筆を提示して患者に名前を問うが、答えられないときに「『え』で始まりますよ」、次いで「『えん』で始まりますよ」、さらに「『えんぴ』で始まりますよ」というように検者が徐々に長いヒントを出しても最後まで正答に至らない。逆に患者が「これは『えんぴ』というのですね」と理解し、少なくともその診察中は「えんぴつ」という言葉の代わりに「えんぴ」という言葉を使う様子も観察される。このような呼称や喚語できない単語は病初期ではごく少数に限られるが、進行に伴い徐々に増加する。

#### 2) 単語の理解障害

いわれた名前に対応する物品を複数の物品の中から選択させる語理解課題でも誤りが生じる。上記の呼称課題とこの語理解課題は表裏をなす課題であるため、1つの単語に対して呼称も理解もできなかった場合、双方向性の障害と表現する。そしてこの双方向性の障害が SD の特徴とされている。また、SD 患者では提示された単語 (その音の並び) に既知感を感じない。すなわち「えんぴつ」と患者にいても、その音の並びを聞いたことがないと感じ、しばしば「『えんぴつ』って何ですか」というように逆に質問する。この「○○って何ですか」というフレーズとその言葉を初めて聞くような当惑した様子は SD 患者に特徴的であると筆者は感じており、このフレーズを聞くと SD を疑うほどである。呼称障害と同様、疾患の進行に伴い理解できない単語、双方向性の障害を認める単語も徐々に増加していく。

臨床診察場面では、鉛筆、消しゴム、時計、筆、ホッチキス、懐中電灯などの診察室にある日常物品 5~10 個を用いて呼称と理解を評価することが多い。詳細に調べたいときには失語症語彙検査<sup>9)</sup>が有用である。この検査では、カテゴリー (動物、野菜果物、色、身体部位など

10 カテゴリー)と親密度(どの程度なじみがあると感じられるかを表した指標で、高と低の2分類されている)が統制された200個の名詞が用意されている。

### 3) 障害されやすい単語

SDでは親密度の低い単語ほど障害されやすい。また単語のカテゴリーによっても障害のされやすさが異なる可能性があり、色や身体部位に関する単語は障害されにくい<sup>10)</sup>。また名詞よりも動詞のほうが障害されにくいことも報告されている<sup>11)</sup>。

### 4) 障害される単語の一貫性

SD患者に複数回、呼称課題や語理解課題を行うと、呼称障害、語理解障害、双方向性の障害を認める単語、および既知感を有さない単語が一貫する傾向がある<sup>12)</sup>。これは失語症患者一般で、障害される単語に浮動性を認めることとは対照的である。しかし、病初期であればSDでも浮動性を認めることもある。経過を観察していくと、全体としては障害を認める単語が増えていく。その中で、障害に浮動性を認める単語が混在しながら、一貫して障害される単語が増えていく。

### 5) 表層性錯読、類音的錯読 (surface dyslexia)

欧米の研究で、SD患者では、綴りから予想される発音とは異なる特別な発音をする不規則発音単語を、一般的な規則に従って発音する傾向があることが報告されている。このことから個々の特別な単語をどのように発音するかという知識は障害されるが、一般的な綴りと発音の規則は保たれやすいと考えられる。

わが国の患者においてもこの症状が観察される。ただし、音と文字形態との対応が規則的な仮名では認めにくく、音が複数あり、かつ場合によって意味や読み方が異なる漢字で観察されやすい。例えば、「団子」を「だんし」、「時計」を「じけい」と読む。さらにこの症状は熟字訓と呼ばれる特別な読み方をする熟語で認めやすい。例えば「海老」を「かいろう」、「土産」を「どさん」と読む。文字と線画を対応させるよう指示する課題では、正しく読めなかった物を正しく指示することはまずない。熟語の意味はわからなくても、個々の漢字の意味はわかっていることがあり、例えば「団子」を「だんし」と読んでから、「子」の文字に引きずられるかのように「子供」の線画を指すというような反応はしばしば認められる。この表層性錯読の程度は意味記憶障害の程度とよく相関することが報告されている<sup>13)</sup>。

### 6) 類音的錯書

SD患者の漢字の書字で類音的錯書という特徴的な症状を認める。漢字には音と意味とがあるが、漢字の意味は無視し、音のみを使うのである。例えば「汽車」を「寄

Table 語義失語と意味記憶障害の分類 — 鉛筆の場合

	語義失語	→	意味記憶障害
呼称 (語頭音効果)	×		×
指示	×		×
名前を聞いたことがあるか	×		×
みたことがあるか	○		×
使い方を説明できるか	○		×
使えるか	○		×
関連する物を選択できるか	○		×
一般的知識を有しているか	○		×

この分類は鉛筆のような使用可能な物品に適用可能

社」と、「新聞」を「新文」というように漢字の音を借りてあたかも仮名のように使う。

### 7) 諺の補完障害と字義的解釈

諺の補完現象とは、例えば、「『ちりも積もれば』の後には何と続きますか」というように、質問したときに、「山となる」と正しく補える現象である。諺の意味を答えることができなくても、よく知られた諺なら重度の失語症患者でも補完は自動的になされることがほとんどで、プライミングの一種と考えられる。しかし、SD患者では「ちりも積もれば山」、次いで「ちりも積もれば山とな」までヒントを出しても補完できない<sup>14)</sup>。また諺や比喩の意味を問うたときには字義的解釈にとどまり、諺や比喩の持つ特別の意味は答えられない。例えば、「腹が立つ」の意味を問うたときに、腹を押さえて、次に立つ動作をするというように、「腹」、「立つ」という言葉それぞれの字義的な意味理解にとどまる。

### 8) 語義失語から意味記憶障害へ

縦断的にSD患者の単語の障害を観察していると、当初は語義の障害レベルにとどまっていた単語が、進行に伴い意味記憶障害ととらえられる状態へと進行する。そして、意味記憶障害レベルにある単語が進行に伴い増加する。ここで語義の障害にとどまっているレベルとは、Tableのように鉛筆を例にとると以下のような状態である。Tableの上段3つの項目に障害を認める、すなわち呼称と了解ができない双方向性の障害を認め、かつ名前を聞いたことがないと既知感も有さない。しかし、下段の5つの項目に関しては障害されない。すなわち鉛筆自体はみたことがあるといい、使い方や用途を説明でき、また正しく使える。さらに、ノートや消しゴム、鉛筆削りを関連する物と認識でき、一般的な知識、三菱鉛筆やトンボ鉛筆があり、六角柱をしていることが多いなどを答えることができる。

一方、意味記憶障害レベルとは、その対象物を同定するために一般的に考えることができるモダリティーすべ

てから迫ってもその対象物を同定できない状態で、Table では全項目が障害された状態である。当然、鉛筆に関連する一般的な知識をどれくらい詳細に評価するかによって語義の障害レベルか意味記憶障害のレベルかの判断は変わってくるが、臨床的に語義失語のレベルか意味記憶障害のレベルかの区別を厳格にしなければならない場面は少ない。また、同時期に語義失語のレベルにとどまる単語と意味記憶障害のレベルに達している単語が混在することもよくある。また、単語の属性によっても意味記憶障害レベルのとらえ方は異なる。言語情報や視覚情報だけでなく嗅覚情報や触覚情報がその対象物を規定するために有用なこともある。また、「利き手」などの抽象的な言葉もあり、このような単語については語義失語レベルか意味記憶障害レベルかを区別することは困難である。

## 2. 言語以外の意味記憶障害

### 1) 視覚イメージの意味記憶障害

SD 患者では両側側頭葉の萎縮が特徴的な所見であるが、通常、萎縮の程度には左右差がある。どちらが優位に萎縮しているかによって左側頭葉優位型と右側頭葉優位型とに分類されるが、臨床的には左側頭葉優位型のほうが多い。どちらの萎縮が優位であっても語義失語と意味記憶障害を呈する。SD では一般的に視空間認知機能は保たれ無意味図形の模写や弁別に障害はない。しかし、以下のような視覚イメージが喪失されることがあり、この視覚イメージの喪失は、右側頭葉優位型 SD のほうが早期から顕著となりやすい。

臨床的にも気づきやすいのは相貌の同定障害である。患者自ら、顔がわからないことを自覚していることもある<sup>15)</sup>。有名人、親戚、家族、さらには自分の顔も同定できないし、既知感も有さない。同定できない顔は患者が会っている頻度の影響を受けやすく、毎日会っている家族よりも久しぶりに会う家族の顔のほうがわからなくなりやすい。図形の模写ができるように相貌に関しても、知覚面は保たれ顔写真の異同弁別はできる。また誰の顔であるかはわからなくても「顔」であることはわかっている。

有名な建物や風景の同定障害も認められる。検査としては、絵はがきなどを提示して何であるかを答えさせることが多く、富士山、東京タワー、金閣、国会議事堂などが用いられる。これらの写真に対しても SD 患者はみたことがないといい、何であるかわからない。しかし、それぞれ山、タワー、寺であることはわかる。右側頭葉優位型 SD の病初期では、有名人や家族の顔を同定することはできなくても、その人の名前から自分との関係や

その人の仕事、どんなことをした人かなどその人に関する知識を説明できる場合もある。また、有名な建物や風景をみたことがないといっても、建物の名前からそれがどこにあるのか、どのような建物であるかを答えることができることがある。この段階では視覚失認（連合型）の範疇でとらえることができるが、疾患の進行に伴い、その人、建物についての情報も失っていき、意味記憶障害のレベルへと至る。そのほか、道路標識に関する意味記憶も障害される。

以上のような相貌や有名建物、道路標識のような固有の視覚イメージだけでなく、ホッチキスや懐中電灯のような日常物品に関する視覚イメージも喪失し、みたことがないという。さらにゾウやトラなどの動物の視覚イメージも喪失する。例えば、ライオン、サソリ、クモの写真を見せてもみたことがないと答え、タコの写真をみせたときには、「昔はこんな動物がいたのかな」と発言することもある<sup>15)</sup>。右側頭葉優位型 SD 患者では語義失語による言語情報からの対象物の同定障害に加えて視覚情報からの同定も障害されやすいため、複数モダリティーからの同定障害という意味記憶障害の条件を満たしやすい。

### 2) 象徴的動作の意味記憶障害

象徴的動作とは、「敬礼」、「バイバイ」、「おいでおいで」のような特別な社会的意味を有する動作のことである。SD 患者では象徴的動作をするよう指示してもできない。逆に象徴的動作をみても何を意味する動作であるかを理解できない<sup>16,17)</sup>。しかし、観念運動性失行とは異なり、検者が行う象徴的動作を真似することはできる。以上のことから、SD 患者では象徴的動作の意味記憶が障害されていると考えられる。

### 3) 音の意味記憶障害

特別な意味を包含する音があり、環境音と呼ばれる。例えば救急車のサイレンの音、電話の着信音、動物の鳴き声、波の音などである。このような環境音を聞いても、SD 患者では何を意味する音かわからなくなることがある<sup>18)</sup>。また、SD 患者では環境音が同定できるか否かと音に既知感を持つか否かとは関係があり、既知感を持ってない環境音は同定困難である。さらに環境音の同定ができない対象については、単語や絵からの同定もできない傾向があり、障害が音の認知に限らず意味記憶障害によるものと考えられる。われわれが経験した右側頭葉優位型 SD 患者では、男性の声か女性の声か、声の主の大まかな年齢を判別できたが、家族の声を聞いても既知感を認めず、誰の声かもわからなかった<sup>15)</sup>。

### 3. SDの意味記憶障害以外の症候

#### 1) 精神行動障害

精神行動障害としては脱抑制, 考え無精, 常同行動, 易怒性, 意欲低下などを認める。これらは前頭側頭型認知症 (frontotemporal dementia: FTD) でも認める症状であるが, 脱抑制や意欲低下などの明らかな人格変化はFTDに比べるとやや遅れ, 初期には礼節が保たれている場合が多く<sup>19)</sup>, 日常生活は概ね自立している。SD患者で目立つ症状は常同行動である。同じ話を繰り返す滞続言語, 同じ食べ物ばかり食べたがる常同的食行動異常, 同じルートを散歩せずにはいられない常同的周遊, 自分で決めた時間に決めたことをせずにはおれない時刻表的生活などがしばしば認められる。これらの常同行動を評価する評価尺度としてはStereotypy Rating Inventory (SRI)<sup>20)</sup>が有用である。

#### 2) 病識

病識については, 少なくとも初期には比較的保たれている印象を持つ。患者は言葉が思い出しにくいことを自覚し, 思い悩んでいる様子がみられる。そして自ら単語帳を作成する患者もいる。ある患者は, 名前がわからなかった物について線画を描き, その横に名前を書いていた。そしてこのような単語がA4用紙数枚にぎっしりと書かれていた。さらにそれらの単語を別の紙にア行, カ行というように行ごとにまとめ直していた。診察のときに物の名前を思い出せないとその用紙を出してきて, 答えようとすることもあった。また, 顔がわからないことも自覚していることが多く, このことを訴える。自分の顔を鏡でみて自分がこんな顔をしているのかと毎日納得するといっていた患者もいた<sup>15)</sup>。

#### 3) 芸術的才能の開花

芸術的活動をほとんどしていなかったSD患者が, 臨床症状が明らかになった後に素晴らしい絵や写真, 彫刻を作成するようになった事実が報告されている<sup>21)</sup>。作品は視覚的作品に限られ, その特徴は写実性であり, 抽象性, 象徴性という要素はなかった。視空間認知機能とエピソード記憶が保たれていたこと, 前頭葉の障害が比較的軽度であったことが前提条件であると思われるが, 常同行動と疾患に伴う社会との隔絶によるさらなる作品への集中が作品形成を促進したと考えられる。報告者は側頭葉前部部の障害が芸術的才能を, 直接, 活性化させた可能性も示唆している。さらに芸術的才能の開花はFTLD (SD) の診断に役立つとしている。

### 4. SDの進行に伴う症候の推移

14例の左側頭葉優位型SD患者と5例の右側頭葉優

位型SD患者の経過を長期間縦断的に観察した研究<sup>22)</sup>では, 19例中16例で言語障害が初発症状であった。言語障害の中では, 喚語困難・呼称障害が最も早く, 発症後平均1.3年の時点で認めた。次いで語理解障害を認め(発症後平均2.1年), その後錯語や読字・書字障害(約2.5年後)を認めた。右側頭葉優位型SD患者では, 相貌の同定障害が最も早く出現する症状で発症後平均0.9年の時点であった。これは左側頭葉優位型(4.5年後)と比較して有意に早い時期であった。

常同行動, 脱抑制, 攻撃性, 意欲低下などの精神行動障害は発症後3~5年の時点で認め始めた。その中で最も早期に出現する症状は常同行動であった。易刺激性・攻撃性は右側頭葉優位型患者(平均2.9年後)で左側頭葉優位型患者(平均4.4年後)と比較して有意に早く出現した。

日常生活動作 (activities of daily living: ADL) の障害については, 平均5年以上の経過で認め始め, 道迷い(平均5.4年後), 食事の介助(平均6.6年後), 尿失禁(平均7.0年後), 無言症(平均7.1年後), 更衣障害(平均7.1年後), 臥床傾向(平均7.4年後)であった。

### 5. 運動ニューロン疾患を伴うSD

FTLDには運動ニューロン疾患 (motor neuron disease: MND) を伴う症例が存在することが知られているが, MNDを伴う症例の臨床型はMND with dementia (D-MND)として報告された症例を含めてもほとんどFTD (FTD-MND)である<sup>23,24)</sup>。そして, FTD-MNDはMNDを伴わないFTDよりも, 生存期間が短く, 障害が前頭葉に限局しやすいなど異なる点があることが報告されている<sup>25)</sup>。一方, SDにMNDを伴うSD-MNDの報告は日本から3例<sup>26-28)</sup>, 海外から2例<sup>29,30)</sup>にとどまり報告例は少ない。

しかし, SDの連続剖検18例での検討では, 臨床症状の評価時にMNDの症状を呈していた症例はいなかったが, 免疫組織学的には18例中13例にMNDに特徴的なタウ陰性ユビキチン陽性封入体を認め, さらにそのうち5例においては運動神経系に病理学的異常所見を認めた<sup>31)</sup>。したがって, SDでもMNDを伴う症例が一定数存在すると予想される。報告数が少ないのは, MNDの全経過が短いこと, 球麻痺を呈すると言語症状が評価困難であること, SDそのものの頻度が少ないことなどが理由と考えられる。

またSD-MNDでは, 仮名の錯書や脱落などの仮名書字障害と色名呼称障害が目立つなど一般的なSDとは異なる症状を有する可能性が報告されている。特に仮名書

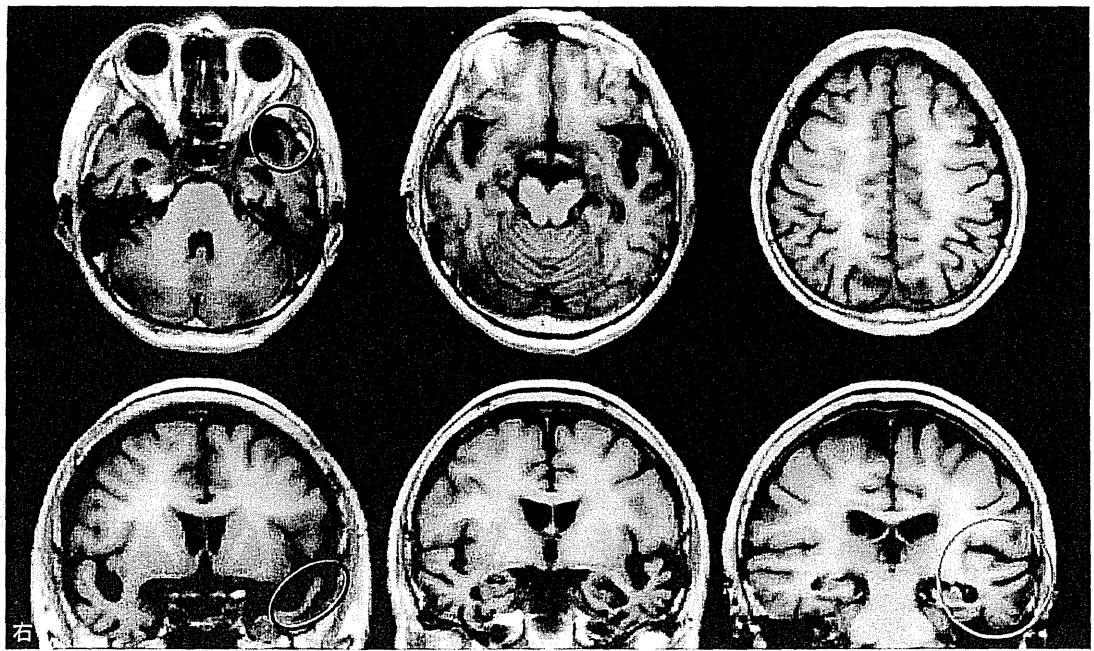


Fig. 1 左側頭葉優位型 SD 患者の MR 画像

左側頭葉前方部に楔形の強い脳萎縮を認める (赤い円)。側頭葉でも後方部になると萎縮は軽度となる (青い円)。また、前頭葉の萎縮は目立たない。

字障害は FTD-MND の特徴的症候とされており、臨床病型によらず D-MND で高率に認める特徴である可能性がある<sup>20)</sup>。これらの症状が出現しやすいのは、SD-MND において、仮名書きに関連する左中前頭回と色の呼称に関連する左中・下前頭回を含む側頭葉が一般的な SD よりも早期から障害されるからではないかと考えられている。

### III. SD の病巣

#### 1. 神経画像検査所見

SD の診断には、magnetic resonance imaging (MRI) が有用で、初期には左右非対称性の側頭葉の限局性萎縮を認める (Fig. 1)。特に、側頭極、嗅内皮質、海馬傍回、紡錘状回、下側頭回の皮質の萎縮が顕著である<sup>21)</sup>。他に前頭葉眼窩面、島皮質、帯状皮質における萎縮が認められる。進行は当初の障害が目立つのと対側の側頭葉でより速く進行し、末期には側頭葉萎縮の左右差はほとんどなくなる事がわかっている<sup>22)</sup>。Positron emission tomography (PET) や single photon emission computed tomography (SPECT) といった脳機能画像検査においては、MRI でみられる脳萎縮と同部位に機能低下が認められる<sup>23)</sup> (Fig. 2)。

#### 2. 意味記憶障害の脳内責任部位

意味記憶は脳内のさまざまな領域に分散して保存され、これらの情報をつなぐ中心が側頭葉前方部にあると考えられている<sup>24)</sup>。そして SD 患者では、前方部を含む側頭葉の萎縮のため意味記憶障害を呈すると考えられている。SD 患者に対してトラクトグラフィィを行い白質線維の障害について検討した研究では、SD 患者では側頭葉を通る下縦束、鉤状束、弓状束が障害され、特に側頭極に位置する下縦束の前方部と鉤状束の障害が強い。しかし脳梁膨大部、前頭葉と頭頂葉を結ぶ上縦束の障害は軽度である<sup>25)</sup>。この知見から、SD の意味記憶障害は側頭葉前方部と他領域との線維連絡が障害されることによって生じる、すなわち離断症候ととらえることも可能である。例えば視覚野からの視覚情報が下縦束の障害によって側頭葉前方部に至らないという考え方である。一方、上縦束などを介する前頭葉と頭頂葉との線維連絡は相対的に保たれるため、流暢性、文法、語彙と音韻の関連は保たれる。この研究ではさらに単語を読む際の脳活動を機能的 MRI で計測している。その結果、SD 患者では紡錘状回の中央と上側頭回の活動が健常者と比較すると低下していた。

#### 3. 表層性錯読時の脳活動

SD 患者に対して表層性錯読の出現メカニズムを調べ

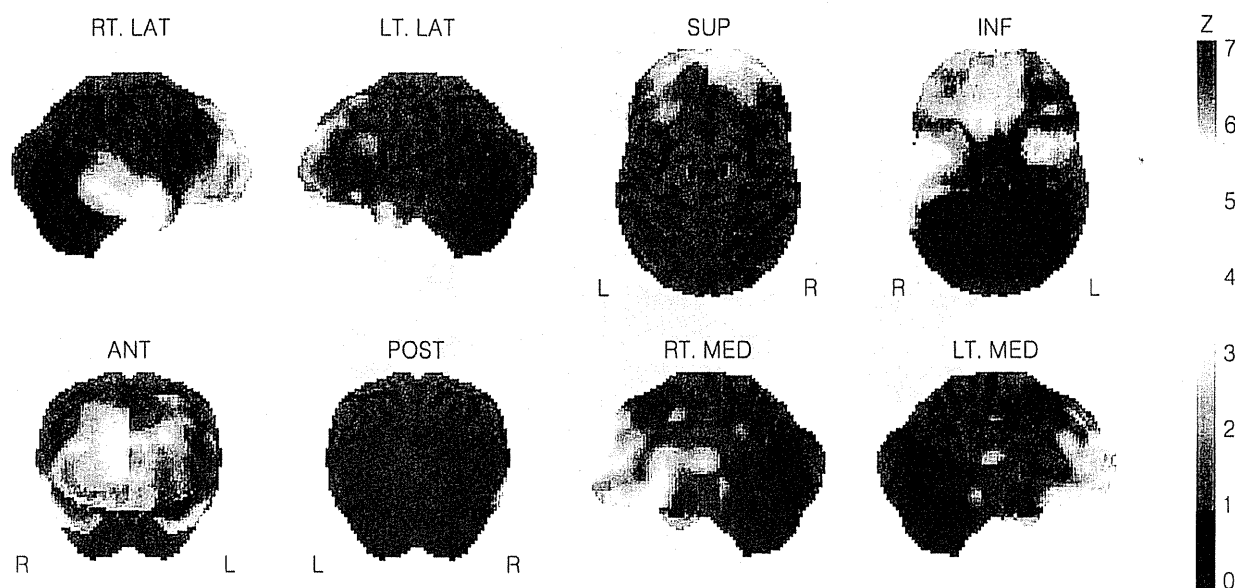


Fig. 2 右側頭葉優位型 SD 患者の 3D-SSP 解析画像

IMP-SPECT 画像を 3D-SSP で解析した Z-score 像。解析には自施設健常者データベースを使用し、参照部位は全脳、Z-score > 1 の領域をカラー表示している。カラーバーは Z-score を表す。

略語 RT. LAT: right lateral, LT. LAT: left lateral, SUP: superior, INF: inferior, ANT: anterior, POST: posterior; RT. MED: right medial, LT. MED: left medial

た研究がある<sup>32)</sup>。この研究で用いられた単語は、一般的な規則に従って綴りから発音が推測できる規則単語、一般的な規則に従わない発音をする不規則単語、実在しない偽単語で、規則的単語と不規則的単語については低頻度語と高頻度語とが用意された。そして SD 患者と健常者がこれらの単語を読むときの脳活動を機能的 MRI で測定したところ、被験者群と単語間で賦活に最も大きな差を認めた領域は左の頭頂間溝で、健常者が偽単語を読んでいるときに、SD 患者が低頻度の不規則単語を読んでいるときに強く賦活された。さらに SD 患者においては、不規則単語を一般的な規則に従って読もうとした度合いが強いほどこの部位の賦活が大きかった。子供は大人よりも読字の際にこの領域が強く賦活するとの発達研究もあることから、この領域が綴り(文字形態)から音に変換するのに重要な部位で、SD 患者では、意味記憶が障害された不規則単語を読むときに、綴り→音変換機能を用いて代替するため、この領域が強く賦活されたと解釈されている。

#### IV. SD の神経病理学的異常

FtLD は以前、Pick 病と呼ばれていた疾患を含む概念である。Pick 病は Onari と Spatz<sup>33)</sup> によって、前頭側頭葉に高度の限局性萎縮を呈する疾患として初めて病理学的に定義されたが、この際、Pick 球(嗜銀性神経細胞

内封入体)の有無は問わなかった。したがって、Pick 球を伴う Pick 病と伴わない Pick 病が存在することになった。近年、免疫組織学的研究が進み、Pick 球を伴う Pick 病は、タウ陽性の封入体を有する FTLD (FTLD-tau)に含まれ、Pick 球を伴わない Pick 病がユビキチン陽性タウ陰性封入体を有する FTLD に関連することがわかってきた<sup>34)</sup>。また、ユビキチン陽性封入体を構成する蛋白が TAR DNA-binding protein of 43kDa (TDP-43) であることが明らかにされ<sup>35)</sup>、この封入体を有する FTLD を FTLD-TDP と呼ぶようになった。

そして Mackenzie ら<sup>36)</sup>や Sampathu ら<sup>37)</sup>がユビキチン陽性タウ陰性封入体の形態によって FTLD-TDP をタイプ分類した。その後、この病理学的タイプ分類と臨床型との関連が報告されている。病理学的に FTLD-TDP と診断された 42 例の患者を Mackenzie らの分類に基づいて分類した研究では、タイプ 1 が 22 例、タイプ 2 が 9 例、タイプ 3 が 11 例で、タイプ 2 は全例臨床的には SD であった<sup>36)</sup>。また、病理学的に FTLD-TDP と診断された 28 例を Sampathu らの分類に従って分類した別の研究では、タイプ 1 が 9 例、タイプ 2 が 5 例、タイプ 3 が 10 例、分類不能が 4 例であった<sup>37)</sup>。そして臨床的に SD であった症例はすべてタイプ 1 であった。

Mackenzie らの分類のタイプ 2 は Sampathu らの分類ではタイプ 1 に相当するため、両研究の結果は一致し

ており、SD は病理学的に均一な疾患である可能性が高くなってきた。すなわち、SD では病理学的に、前頭側頭葉皮質表層のユビキチン陽性変性神経突起を主体とし、ユビキチン陽性神経細胞質内封入体や核内封入体はほとんどみられないという特徴<sup>46)</sup>を有する。

前述したSD-MNDについては、病理報告例が1例あり、この症例はMackenzieらの分類のタイプ3であった<sup>30)</sup>。このタイプ3は前頭葉の後方に目立つ萎縮を有し、臨床的にはFTD-MNDであることが多いとされている<sup>44)</sup>。したがって、SD-MNDは病理学的にはSDよりもFTD-MNDに近いのかもしれない。

## おわりに

SDは、頻度は多くないが、特徴的な神経心理学的・精神行動学的症状と神経画像学的所見を呈するため、この疾患を疑いさえすれば、臨床診断は容易な疾患である。また、病理学的にも均一な疾患であることが明らかになってきたため、今後、病態解明が進み、治療薬の開発研究も進むと予想される。同時に本疾患の病態解明により、意味記憶の脳内メカニズムについての新たな知見も得られると思う。今後の研究が期待される。

## 謝辞

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## 文 献

- Pick A: Über einen weiteren Symptomenkomplex im Pahnmen der Dementia senilis, bedingt durch umschriebebe stärkere Hirnatrophie (gemischte Apraxie). *Monatsschr Psychiatr Neurol* 19: 97-108, 1906
- Snowden JS, Goulding PJ, Neary D: Semantic dementia: a form of circumscribed cerebral atrophy. *Behav Neurol* 2: 167-182, 1989
- Hodges JR, Patterson K, Oxbury S, Funnell E: Semantic dementia. Progressive fluent aphasia with temporal lobe atrophy. *Brain* 115 (Pt 6): 1783-1806, 1992
- Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, et al: Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 51: 1546-1554, 1998
- Ikeda M, Ishikawa T, Tanabe H: Epidemiology of frontotemporal lobar degeneration. *Dement Geriatr Cogn Disord* 17: 265-268, 2004
- Ratnavalli E, Brayne C, Dawson K, Hodges JR: The prevalence of frontotemporal dementia. *Neurology* 58: 1615-1621, 2002
- Goldman JS, Farmer JM, Wood EM, Johnson JK, Boxer A, et al: Comparison of family histories in FTLT subtypes and related tauopathies. *Neurology* 65: 1817-1819, 2005
- 井村恒郎: 失語—日本語に於ける特性—。 *精神経誌* 47: 196-218, 1943
- 藤田郁代, 物井寿子, 奥平奈保子, 植田 恵, 小野久里子, 他: 失語症語彙検査 単語の情報処理の評価。 *エスコール*, 君津, 2000
- 伊藤皇一, 中川賀嗣, 池田 学, 山田典史, 橋本 衛, 他: 語義失語における語の意味カテゴリー—特異性障害。 *失語症研究* 14: 221-229, 1994
- 田辺敬貴, 池田 学, 中川賀嗣, 山本晴子, 池尻義隆, 他: 語義失語と意味記憶障害。 *失語症研究* 12: 153-167, 1992
- Hodges JR, Graham N, Patterson K: Charting the progression in semantic dementia: implications for the organisation of semantic memory. *Memory* 3: 463-495, 1995
- Wooliams AM, Ralph MA, Plaut DC, K P: SD-squared: on the association between semantic dementia and surface dyslexia. *Psychol Rev* 114: 316-339, 2007
- Nakagawa Y, Tanabe H, Ikeda M, Kazui H, Ito K, et al: Completion phenomenon in transcortical sensory aphasia due to various etiologies. *Behav Neurol* 6: 135-142, 1993
- 数井裕光, 田辺敬貴, 池田 学, 橋本 衛, 山田典史: 特異な人物の同定障害を呈した限局性脳萎縮の1例。 *No To Shinkei* 47: 77-85, 1995
- Nishio Y, Kazui H, Hashimoto M, Shimizu K, Onouchi K, et al: Actions anchored by concepts: defective action comprehension in semantic dementia. *J Neurol Neurosurg Psychiatry* 77: 1313-1317, 2006
- 近藤正樹, 望月 聡, 小早川睦貴, 鶴谷奈津子, 河村 満: 側頭葉型 Pick 病 (意味性認知症) における行為表出・行為理解障害。 *高次脳機能研究* 29: 268-276, 2009
- Bozeat S, Lambon Ralph MA, Patterson K, Garrard P, Hodges JR: Non-verbal semantic impairment in semantic dementia. *Neuropsychologia* 38: 1207-1215, 2000
- 田辺敬貴: 語義失語症者・その人となり—器質性病変と性格の変容—。 *神経心理* 8: 34-42, 1992
- Shigenobu K, Ikeda M, Fukuhara R, Maki N, Hokoishi K, et al: The Stereotypy Rating Inventory for frontotemporal lobar degeneration. *Psychiatry Res* 110: 175-187, 2002
- Miller BL, Cummings J, Mishkin F, Boone K, Prince F, et al: Emergence of artistic talent in frontotemporal dementia. *Neurology* 51: 978-982, 1998
- Kashibayashi T, Ikeda M, Komori K, Shinagawa S,

- Shimizu H, et al: Transition of distinctive symptoms of semantic dementia during longitudinal clinical observation. *Dement Geriatr Cogn Disord* 29: 224-232, 2010
- 23) Mitsuyama Y: Presenile dementia with motor neuron disease in Japan: clinico-pathological review of 26 cases. *J Neurol Neurosurg Psychiatry* 47: 953-959, 1984
- 24) Neary D, Snowden JS, Mann DM, Northen B, Goulding PJ, et al: Frontal lobe dementia and motor neuron disease. *J Neurol Neurosurg Psychiatry* 53: 23-32, 1990
- 25) Jeong Y, Park KC, Cho SS, Kim EJ, Kang SJ, et al: Pattern of glucose hypometabolism in frontotemporal dementia with motor neuron disease. *Neurology* 64: 734-736, 2005
- 26) 色井淳子, 大熊泰之, 深江治郎, 藤島健次, 後藤啓五: 漢字の失読と語義失語を伴った筋萎縮性側索硬化症の1例. *No To Shinkei* 54: 903-907 2002
- 27) Yokota O, Tsuchiya K, Itoh Y, Ishizu H, Akiyama H, et al: Frontotemporal lobar degeneration with ubiquitin pathology: an autopsy case presenting with semantic dementia and upper motor neuron signs with a clinical course of 19 years. *Acta Neuropathol* 112: 739-749, 2006
- 28) 木藤友実子, 數井裕光, 吉田哲彦, 久保嘉彦, 高屋雅彦, 他: 経時的に詳細な言語機能評価をした運動ニューロン疾患を伴う意味性認知症の1例. *Brain Nerve* 62: 625-630, 2010
- 29) Gainotti G, Ferraccioli M, Quaranta D, Marra C: Cross-modal recognition disorders for persons and other unique entities in a patient with right frontotemporal degeneration. *Cortex* 44: 238-248, 2008
- 30) Ostberg P, Bogdanovic N: Semantic dementia with lower motor neuron disease showing FTL-D-TDP type 3 pathology (sensu Mackenzie). *Neuropathology* 31: 271-279, 2010
- 31) Davies RR, Hodges JR, Kril JJ, Patterson K, Halliday GM, et al: The pathological basis of semantic dementia. *Brain* 128: 1984-1995, 2005
- 32) Rohrer JD, Warren JD, Modat M, Ridgway GR, Douiri A, et al: Patterns of cortical thinning in the language variants of frontotemporal lobar degeneration. *Neurology* 72: 1562-1569, 2009
- 33) Rohrer JD, McNaught E, Foster J, Clegg SL, Barnes J, et al: Tracking progression in frontotemporal lobar degeneration: serial MRI in semantic dementia. *Neurology* 71: 1445-1451, 2008
- 34) Desgranges B, Matuszewski V, Piolino P, Chetelat G, Mezenge F, et al: Anatomical and functional alterations in semantic dementia: a voxel-based MRI and PET study. *Neurobiol Aging* 28: 1904-1913, 2007
- 35) Patterson K, Nestor PJ, Rogers TT: Where do you know what you know? The representation of semantic knowledge in the human brain. *Nat Rev Neurosci* 8: 976-987, 2007
- 36) Agosta F, Henry RG, Migliaccio R, Neuhaus J, Miller BL, et al: Language networks in semantic dementia. *Brain* 133: 286-299, 2010
- 37) Wilson SM, Brambati SM, Henry RG, Handwerker DA, Agosta F, et al: The neural basis of surface dyslexia in semantic dementia. *Brain* 132: 71-86, 2009
- 38) Onari K, Spatz H: Anatomische Beiträge zur Lehre von der Pickschen umschriebenen Grosshirnrinden-Atrophie "Picksche Krankheit". *Ztschr Ges Neurol Psychiatr* 101: 470-511, 1926
- 39) Mackenzie IR, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, et al: Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. *Acta Neuropathol* 117: 15-18, 2009
- 40) Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, et al: TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 351: 602-611, 2006
- 41) Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, et al: Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314: 130-133, 2006
- 42) Mackenzie IR, Baborie A, Pickering-Brown S, Du Plessis D, Jaros E, et al: Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. *Acta Neuropathol* 112: 539-549, 2006
- 43) Sampathu DM, Neumann M, Kwong LK, Chou TT, Micsenyi M, et al: Pathological heterogeneity of frontotemporal lobar degeneration with ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. *Am J Pathol* 169: 1343-1352, 2006
- 44) Whitwell JL, Jack CR, Jr., Parisi JE, Senjem ML, Knopman DS, et al: Does TDP-43 type confer a distinct pattern of atrophy in frontotemporal lobar degeneration? *Neurology* 75: 2212-2220, 2010
- 45) Rohrer JD, Geser F, Zhou J, Gennatas ED, Sidhu M, et al: TDP-43 subtypes are associated with distinct atrophy patterns in frontotemporal dementia. *Neurology* 75: 2204-2211, 2010
- 46) 吉田眞理: FTL-D-U の病理. *Brain Nerve* 61: 1308-1318, 2009

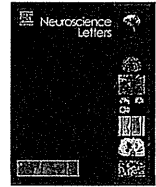




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## Association between CAG repeat length in the *PPP2R2B* gene and Alzheimer disease in the Japanese population

Ryo Kimura<sup>a,\*</sup>, Takashi Morihara<sup>b</sup>, Takashi Kudo<sup>b</sup>, Kouzin Kamino<sup>c</sup>, Masatoshi Takeda<sup>b</sup>

<sup>a</sup> Department of Psychiatry, Osaka General Medical Center, 3-1-56 Bandai Higashi, Sumiyoshi-ku, Osaka 558-8558, Japan

<sup>b</sup> Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan

<sup>c</sup> National Hospital Organization, Shoraiso Hospital, Nara, Japan

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### ABSTRACT

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

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Alzheimer disease (AD) is the most common cause of dementia in the elderly, and is characterized by progressive cognitive decline and cerebral atrophy. The primary pathological feature of AD is the presence of neurofibrillary tangles and senile plaques in the brain [26]. The presence of the  $\epsilon 4$  allele of the apolipoprotein E (*APOE*) gene (*APOE4*) confers a heightened risk of late-onset AD in multiple genetic backgrounds [4]. Although trinucleotide repeats are common features of the human genome, the trinucleotide repeat number varies among individuals and the lengths of these repeats is associated with many genetic diseases, including Huntington disease (HD) and Dentatorubral-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].

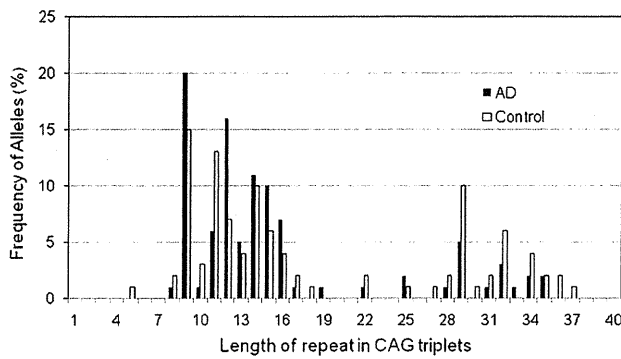
The *PPP2R2B* gene, which encodes a brain-specific regulatory B subunit of the serine/threonine protein phosphatase 2A (PP2A), is located on chromosome 5q31–33 and is widely expressed in brain neurons [21]. PP2A has been implicated in cell cycle and proliferation and development and regulation of multiple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

\* Corresponding author. Tel.: +81 6 6692 1201; fax: +81 6 6606 7000.  
E-mail address: [kimura@psy.med.osaka-u.ac.jp](mailto:kimura@psy.med.osaka-u.ac.jp) (R. Kimura).



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

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

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

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

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

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

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

**Table 2**

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

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

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

\* *p* < 0.05, statistically significant.  
OR, odds ratio.

Because the mean CAG repeat length among all subjects was 15, we dichotomized the alleles into short ( $\leq 15$ ) 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

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  $\epsilon 4$  allele of *APOE* gene confers a heightened risk of late-onset AD [4]. As compared to individuals without the  $\epsilon 4$  alleles, the risk for AD is 2- to 3-fold and about 12-fold higher in individuals carrying one and two  $\epsilon 4$  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 ( $\leq 15$ ) 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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### References

- [1] L. Bertram, M.B. McQueen, K. Mullin, D. Blacker, R.E. Tanzi, Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database, *Nat. Genet.* 39 (2007) 17–23.
- [2] A. Brusco, C. Cagnoli, A. Franco, E. Dragone, A. Nardacchione, E. Grosso, P. Mortara, R. Mutani, N. Migone, L. Orsi, Analysis of SCA8 and SCA12 loci in 134 Italian ataxic patients negative for SCA1–3, 6 and 7 CAG expansions, *J. Neurol.* 249 (2002) 923–929.
- [3] C.M. Chen, Y.T. Hou, J.Y. Liu, Y.R. Wu, C.H. Lin, H.C. Fung, W.C. Hsu, Y. Hsu, S.H. Lee, H.M. Hsieh-Li, M.T. Su, S.T. Chen, H.Y. Lane, G.J. Lee-Chen, *PPP2R2B* CAG repeat length in the Han Chinese in Taiwan: association analyses in neurological and psychiatric disorders and potential functional implications, *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* 150B (2009) 124–129.
- [4] E.H. Corder, A.M. Saunders, W.J. Strittmatter, D.E. Schmechel, P.C. Gaskell, G.W. Small, A.D. Roses, J.L. Haines, M.A. Pericak-Vance, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families, *Science* 261 (1993) 921–923.
- [5] H. Fujigasaki, I.C. Verma, A. Camuzat, R.L. Margolis, C. Zander, A.S. Lebre, L. Jamot, R. Saxena, I. Anand, S.E. Holmes, C.A. Ross, A. Durr, A. Brice, SCA12 is a rare locus for autosomal dominant cerebellar ataxia: a study of an Indian family, *Ann. Neurol.* 49 (2001) 117–121.
- [6] C.X. Gong, I. Grundke-Iqbal, K. Iqbal, Dephosphorylation of Alzheimer's disease abnormally phosphorylated tau by protein phosphatase-2A, *Neuroscience* 61 (1994) 765–772.
- [7] C.X. Gong, T. Lidsky, J. Wegiel, L. Zuck, I. Grundke-Iqbal, K. Iqbal, Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease, *J. Biol. Chem.* 275 (2000) 5535–5544.
- [8] C.X. Gong, S. Shaikh, J.Z. Wang, T. Zaidi, I. Grundke-Iqbal, K. Iqbal, Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain, *J. Neurochem.* 65 (1995) 732–738.
- [9] F.M. Harris, W.J. Brecht, Q. Xu, R.W. Mahley, Y. Huang, Increased tau phosphorylation in apolipoprotein E4 transgenic mice is associated with activation of extracellular signal-regulated kinase: modulation by zinc, *J. Biol. Chem.* 279 (2004) 44795–44801.
- [10] S.E. Holmes, E.O. Hearn, C.A. Ross, R.L. Margolis, SCA12: an unusual mutation leads to an unusual spinocerebellar ataxia, *Brain Res. Bull.* 56 (2001) 397–403.
- [11] S.E. Holmes, E.E. O'Hearn, M.G. McInnis, D.A. Gorelick-Feldman, J.J. Kleiderlein, C. Callahan, N.G. Kwak, R.G. Ingersoll-Ashworth, M. Sherr, A.J. Sumner, A.H. Sharp, U. Ananth, W.K. Seltzer, M.A. Boss, A.M. Vieria-Saecker, J.T. Epplen, O. Riess, C.A. Ross, R.L. Margolis, Expansion of a novel CAG trinucleotide repeat in the 5' region of *PPP2R2B* is associated with SCA12, *Nat. Genet.* 23 (1999) 391–392.
- [12] K. Iqbal, C. Alonso Adel, S. Chen, M.O. Chohan, E. El-Akkad, C.X. Gong, S. Khattoon, B. Li, F. Liu, A. Rahman, H. Tanimukai, I. Grundke-Iqbal, Tau pathology in Alzheimer disease and other tauopathies, *Biochim. Biophys. Acta* 1739 (2005) 198–210.
- [13] K. Iqbal, F. Liu, C.X. Gong, C. Alonso Adel, I. Grundke-Iqbal, Mechanisms of tau-induced neurodegeneration, *Acta Neuropathol.* 118 (2009) 53–69.
- [14] J. Kim, J.M. Basak, D.M. Holtzman, The role of apolipoprotein E in Alzheimer's disease, *Neuron* 63 (2009) 287–303.
- [15] R. Kimura, K. Kamino, M. Yamamoto, A. Nuripa, T. Kida, H. Kazui, R. Hashimoto, T. Tanaka, T. Kudo, H. Yamagata, Y. Tabara, T. Miki, H. Akatsu, K. Kosaka, E. Funakoshi, K. Nishitomi, G. Sakaguchi, A. Kato, H. Hattori, T. Uema, M. Takeda, The *DYRK1A* gene, encoded in chromosome 21 Down syndrome critical region, bridges between beta-amyloid production and tau phosphorylation in Alzheimer disease, *Hum. Mol. Genet.* 16 (2007) 15–23.
- [16] Z. Korade, A.K. Kenworthy, Lipid rafts, cholesterol, and the brain, *Neuropharmacology* 55 (2008) 1265–1273.
- [17] V.M. Lee, M. Goedert, J.Q. Trojanowski, Neurodegenerative tauopathies, *Annu. Rev. Neurosci.* 24 (2001) 1121–1159.
- [18] F. Liu, I. Grundke-Iqbal, K. Iqbal, C.X. Gong, Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation, *Eur. J. Neurosci.* 22 (2005) 1942–1950.
- [19] R.W. Mahley, B.P. Nathan, R.E. Pitas, E. Apolipoprotein, Structure, function, and possible roles in Alzheimer's disease, *Ann. N. Y. Acad. Sci.* 777 (1996) 139–145.
- [20] M.U. Manto, The wide spectrum of spinocerebellar ataxias (SCAs), *Cerebellum* 4 (2005) 2–6.
- [21] R.E. Mayer, P. Hendrix, P. Cron, R. Matthies, S.R. Stone, J. Goris, W. Merlevede, J. Hofsteenge, B.A. Hemmings, Structure of the 55-kDa regulatory subunit of protein phosphatase 2A: evidence for a neuronal-specific isoform, *Biochemistry* 30 (1991) 3589–3597.
- [22] G. McKhann, D. Drachman, M. Folstein, R. Katzman, D. Price, E.M. Stadlan, Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease, *Neurology* 34 (1984) 939–944.
- [23] E. O'Hearn, S.E. Holmes, P.C. Calvert, C.A. Ross, R.L. Margolis, SCA-12: tremor with cerebellar and cortical atrophy is associated with a CAG repeat expansion, *Neurology* 56 (2001) 299–303.
- [24] A.D. Roses, Apolipoprotein E alleles as risk factors in Alzheimer's disease, *Annu. Rev. Med.* 47 (1996) 387–400.
- [25] C.A. Ross, Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders, *Neuron* 35 (2002) 819–822.
- [26] D.J. Selkoe, Alzheimer's disease is a synaptic failure, *Science* 298 (2002) 789–791.
- [27] A.K. Srivastava, S. Choudhry, M.S. Gopinath, S. Roy, M. Tripathi, S.K. Brahmachari, S. Jain, Molecular and clinical correlation in five Indian families with spinocerebellar ataxia 12, *Ann. Neurol.* 50 (2001) 796–800.

- [28] A. Sulek, D. Hoffman-Zacharska, M. Bednarska-Makaruk, W. Szirkowiec, J. Zaremba, Polymorphism of trinucleotide repeats in non-translated regions of SCA8 and SCA12 genes: allele distribution in a Polish control group, *J. Appl. Genet.* 45 (2004) 101–105.
- [29] H.F. Tsai, C.S. Liu, T.M. Leu, F.C. Wen, S.J. Lin, C.C. Liu, D.K. Yang, C. Li, M. Hsieh, Analysis of trinucleotide repeats in different SCA loci in spinocerebellar ataxia patients and in normal population of Taiwan, *Acta Neurol. Scand.* 109 (2004) 355–360.
- [30] D.M. Virshup, Protein phosphatase 2A: a panoply of enzymes, *Curr. Opin. Cell. Biol.* 12 (2000) 180–185.
- [31] Y. Xu, Y. Chen, P. Zhang, P.D. Jeffrey, Y. Shi, Structure of a protein phosphatase 2A holoenzyme: insights into B55-mediated Tau dephosphorylation, *Mol. Cell* 31 (2008) 873–885.

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

Noriyuki Hayashi<sup>a</sup> Hiroaki Kazui<sup>a</sup> Kouzin Kamino<sup>a,f</sup> Hiromasa Tokunaga<sup>a</sup>  
Masahiko Takaya<sup>a</sup> Mikiko Yokokoji<sup>a</sup> Ryo Kimura<sup>c</sup> Yumiko Kito<sup>a</sup> Tamiki Wada<sup>a</sup>  
Keiko Nomura<sup>a</sup> Hiromichi Sugiyama<sup>a</sup> Daisuke Yamamoto<sup>a</sup> Tetsuhiko Yoshida<sup>d</sup>  
Antonio Currais<sup>g</sup> Salvador Soriano<sup>h</sup> Toshimitsu Hamasaki<sup>b</sup> Mitsuko Yamamoto<sup>a</sup>  
Yuka Yasuda<sup>a</sup> Ryota Hashimoto<sup>a,e</sup> Hitoshi Tanimukai<sup>a</sup> Shinji Tagami<sup>a</sup> Masayasu Okochi<sup>a</sup>  
Toshihisa Tanaka<sup>a</sup> Takashi Kudo<sup>a</sup> Takashi Morihara<sup>a</sup> Masatoshi Takeda<sup>a</sup>

Departments of <sup>a</sup>Psychiatry and <sup>b</sup>Biomedical Statistics, Osaka University Graduate School of Medicine, <sup>c</sup>Osaka General Medical Center, <sup>d</sup>Osaka National Hospital, and <sup>e</sup>Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Osaka, and <sup>f</sup>National Hospital Organization, Shoraiso Hospital, Nara, Japan; <sup>g</sup>Department of Neuroscience, MRC Centre for Neurodegeneration Research, Institute of Psychiatry, King's College London, London, UK; <sup>h</sup>Department of Human Anatomy and Pathology, Loma Linda University Medical School, Loma Linda, Calif., USA

### 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, diag-

nosed 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. Copyright © 2010 S. Karger AG, Basel

### Introduction

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

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 $\pm$ SD, years	68.5 $\pm$ 10.0	72.2 $\pm$ 8.1	0.267
Mean age of first abnormal memory loss episode reported by caregivers $\pm$ SD, years	63.8 $\pm$ 2.6	69.3 $\pm$ 2.0 <sup>1</sup>	0.104
Male/female, n	5/7	8/12	1.000
<i>APOE</i> $\epsilon$ 4+/-, 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 $\pm$ SD	17.8 $\pm$ 2.7	20.4 $\pm$ 4.6	0.093
Mean ADAS score $\pm$ SD	20.0 $\pm$ 5.8	17.6 $\pm$ 7.1 <sup>1</sup>	0.335
Years of education			
Median	12	10	0.151
IQR	10.25–15	9–14 <sup>1</sup>	

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

<sup>1</sup> 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  $\pm$  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  $\chi^2$  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*  $\epsilon$ 4, 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*  $\epsilon$ 4, 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*  $\epsilon$ 4, 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*  $\epsilon$ 4 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*  $\epsilon$ 4, years of education and ADAS score (this model is appropriate for total profile score); model 2: adjusted for *APOE*  $\epsilon$ 4, 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 $\pm$ SD, years	75.5 $\pm$ 4.9	75.2 $\pm$ 8.6	0.600
Male/female, n	170/205	110/236	<0.001
<i>APOE</i> $\epsilon$ 4+/-, 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 <sup>a</sup>	p <sup>b</sup>	p <sup>c</sup>
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.

<sup>a</sup> p for Hardy-Weinberg equilibrium tests (Pearson  $\chi^2$  test).

<sup>b</sup> p for genotype distribution (Fisher's exact test).

<sup>c</sup> 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*  $\epsilon$ 4 (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).

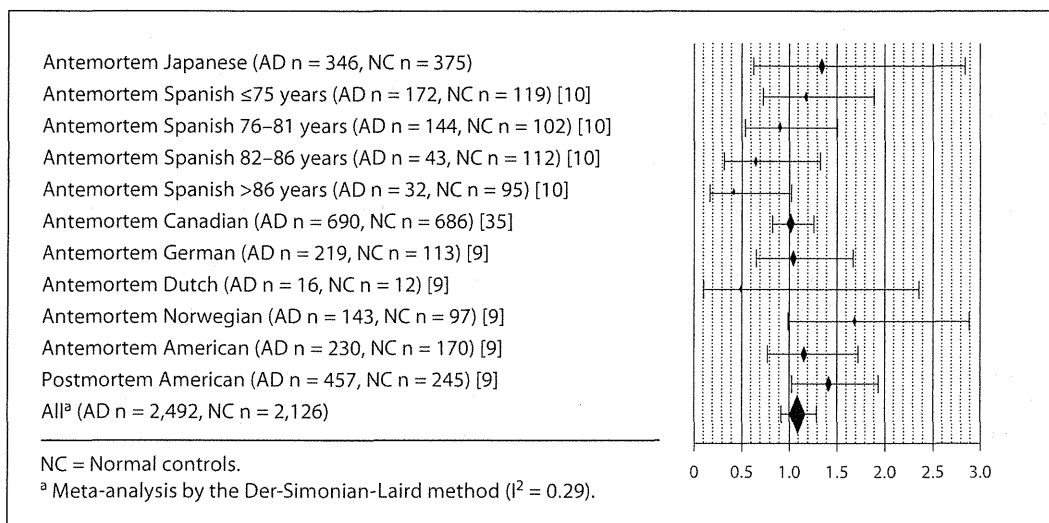
## Discussion

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 ( $2.6 \pm 1.7$  vs.  $4.7 \pm 1.2$  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*  $\epsilon 4$  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*  $\epsilon 4$ –:  $3.40 \pm 2.12$  vs. *APOE*  $\epsilon 4$ + :  $3.64 \pm 3.16$ ;  $p = 0.831$ ) and RBMT total screening score (*APOE*  $\epsilon 4$ –:  $0.80 \pm 0.63$  vs. *APOE*  $\epsilon 4$ + :  $0.86 \pm 1.17$ ;  $p = 0.873$ ) between *APOE*  $\epsilon 4$  carriers and non-*APOE*- $\epsilon 4$  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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### References

- Kremerskothen J, Plaas C, Buther K, Finger I, Veltel S, Matanis T, Liedtke T, Barnekow A: Characterization of *KIBRA*, a novel WW domain-containing protein. *Biochem Biophys Res Commun* 2003;300:862–867.
- Buther K, Plaas C, Barnekow A, Kremerskothen J: *KIBRA* is a novel substrate for protein kinase Czeta. *Biochem Biophys Res Commun* 2004;317:703–707.
- Genevet A, Wehr MC, Brain R, Thompson BJ, Tapon N: *KIBRA* is a regulator of the Salvador/Warts/Hippo signaling network. *Dev Cell* 2010;18:300–308.
- Papassotiropoulos A, Stephan DA, Huentelman MJ, Hoerndli FJ, Craig DW, Pearson JV, Huynh KD, Brunner F, Corneveaux J, Osborne D, Wollmer MA, Aerni A, Coluccia D, Hanggi J, Mondadori CR, Buchmann A, Reiman EM, Caselli RJ, Henke K, de Quervain DJ: Common *KIBRA* alleles are associated with human memory performance. *Science* 2006;314:475–478.
- Schaper K, Kolsch H, Popp J, Wagner M, Jessen F: *KIBRA* gene variants are associated with episodic memory in healthy elderly. *Neurobiol Aging* 2008;29:1123–1125.
- Yasuda Y, Hashimoto R, Ohi K, Fukumoto M, Takamura H, Iike N, Yoshida T, Hayashi N, Takahashi H, Yamamori H, Morihara T, Tagami S, Okochi M, Tanaka T, Kudo T, Kamino K, Ishii R, Iwase M, Kazui H, Takeda M: Association study of the *KIBRA* gene with memory performance in a Japanese population. *World J Biol Psychiatry* 2010, E-pub ahead of print.
- Almeida OP, Schwab SG, Lautenschlager NT, Morar B, Greenop KR, Flicker L, Wildenauer D: *KIBRA* genetic polymorphism influences episodic memory in later life, but does not increase the risk of mild cognitive impairment. *J Cell Mol Med* 2008;12:1672–1676.
- Yano M, Umeda S, Mimura M: Preserved priming but insensitivity to perceptual fluency on recognition judgments in Alzheimer's disease. *Psychogeriatrics* 2008;8:178–187.
- Corneveaux JJ, Liang WS, Reiman EM, Webster JA, Myers AJ, Zismann VL, Joshipura KD, Pearson JV, Hu-Lince D, Craig DW, Coon KD, Dunckley T, Bandy D, Lee W, Chen K, Beach TG, Mastroeni D, Grover A, Ravid R, Sando SB, Aasly JO, Heun R, Jessen F, Kolsch H, Rogers J, Hutton ML, Melquist S, Petersen RC, Alexander GE, Caselli RJ, Papassotiropoulos A, Stephan DA, Huentelman MJ: Evidence for an association between *KIBRA* and late-onset Alzheimer's disease. *Neurobiol Aging* 2010;31:901–909.
- Rodriguez-Rodriguez E, Infante J, Llorca J, Mateo I, Sanchez-Quintana C, Garcia-Gorostia I, Sanchez-Juan P, Berciano J, Combarros O: Age-dependent association of *KIBRA* genetic variation and Alzheimer's disease risk. *Neurobiol Aging* 2009;30:322–324.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984;34:939–944.
- Wilson B, Cockburn J, Baddeley A, Hiorns R: The development and validation of a test battery for detecting and monitoring everyday memory problems. *J Clin Exp Neuropsychol* 1989;11:855–870.
- Wilson B, Cockburn J, Baddeley A: The Rivermead Behavioural Memory Test. Elury, Thames Valley Test Company, 1991.
- Morris P: The Validity of Subjective Reports on Memory: Actions and Absentmindedness. London, Academic Press, 1984, pp 153–172.
- Watomori T, Hata H, Miyamori T, Eto F: Japanese version of the Rivermead Behavioural Memory Test. Tokyo, Chiba Test Center Co Ltd, 2002.
- Kazui H, Watomori T, Honda R, Tokimasa A, Hirono N, Mori E: The validation of Japanese version of the Rivermead Behavioural Memory Test: a test for everyday memory (in Japanese). *Shinkei Kenkyu No Shinpo* 2002; 46:307–318.
- Kazui H, Watomori T, Honda R, Mori E: The validation of a Japanese version of the Everyday Memory Checklist (in Japanese). *No To Shinkei* 2003;55:317–325.
- Matsuda A, Kazui H, Hirono N, Mori E: Validity of the Japanese version of Rivermead Behavioural Memory Test for evaluation of everyday memory function in patients with mild Alzheimer's disease (in Japanese). *No To Shinkei* 2002;54:673–678.
- Kazui H, Matsuda A, Hirono N, Mori E, Miyoshi N, Ogino A, Tokunaga H, Ikejiri Y, Takeda M: Everyday memory impairment of patients with mild cognitive impairment. *Dement Geriatr Cogn Disord* 2005;19:331–337.
- Kimura R, Kamino K, Yamamoto M, Nuripa A, Kida T, Kazui H, Hashimoto R, Tanaka T, Kudo T, Yamagata H, Tabara Y, Miki T, Akatsu H, Kosaka K, Funakoshi E, Nishitomi K, Sakaguchi G, Kato A, Hattori H, Uema T, Takeda M: The *DYRK1A* gene, encoded in chromosome 21 Down syndrome critical region, bridges between beta-amyloid production and tau phosphorylation in Alzheimer disease. *Hum Mol Genet* 2007;16:15–23.

- 21 Kimura R, Yamamoto M, Morihara T, Akatsu H, Kudo T, Kamino K, Takeda M: SORL1 is genetically associated with Alzheimer disease in a Japanese population. *Neurosci Lett* 2009;461:177-180.
- 22 Aidaraliev N, Kamino K, Kimura R, Yamamoto M, Morihara T, Kazui H, Hashimoto R, Tanaka T, Kudo T, Kida T, Okuda J, Uema T, Yamagata H, Miki T, Akatsu H, Kosaka K, Takeda M: Dynamin 2 gene is a novel susceptibility gene for late-onset Alzheimer disease in non-APOE-epsilon4 carriers. *J Hum Genet* 2008;53:296-302.
- 23 Taguchi K, Yamagata HD, Zhong W, Kamino K, Akatsu H, Hata R, Yamamoto T, Kosaka K, Takeda M, Kondo I, Miki T: Identification of hippocampus-related candidate genes for Alzheimer's disease. *Ann Neurol* 2005;57:585-588.
- 24 Akaike H: A new look at the statistical model identification. *IEEE Trans Automat Contr* 1974;AC-19:716-723.
- 25 Rosenberg SJ, Ryan JJ, Prifitera A: Rey Auditory-Verbal Learning Test performance of patients with and without memory impairment. *J Clin Psychol* 1984;40:785-787.
- 26 Wechsler D: Wechsler Memory Scale: Revised. Toronto, Psychological Corporation, Harcourt Brace, and Jovanovich, 1997.
- 27 Osterrieth PA: Le test de copie d'une figure complexe. *Arch Psychol* 1944;30:206-356.
- 28 Takeda S, Nakagome K, Tajime K, Saito M: Effectiveness of the Takeda Three Colors Combination Test as a screening test for dementia. *Psychogeriatrics* 2009;9:4-10.
- 29 Bates TC, Price JF, Harris SE, Marioni RE, Fowkes FG, Stewart MC, Murray GD, Whalley LJ, Starr JM, Deary IJ: Association of KIBRA and memory. *Neurosci Lett* 2009;458:140-143.
- 30 Katzman R, Aronson M, Fuld P, Kawas C, Brown T, Morgenstern H, Frishman W, Gidez L, Eder H, Ooi WL: Development of dementing illnesses in an 80-year-old volunteer cohort. *Ann Neurol* 1989;25:317-324.
- 31 Stern Y: What is cognitive reserve? Theory and research application of the reserve concept. *J Int Neuropsychol Soc* 2002;8:448-460.
- 32 Roe CM, Xiong C, Miller JP, Morris JC: Education and Alzheimer disease without dementia: support for the cognitive reserve hypothesis. *Neurology* 2007;68:223-228.
- 33 Bruandet A, Richard F, Bombois S, Maurage CA, Masse I, Amouyel P, Pasquier F: Cognitive decline and survival in Alzheimer's disease according to education level. *Dement Geriatr Cogn Disord* 2008;25:74-80.
- 34 Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, Ahern GL, Baxter LC, Rapcsak SZ, Shi J, Woodruff BK, Locke DE, Snyder CH, Alexander GE, Rademakers R, Reiman EM: Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. *N Engl J Med* 2009;361:255-263.
- 35 Li H, Wetten S, Li L, St Jean PL, Upmanyu R, Surh L, Hosford D, Barnes MR, Briley JD, Borrie M, Coletta N, Delisle R, Dhalla D, Ehm MG, Feldman HH, Fornazzari L, Gauthier S, Goodgame N, Guzman D, Hammond S, Hollingworth P, Hsiung GY, Johnson J, Kelly DD, Keren R, Kertesz A, King KS, Lovestone S, Loy-English I, Matthews PM, Owen MJ, Plumpton M, Pryse-Phillips W, Prinjha RK, Richardson JC, Saunders A, Slater AJ, St George-Hyslop PH, Stinnett SW, Swartz JE, Taylor RL, Wherrett J, Williams J, Yarnall DP, Gibson RA, Irizarry MC, Middleton LT, Roses AD: Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease. *Arch Neurol* 2008;65:45-53.



## Research report

## Frontal cortex activation associated with speeded processing of visuospatial working memory revealed by multichannel near-infrared spectroscopy during Advanced Trail Making Test performance

Takayuki Nakahachi<sup>a,b</sup>, Ryouhei Ishii<sup>a,\*</sup>, Masao Iwase<sup>a</sup>, Leonides Canuet<sup>a</sup>, Hidetoshi Takahashi<sup>a</sup>, Ryu Kurimoto<sup>a</sup>, Koji Ikezawa<sup>a</sup>, Michiyo Azechi<sup>a</sup>, Osami Kajimoto<sup>c,d</sup>, Masatoshi Takeda<sup>a</sup>

<sup>a</sup> Department of Clinical Neuroscience and Psychiatry, Osaka University Graduate School of Medicine, D3 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

<sup>b</sup> Faculty of Nursing and Rehabilitation, Konan Women's University, 6-2-23 Morikita-cho, Higashinada-ku, Kobe 658-0001, Japan

<sup>c</sup> Department of Medical Science on Fatigue, Osaka City University Graduate School of Medicine, 1-5-7 Asahimachi, Abeno-ku, Osaka 545-8585, Japan

<sup>d</sup> Soiken Incorporation, 1-4-2 Shinsenrihigashimachi, Toyonaka, Osaka 560-0082, Japan

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## ABSTRACT

Although visuospatial working memory (VSWM) is commonly used during speeded and unconscious memory processing in daily life, most neuroimaging studies on VSWM use tasks that impose motor restrictions onto the examinees to avoid movement-related artifacts. Multichannel near-infrared spectroscopy (NIRS), however, can measure cortical activation during cognitive processing without interfering with task procedure. The purpose of this study is to determine whether multichannel NIRS can detect VSWM-induced frontal cortex activation similar to that seen in VSWM performance in daily-life activity. Using NIRS, we measured relative changes in the concentration of oxygenated hemoglobin as an index of frontal activation in 52 measurement points (channels) on the frontal area during the Advanced Trail Making Test (ATMT), a tool used to assess VSWM. The ATMT consists of two tasks, R and F, with the former assessing motor factors and the latter relating to both motor and cognitive factors involved in speeded and unconscious VSWM operations. Twenty-six healthy volunteers were enrolled in this study. Channel activation during Task F performance was observed bilaterally over the dorsolateral and ventrolateral prefrontal cortex. This distribution may reflect central executive function of working memory. Channel activation during Task R was circumscribed to part of the left ventrolateral prefrontal cortex partially overlapping with areas active during Task F performance, likely representing task-related motor factor activation. Our findings suggest that multichannel NIRS during ATMT performance is an appropriate means of measuring cortical activation induced by VSWM operations during daily activity.

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## 1. Introduction

Visuospatial working memory (VSWM) is a cognitive function involved in temporal storage and effective manipulation of optical information related to object identification and spatial location [7,35,41]. It has been reported that several brain regions within the frontal lobe such as the dorsolateral prefrontal cortex (DLPFC), the ventrolateral prefrontal cortex (VLPFC), the frontal eye field (FEF) cortex, and the supplementary motor area (SMA) are involved in VSWM processing, with variation depending on task-specific features and modes of reaction [1,12,14,15,20,22,29,30,31,39,40,41]. The majority of these data stem from studies using static and conscious memory-demanding tasks, such as the delayed response task

and the n-back task. Clinically convenient, these tasks use controlled stimuli and require little space and time, but they impose motor restrictions onto the examinees to avoid movement-related neuroimaging artifacts. This is an important limitation, as in daily activity, memory representation is continuously updated during fast and unconscious VSWM operations. It is therefore unclear whether aforementioned brain areas truly reflect those activated during VSWM operations in daily activity.

The Advanced Trail Making Test (ATMT) is a derivative from the Trail Making Test Part A (TMT-A), which is a simple and standardized neuropsychological test that is widely used in clinical practice [3,42]. In the TMT-A the subject is asked to connect on paper the encircled numbers 1 through 25 in ascending order by drawing a line with a pencil as rapidly as possible. In speeded memory processing, this task requires the subject to use motor factors, including visual search and visuomotor coordination as well as cognitive factors, especially VSWM processing, to memorize locations

\* Corresponding author. Tel.: +81 6 6879 3051; fax: +81 6 6879 3059.  
E-mail address: [ishii@psy.med.osaka-u.ac.jp](mailto:ishii@psy.med.osaka-u.ac.jp) (R. Ishii).