②セレギリン（エフピー）

抗パーキンソン剤として発売されている。MAO-B阻害作用が中心だが，抗酸化作用およびAChE阻害作用も持っている。

③銀杏葉抽出液（EGb761）

イチョウの緑葉と小枝のエキスである。健康食品としても入手可能だが，自分でも簡単に作ることができる。抗酸化作用とアセチルコリン系の賦活作用がある。

④当帰芍薬散

漢方薬として承認されている。エストロゲンの分泌亢進，nAChの増加および抗酸化作用[※3]がある。

2）グルタミンレセプター阻害薬（第二世代）

グルタミン酸受容体は，グルタミンレセプター（NMDA）受容体と，非NMDA受容体（カイニン酸〈KA〉受容体とグルタミンに反応するキスカル酸受容体〈AMPA〉）に区分され，さらに，NMDA受容体はグリシン結合部位とグルタミン酸結合部位を持っている。

（1）メマンチン（ナメンダ）

NMDA受容体の拮抗薬としてアメリカで承認され，アリセプトと併用してアルツハイマー型痴呆に使用できるようになった。現在でも通信販売で入手可能である。

（2）サイクロセリン（サイクロセリン）

NMDA受容体グリシン結合部位作動薬。すでに抗結核薬として日本でも発売されている。頭部外傷後遺症の患者や動物（ネズミ）実験では記憶障害に有効との報告があるが，抗痴呆薬としての承認はされていない。

3）抗炎症薬（第三世代に準じる薬物）

抗炎症薬によるアルツハイマー型痴呆の進行防止作用は，COX-2阻害と考えられている。これは，ミクログリアの活性を抑制し，フリーラジカルやサイトカインの放出を抑えるものである（図1）。

さらに，抗炎症薬にはAβx-42産生を直接抑制する作用が見つかり，γ-セクレターゼへの作用と考えられている。なお，この作用が見られる抗炎症薬には，イブプロフェン（イブプロフェン），インドメタシン（インダシン），フルルビプロフェン（ロピオン），スリンダク（クリノリル）などが報告されている。しかし，Aβ産生の抑制はない抗炎症薬も存在する。具体的には，ジクロフェナク（ソファリン，ボルタレン），ナプロキセン（ナイキサン），セレコキシブ（未発売），メロキシカム（モービック）などであるが，前者との違いは不明である。

※3　抗酸化作用：SOD活性の亢進，8-ヒドロキシ-2'-デオキシグアノシン：8-OHdG生成を抑制する作用。

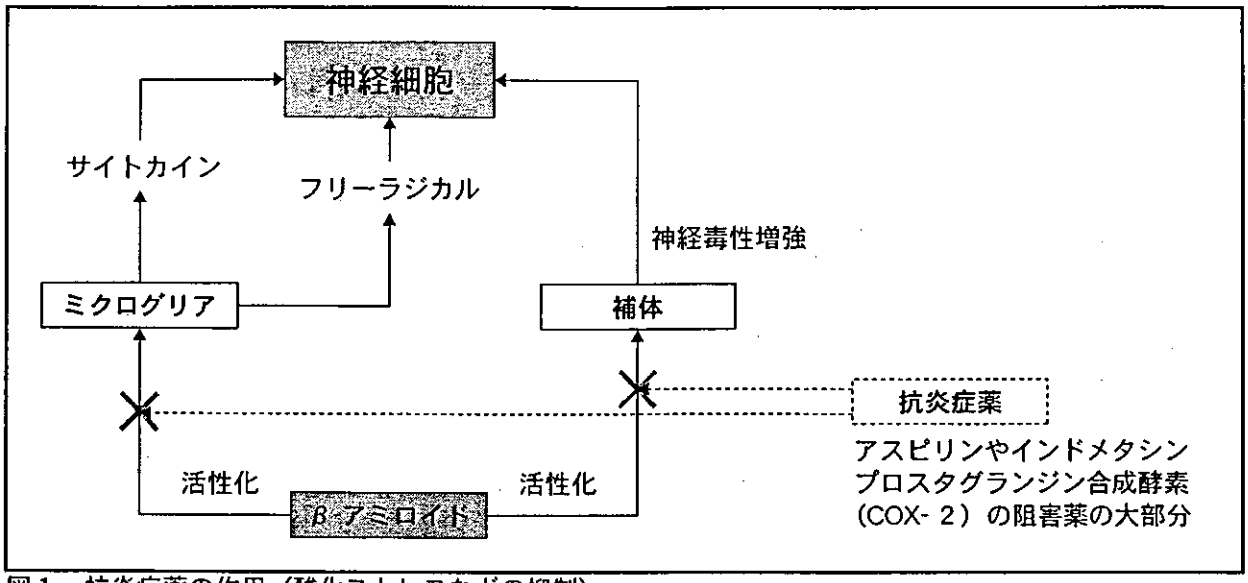

図1　抗炎症薬の作用（酸化ストレスなどの抑制）

4）コレステロール降下薬（第三世代に準じる薬物）

今まで，高コレステロール血症とアルツハイマー型痴呆に有意の相関があること，アポ蛋白（APO）E4が高コレステロール血症を生じることなどの報告はあった。その後，スタチンによるアルツハイマー病の発症率の低下の報告があり，以下のような機序が提案された。

すなわち，神経細胞膜には，グリコスフィンゴリピドやコレステロールの部分に対応した，界面活性剤に対する不溶性の膜部分があり，これはLipid raftsと呼ばれている。この部分には，アミロイド前駆体蛋白質（APP），Aβ，γ-セクレターゼ，β-セクレターゼ（BACE1）が存在し，Aββ分裂が起こるといわれる（ちなみに，α-セクレターゼはこの部分には存在しない）。そのため，総コレステロールを低下させると，コレステロールを含むLipid raftsの大きさが減少し，α-セクレターゼの活性を増強し，水溶性のα-APPを増加させ，逆に不溶性のβ-APP産生量を低下させることになる。

ただし，スタチンのなかで，ロバスタチン，プラバスタチン（メバロチン）にはアルツハイマー型痴呆発症抑制効果があるが，シンバスタチン（リポバス）には抑制作用がない。この機序の違いについては不明である。

5）女性ホルモン（エストロゲン：第三世代に準じる薬物）

エストロゲンがアルツハイマー型痴呆発症を抑制すると報告されているが，これはエストロゲンのα-セクレターゼ活性の亢進と抗酸化作用に基づく。閉経直後からのエ

ストロゲンの早期投与はアルツハイマー型痴呆の発症を減少させるが，高齢になってからの投与は効果が少ないといわれる。これは，エストロゲンに対する反応性の減弱が原因といわれる。ただし，エストロゲンによる治療トライアルは，乳がん発症率が高くなるという理由で，アメリカにおいては中止された。現在日本では，合成の17α-エストラジオール派生物（J-861）の治験が行われている。

6）ワクチン療法（第三世代）

研究の発端は，変異型ヒトアミロイド前駆体蛋白質（APP）発現マウスにAβ-42の注射で抗Aβ-42抗体作成を試みていたところ，アミロイドの新規沈着防止と，沈着したアミロイドの除去が認められたことに始まる。

マウスへの有効性が確認された後，ヒトに対して合成Aβ-42（AN-1792/Betabloc）による臨床治験が開始されたが，2002（平成14）年フェーズⅡ段階で脳炎と髄膜炎患者が15名ほど発生したため，中止になった。しかし，脳炎患者の剖検例でアミロイド沈着の防止が確認されたことなどもあり，現在は接種方法や抗原が変更されて，新たな臨床治験が開始されている。具体的には，Aβ-42のN（1－11）部位のペプチドによるワクチン療法（注射，経口）であるが，今後は作成された抗体の投与（受動免疫）も考えられている。

3．痴呆治療および予防のための戦略

65歳以上に限れば，痴呆と診断される疾患の種類は大きく2つに分けられる。1つは血管の変化に基づくもの，もう1つは異常蛋白の蓄積に基づくものである。前者は，脳血管性痴呆（VD）としてまとめられ，後者はアルツハイマー型痴呆や前頭側頭型痴呆（FTD）に代表される変性疾患にまとめられる。なお，有病率でいえば，脳血管性痴呆3％，アルツハイマー型痴呆（Aβとタウの蓄積）3％，前頭側頭型痴呆（タウの蓄積）1％，レビー小体病（α-シヌクレインの蓄積）0.1％である。ここでは，これらのうち脳血管性痴呆とアルツハイマー型痴呆について述べる。

1）血管性痴呆の治療と予防

脳血管性痴呆の発生原因は，脳血管障害（脳梗塞や脳出血）によって血流が停止し，神経細胞が酸素欠乏や栄養不足に陥って死滅することにある。脳血管性痴呆は，障害される血管の部位や分布により種々に分類されるが，脳血管の障害に統一される。脳血管性痴呆の治療は，脳梗塞や脳出血の再発を防止することにある。これらに有効なものは，血管拡張を目的とした薬剤（脳血管拡張薬），血流の凝固を阻害する薬剤（抗凝固薬）および血管の狭窄を防止する

薬剤（コレステロール降下薬）である。

すなわち，予防ないし治療は戦略１（図２）を用いることになるが，脳血管障害の原因となる各種の生活習慣病（高血圧，糖尿病，高脂血症，肥満）の治療や予防こそ，本来は脳血管性痴呆の予防に最も重要な因子である。

２）アルツハイマー型痴呆の治療と予防

アルツハイマー型痴呆の原因は，β-アミロイド蛋白による細胞毒性である。β-アミロイドの毒性を軽減するためには，活性酸素やインターロイキンを放出するミクログリアの反応性を低下させたり，補体の活性を低下させたりするような，抗炎症薬の投与がある（図１）。

また，根本的なアルツハイマー型痴呆の予防ないし治療は，β-アミロイドの蓄積の阻止と蓄積したものの除去にある。蓄積の阻止は，α-セクレターゼの活性を増強して可溶性βアミロイドを増加すること（M１，エストロゲンなど）と，β-セクレターゼの活性を減少させて，不溶性β-アミロイドの産生を低下すること（セクレターゼ阻害薬など）にある。また，蓄積物の除去には，β-アミロイドの分解と，ミクログリアによる呑食能の亢進，すなわちワクチン療法が有効であろう（図３）。

３）補助療法

第三世代ないし第三世代に準じる治療が可能になるまでの治療として，また当面補助的に用いて有用と思われる療法について述べる。これは，現在の治療法のアプローチを逆にしたもので，筆者が考えた視点である。

（１）神経細胞の機能保持

アルツハイマー病などの異常蛋白蓄積病について，蓄積した異常蛋白の面からでなく，それに曝される神経細胞の面からみる

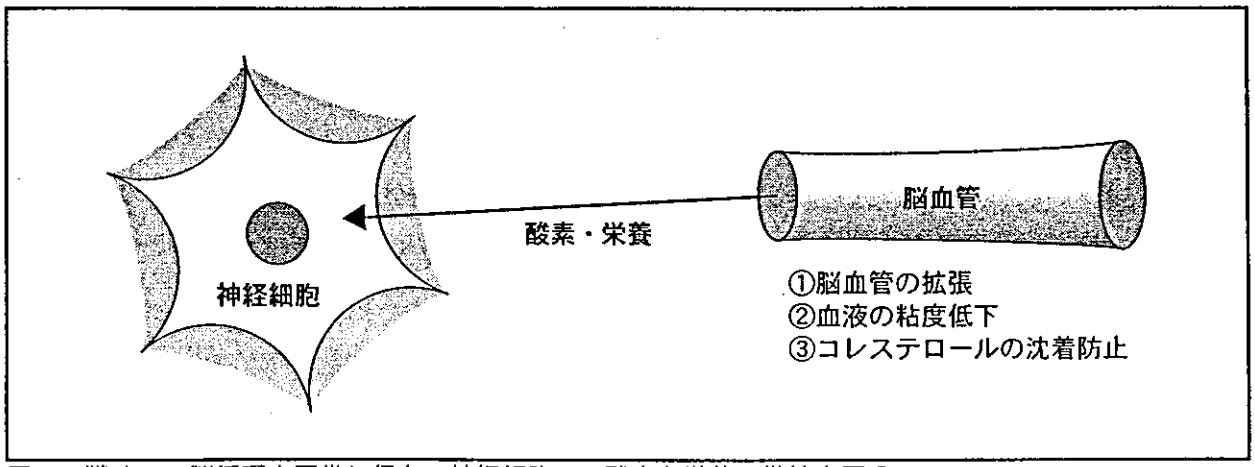

図２　戦略１：脳循環を正常に保ち，神経細胞への酸素と栄養の供給を図る

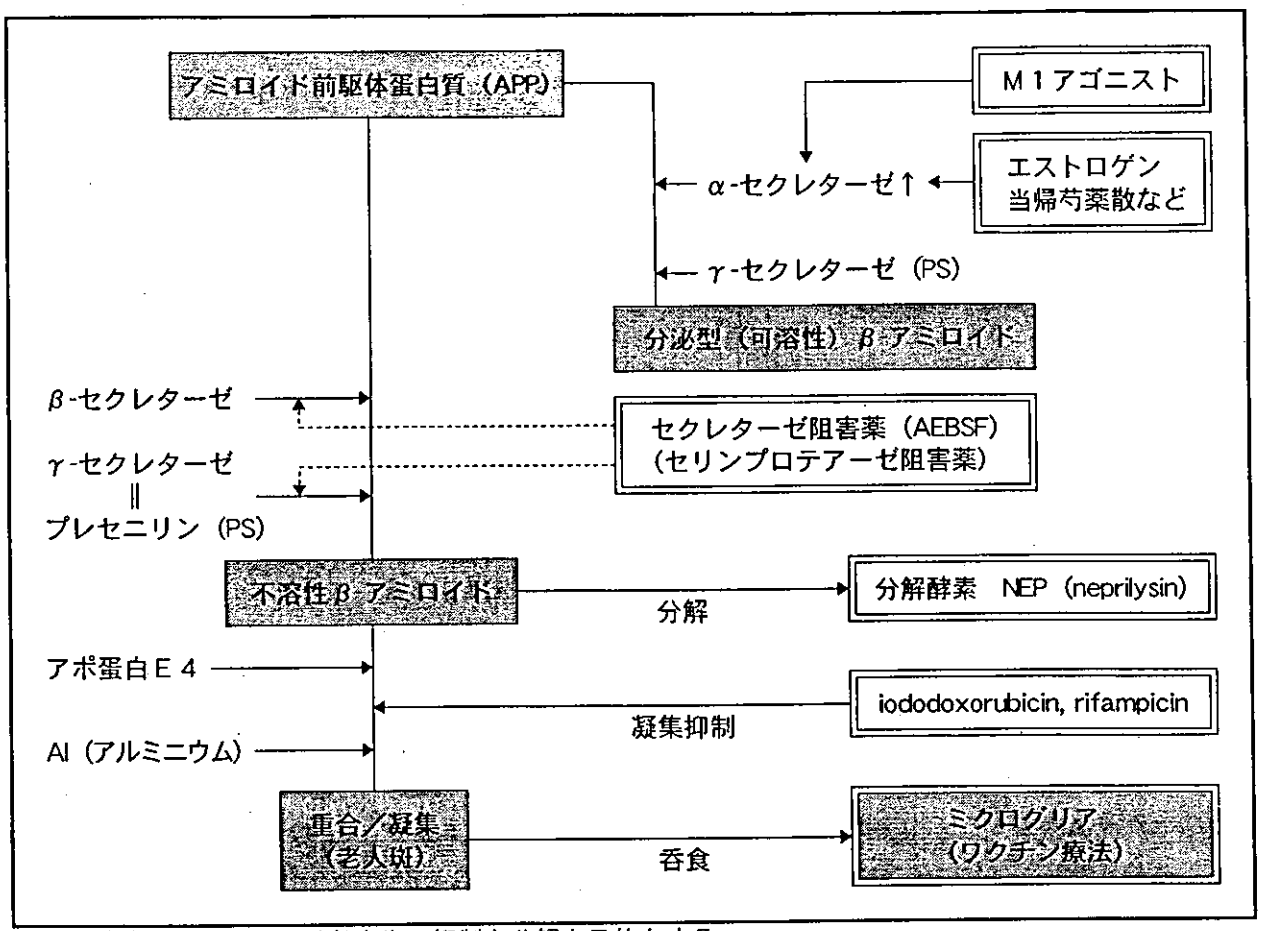

図3　戦略2：βアミロイド産生の抑制と分解を目的とする

と，別の戦略が考えられる。すなわち，神経細胞の変性や消失は，すでに述べたようにβ-アミロイドによるミクログリアの活性化によるフリーラジカルやインターロイキンなどのサイトカインの発生および凝集した老人斑以前のアミロスフェロイド（真球状物質Aβ-40，42）の細胞膜への付着が原因とされる。そのため，β-アミロイド除去を第一義とすべきだが，細胞膜の強化により毒性に対抗する形で，神経細胞の機能保持を図ることも可能ではないかと考えられる（図4）。

①膜成分の必須アミノ酸と必須脂肪酸の摂取

例えば，神経細胞膜上の脂質の25～30%を占めるDHA（Docosahexaeic acid）を含むn 3系多価不飽和脂肪酸を適度に摂取し，膜の機能改善を目指す方法である。

②膜の酸化防止

ミクログリアの活性を抑えるというより，抗酸化物質（ビタミンA，C，E，ポリフェノール，フラボノイド）摂取により，ミクログリアから発生したフリーラジカルなどを除去して，細胞膜を保護する方法である。

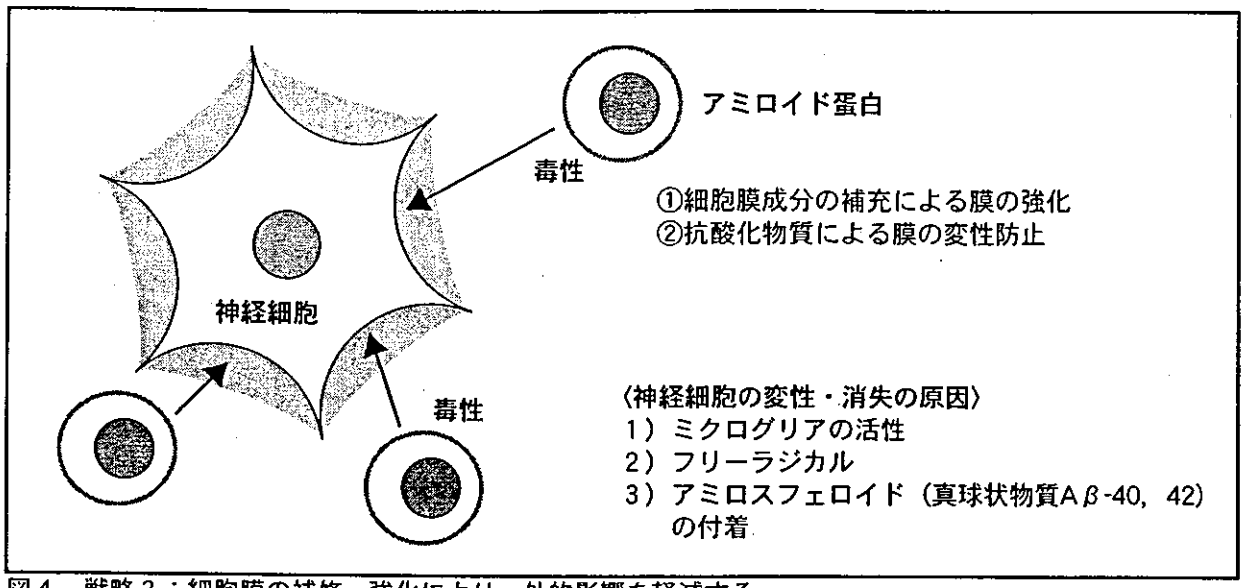

図4　戦略3：細胞膜の補修・強化により，外的影響を軽減する

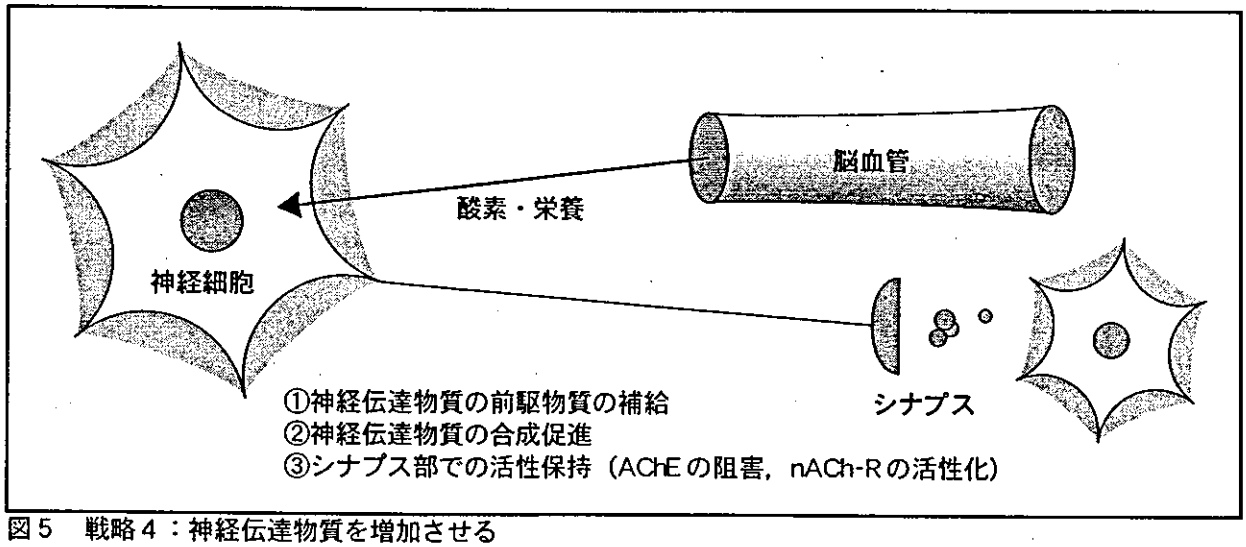

図5　戦略4：神経伝達物質を増加させる

（2）神経伝達物質の供給

記憶障害は，アセチルコリン系の神経伝達物質の減少による。そのため，以前より行われていた前駆物質や変換促進物質の摂取を通じて，神経細胞内のアセチルコリンの増加を目指す方法である（図5）。前駆物質の代表的なものとして，レシチンやホスファジル・セリンがある。また，コリンからアセチルコリンへの変換促進物質として，ビタミンB_1，女性ホルモン，葉酸などが挙げられる。さらに，アセチルコリン性受容体の活性を増加させるものには，AChE阻害薬，セビメリン，銀杏葉抽出液，当帰芍薬散などがある。これらについては

前述したとおりである。

①レシチンの摂取

大豆ないし卵黄に多く含まれるリン脂質で，コリンを多く含む。なお，コリンはアセチルコリンの前駆物質である。

②ホスファジル・セリンの摂取

大豆由来のリン脂質。以前からパーキンソン病やアルツハイマー型痴呆に使われている。1日100mg以上の摂取が有効といわれている。

おわりに

抗痴呆薬について，第二世代と第三世代を中心に説明した。治療の原則は，痴呆の原因を除去することであろうが，いずれの痴呆においても，加齢と共に血管因子が合併するため，両者を視野に入れた併用療法を行うべきである。さらに視点を変えて，外的因子（血管障害や異常蛋白の蓄積）の面からでなく，本来の個体が持つ，神経細胞機能の保持という内的因子の面からの治療（図4，5）も考慮すべきであろう。いずれにしても，「痴呆は治らない」という悲観論を捨て，積極的に治療に取り組むべき時代に差しかかっているようだ。

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要点学習

Q1：ドネペジルにはどのような副作用があるのでしょうか。
Q2：今後，抗痴呆薬として期待される薬物とは，何でしょうか。
Q3：脳血管性痴呆の治療と予防について述べましょう。

第46回日本老年医学会学術集会記録
〈ワークショップII：地域に生きる「痴呆」—物忘れ早期発見・早期診断と介護予防〉

4. 痴呆の治療

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Key words：抗痴呆薬，ACh 阻害薬，補助療法，メンタルトレーニング

（日老医誌　2005；42：49—51）

薬物療法の考え方

現時点で痴呆の薬物治療は，中核症状としての記憶障害に作用する薬物と随伴症状に対応する薬物に分類される．前者にはタクリン(Cognex)，ドネペジル(Aricept)，リバスチグミン（Excelon)，ガランタミン（Reminyl）などのアセチルコリンエステラーゼ阻害剤と，血管性痴呆に有効な脳循環改善剤や抗凝固薬がある．後者には表1のような，幻覚妄想，抑うつ，せん妄，徘徊などに使用される向精神薬がある．

抗痴呆薬の区分

痴呆の中核症状に作用する薬剤を抗痴呆薬とすると，歴史的には3つの世代に区分できる．今まで実施されていた脳血流や脳代謝改善を目的とした脳代謝改善薬，脳血管拡張薬，抗凝固薬を第一世代とすると，現在使用可能な神経伝達物質の調整目的としたAChE阻害薬やグルタミンR阻害薬は第二世代の薬物といえる．アルツハイマー病など異常蛋白の蓄積が原因となる疾患では，その蓄積防止を目的とした根治療法は，第三世代といえるが，現時点で使用可能な抗炎症薬，コレステロール降下薬，女性ホルモン，細胞成長因子などは第三世代に準じる薬物といえ，真の意味での第三世代の薬物はワクチン療法が挙げられる．なお，このAβワクチン療法では脳炎などの副作用を抑えるため，Aβ42のN（1～11）部位のペプチドによるワクチン療法（注射，経口）や作成された抗体の投与（受動免疫）が考えられているが，実用化はまだ先である．なお，抗炎症薬のイブプロフェン，インドメサシン，フルルビプロフェン，スリンダクは有効だが，ジクロフェナク，ナプロキセン，セレコキシブ，メロキシカムは無効である．COX2阻害でなく，γセクレターゼへの直接作用と言われるが，効果の有無は何によるかは不明である．また，コレステロール降下剤はLipid rafts（界面活性剤に対する不溶性の膜部分：グリコスフィンゴリピドやコレステロールの部分に対応）に関連し，総コレステロールを低下させるとαセクレターゼ活性の増強，Aβ産生の低下をもたらすというが，スタチンの種類によって，アルツハイマー病の発症抑制があるロバスタチン，プラバスタチンと，発症抑制のないシンバスタチンに別れ，この違いも不明である．

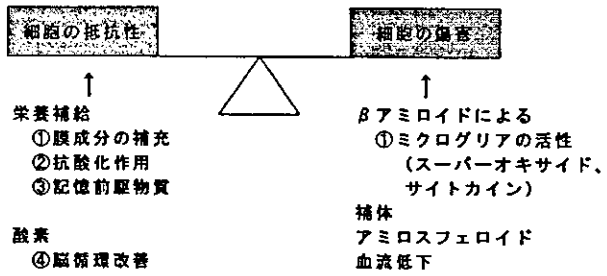

図1　補助療法の考え方

補助療法

現在の薬物療法とは別に，補助療法がある．これは，細胞傷害の種々の原因を取り除く面からでなく，傷害を受ける細胞の抵抗性を高めるもので，薬物以外に食物も関係し，「自分の脳と体の健康は，自分で守る」という予防の意識を高め，また能動的な住民活動になりえるものでもある（図1，薬物療法は，医師が薬剤を選択するため，患者は受け身である）．なお，これは3つの方法に分けられる．1つは神経細胞膜の保護を目指すもので，1）脳細胞膜の構成成分の補給（必須脂肪酸・必須アミノ酸の摂取）には，食物は青魚（DHA），牛乳，食物蛋白，米，そばなど，薬物はサプリメントが，2）抗酸化作用物質の摂取には，薬物はビタミンA，C，E，ポリフェノール，フラボノイドが，食物は緑黄色野菜，茶，

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表1　随伴症状に使用される向精神薬

標的症状＼薬物		抗精神病薬	抗うつ薬	抗不安薬	睡眠薬	脳循環改善薬	その他
1. 幻覚		◎	×	○	○	△	
2. 妄想		○	○	△	△	△	
3. せん妄		◎	○	×	○	○	水分補給
4. 徘徊		◎	×	○	○	○	
5. 興奮・易怒		◎	×	○	○	△	カルバマゼピン
6. 叫声，大声		◎	×	○	○	△	
7. 心気症状		○	○	○	△	×	
8. うつ状態	a. 抑うつ気分	×	◎	○	○	○	メチルフェニデート
	b. 意欲低下	×	◎	○	×	○	甲状腺剤
	c. 不安・焦燥	○	△	◎	○	△	カルバマゼピン
	d. 身体症状	△	◎	○	×	△	漢方薬
9. 睡眠障害		○	○	○	◎	△	抗ヒスタミン剤 漢方薬

表2　非薬物療法の方法

1. メンタルトレーニング（頭の運動）
   ①見当識　：「日時を確認する」　—今日は何月何日か—
   ②短期記憶　：「新しい事柄を記憶する」　—食事内容，数字の逆唱—
   ③会話　：「日常の出来事の確認，理解，まとめて話す」
   ④書字・読字：「手紙や日記を書く，読書する」
   ⑤計算　：「買い物の時など，暗算をする」
   ※刺激が脳血流の増加を促進．
2. 体の運動
   ①散歩，体操，スポーツ：脳全体の覚醒度を高め，脳血流量を増加させる
   ②音楽，歌，カラオケ　：障害の少ない部位を通じて脳に刺激を与える
   ※身体への刺激が脳血流の増加とともに，脳全体の再統合を促進．

紅茶，コーヒー，ココア，赤ワイン，トマト，きのこ，などがある．2つ目は，伝達物質の補給による治療を目指すもので，3）記憶物質の前駆物質の摂取では，薬物はレシチン，コリン，食物は卵黄，大豆類などが，4)記憶物質を増加させる物質の摂取では，薬物は女性ホルモン，ビタミンB1，葉酸，食物は大豆類（イソフラボン＝女性ホルモン），緑黄色野菜などがある．3つ目は，脳血流量の改善と維持を目指すもので，5）脳循環の改善（血管を拡張させて，栄養と酸素を供給する）では，薬物は脳循環改善薬，抗凝固薬，食物は納豆(キナーゼ)，青魚（EPA），銀杏葉などが，6）コレステロールの低下（血管の流れを改善）では，薬物は高脂血症治療薬，食物は食物繊維(こんにゃく，緑黄色野菜など)，魚油，オリーブ油などがある．

非薬物的治療

痴呆の治療及び予防は，メンタルトレーニングと体の運動で対応する．具体的内容は表2のようであるが，作用機序は，種々の刺激により，脳循環を増加させ，結果として神経細胞に栄養と酸素を補給することにある．そのため，単にこれらのトレーニングのみでなく，補給すべき栄養も考慮すべきである．

Abstract

Treatment of dementia

Kazuo Miyanaga

Drugs to treat the primary symptoms of dementia are nootropics (anti-dementia medicine). These can be divided into three stage historically. Drugs of brain metabolic improvement and blood expanssion and anti-coagulation drugs used till now, are the first stage. AChE inhibitor and glutamin receptor inhibitors used at present are second generation drugs. The cause of degenerative diseases like Alzheimer's disease is the accumulation of abnormal protein. So, fundamental medicines to prevent such accumulation are the third generation drugs. Anti-inflammation drugs, anti-cholesterol drugs, female hormones, and nerve growth factors used at present are third generation grugs. In truth, only vaccine treatment is the third stage medicine.

Adjuvant treatment is also available at present. This approach is not based on the elimination of various causes of cell injury, but on increasing resistance to cell injury. These methods include protection of the nerve cell membrane, supply of nerve transmission material, and improvement of brain blood flow.

Non-medicinal methods of treatment and prevention of dementia include mental training and body movement (exercises). These promote supply of nutrition and oxygen to the nerve cells as a result of increases in brain circulation caused by the various stimuli. In addition to training, nutrition should also be suppled at the same time.

Key words: *Nootropics, AChE inhibitor, Assistant treatment, Non-medication, Mental training*
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A novel presenilin 1 mutation (Y154N) in a patient with early onset Alzheimer's disease with spastic paraparesis

Sayoko Hattori[a], Kenji Sakuma[a,b], Yosuke Wakutani[a,*], Kenji Wada[a], Masaru Shimoda[c], Katsuya Urakami[b], Hisanori Kowa[a], Kenji Nakashima[a]

[a] *Department of Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, 36-1 Nishimachi, Yonago-shi 689-8504, Japan*
[b] *Department of Biological Regulation, Section of Environment and Health Science, Faculty of Medicine, School of Health Science, Tottori University, Japan*
[c] *Department of Neurology, Tottori Red Cross Hospital, Japan*

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Abstract

Early onset familial Alzheimer's disease with spastic paraparesis (FAD-SP) has been associated with mutations of the presenilin 1 gene (*PSEN1*). We report a pedigree of FAD-SP due to a novel missense mutation of *PSEN1* (Y154N). The symptoms of the proband were characterized by presenile dementia in her 40s, preceded by spastic paraparesis in her 30s, whereas the mother of the proband presented with spastic paraparesis in her 40s, followed by symptoms of dementia in her mid 60s. The mutation was found only in the proband, and not in a normal family member, normal Japanese control subjects, patients with sporadic Alzheimer's disease or patients with familial spastic paraparesis without dementia. Thus, Y154N is a novel *PSEN1* mutation responsible for FAD-SP of Japanese origin.

Keywords: Presenilin 1; Alzheimer's disease; Spastic paraparesis

Alzheimer's disease (AD) is the most important neurodegenerative disorder leading to dementia. Familial Alzheimer's (FAD), especially the early onset (presenile) type, is inherited in an autosomal dominant fashion. The etiology of FAD is known as mutations in the amyloid precursor protein gene (*APP*) on chromosome 21, the presenilin 1 gene (*PSEN1*) on chromosome 14, and the presenilin 2 gene (*PSEN2*) on chromosome 1. Over 130 mutations of *PSEN1* have been found in FAD pedigrees all over the world (the detailed mutations of *PSEN1* are listed in the AD mutation database: http://molgen-www.uia.ac.be/ADMutations/default.cfm?MT=0&ML=0&Page=Home). Furthermore, *PSEN1* mutations have been found in several pedigrees with alternative clinical phenotypes of frontotemporal dementia and spastic paraparesis with dementia (or FAD with spastic paraparesis; FAD-SP). A deletion mutation (DeltaI83/M84) [5,15], an insertion mutation (InsFI) [12], seven-point mutations (F237I [14], V261F [12], R278T [8], R278K [1], E280G [11], P284L [17], and P436Q [5]) and four independent mutations resulting in deletion of exon 9 (Delta 9) [3–5,13] have been identified in the pedigrees of FAD-SP.

The most characteristic pathological feature of FAD-SP has been reported to be the so-called "cotton wool plaques", which are distinct from classical "senile plaques" [3–5,13,17]. However, the pathomechanisms for the formation of "cotton wool plaques" remain unclear.

Herein, we report a pedigree characterized by spastic paraparesis with dementia bearing a novel *PSEN1* mutation (Y154N).

A 47-year-old Japanese woman (proband, II-4) was admitted to our hospital complaining of progressive gait disturbance followed by gradual cognitive decline. Her first neurological symptom was gait disturbance noticed at the age of 37. At age 42, her family members noticed her first

* Corresponding author. Tel.: +81 859 34 0832; fax: +81 859 34 8083.
E-mail address: ywaku@grape.med.tottori-u.ac.jp (Y. Wakutani).

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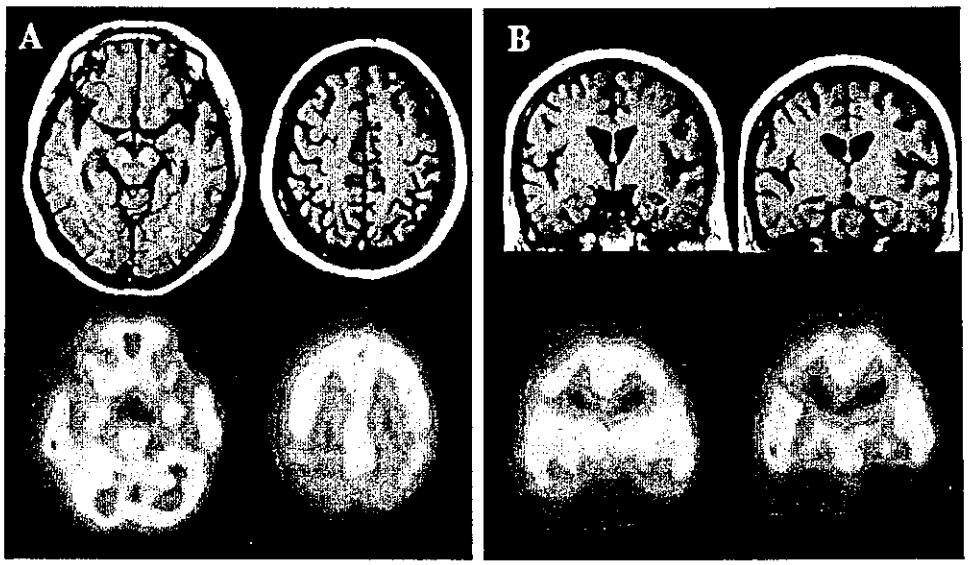

Fig. 1. MRI (T1 weighted image) and SPECT (^{99m}Tc-ECD) findings. (A) Axial T1 weighted images show apparent occipito-parietal atrophy and mild atrophy in internal portion of temporal lobes. Axial SPECT images show marked hypoperfusion in bilateral occipito-parietal areas and internal portion of temporal areas. (B) Coronal images show slight atrophy in bilateral hippocampal areas, whereas coronal SPECT images show apparent hypoperfusion in the internal portion of temporal areas including bilateral hippocampal areas.

amnesic symptoms. She had gradually progressive difficulties with her household chores. She also exhibited a mild slurring dysarthria. Neurological examination on admission at age 47 revealed pyramidal signs in her limbs consistent with spastic paraparesis. Deep tendon reflexes were hyperreactive in all limbs, and muscle tone was markedly spastic in her lower limbs. Pathological reflexes were detected in all limbs. She also had brisk jaw jerk. She showed marked spastic gait, predominantly in her left leg. She also exhibited apparent disorientation and prominent memory disturbance. Scores on the Mini-Mental State Examination and the WAIS were 16/30 and IQ 68, respectively. Levels of CSF amyloid β (Aβ) 42, total Tau protein (hTAU) and phosphorylated Tau protein (pTAU) were 373.4 pg/ml (non-AD controls of our department (nAD) were (n = 27); 1005 ± 248.1), 698.7 pg/ml (nAD (n = 23); 266.1 ± 191.9), 112.0 pg/ml (nAD (n = 23); 31.5 ± 32.8), respectively. The levels of Aβ42, hTAU and pTAU were measured using commercial kits (Innogenetics, Gent, Belgium). The ratios of hTau/Aβ42 and pTau/Aβ42 were 1.84 (nAD (n = 23); 0.31 ± 0.31) and 0.34 (nAD (n = 27); 0.049 ± 32.8), respectively. These results are consistent with previous reports that elevated ratios of hTau/Aβ42 and pTau/Aβ42 are useful CSF biomarkers for AD [6,9].

Cranial MRI showed mild cerebral atrophy in her temporal and parietal lobes (Fig. 1). ^{99m}Tc-ECD SPECT showed hypometabolism in the temporo-parietal and the internal portion of temporal areas (Fig. 1). According to these clinical data, we diagnosed our patient as having AD with spastic paraparesis.

Her family history indicated that the mother of the proband presented in her 40s with progressive gait disturbance and died at age 69. Although her family noticed that she had shown abnormal behavior and cognitive dysfunction consistent with having dementia 2 years before death, she had no episodes indicating her having dementia for at least 10 years after the onset of gait disturbance. Although the clinical manifestations were different between the proband and the mother, we assume that they are in a pedigree of FAD-SP.

After obtaining informed consent, DNA samples were extracted from peripheral blood leukocytes of the proband and her non-symptomatic elder sister. The coding regions and adjacent 5′ and 3′ intron sequences of *APP*, *PSEN1*, and *PSEN2* were screened by PCR-single strand polymorphism analysis (PCR-SSCP) and sequence analysis as previously described [18].

PCR-SSCP demonstrated an abnormal migration pattern in *PSEN1* exon 5 of the proband sample (data not shown), and sequence analysis identified a TAT to AAT substitution at codon 154 of *PSEN1* resulting in an amino acid substitution of tyrosine with asparagine (Y154N) (Fig. 2).

For screening of the Y154N mutation, PCR-restriction endonuclease length polymorphism analysis (PCR-RLFP) using a mismatch primer pair was used. In brief, DNA samples were amplified using a Hot-start PCR kit (TaKaRa, Shiga, Japan) and a primer pair; forward: 5′-GAATCTATACCCCATTCACAGAAGA-3′ and reverse: 5′-TCATGCTCACCTTATAGCACCTGTATTGAT-3′ (underline: mismatch position), followed by PCR-RLFP using *Hin*fI. The A allele of the Y154N mutation gains an artificial *Hin*fI site. In the PCR-RLFP using *Hin*fI, Y154N was detected only in the proband, and not in her non-symptomatic elder sister, 103 healthy controls, 15 patients with early-onset Alzheimer's disease (AD) without spastic paraparesis, 50 patients with late-onset AD and 7 independent patients with

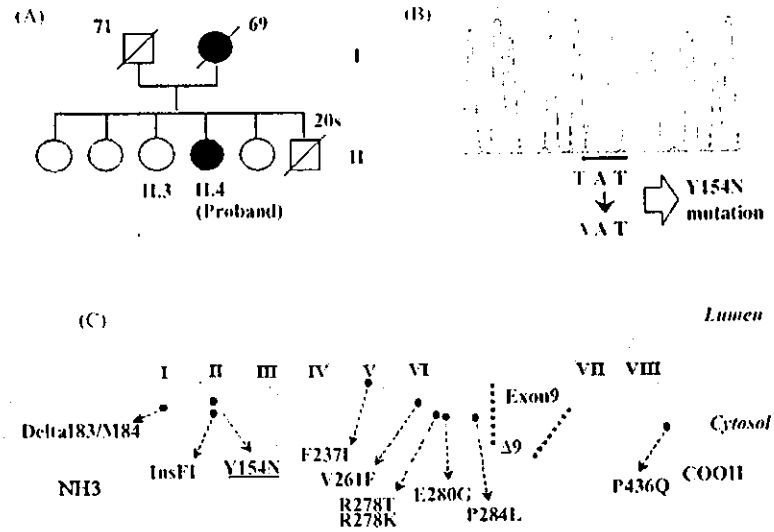

Fig. 2. (A) Pedigree of the family with Y154N *PSEN1* mutation. (B) Sequence diagram of *PSEN1* exon 5. Underlining indicates the heterozygous mutation from the T to A transition, resulting in a change from tyrosine (T) (TAT) to asparagine (N) (AAT) at codon 154 of *PSEN1*. (C) Schematic representation of *PSEN1* mutations for FAD-SP.

familial spastic paraparesis without dementia, indicating that the Y154N is specific to the proband.

Although several other polymorphic migration patterns in *APP*, *PSEN1*, and *PSN2* were detected by PCR-SSCP analysis, sequence analysis revealed that these polymorphic bands reflected the existence of single nucleotide polymorphisms (SNPs) listed in the NCBI SNPs database. APOE genotype of the proband was ε3/3.

We report here a novel PSN1 mutation (Y154N) presenting as FAD-SP. The Y154N mutation is in the cytosol side of predicted transmembrane (TM) domain 2 (Fig. 2) and takes place at the same residue as a previously found mutation (Y154C), which clinically presents as FAD without spastic paraparesis [7].

It has been reported that several *PSEN1* mutations are clinically associated with FAD-SP and cause accumulation of noncongophilic Aβ-positive "cotton wool" plaques in brain parenchyma [3–5,13,15,17]. Pathological analyses have demonstrated that "cotton wool plaques" are homogeneously positive for Aβ42 and negative for Aβ40, whereas amyloid core-like structures are positive for Aβ40 [3,4,15,17]. The deposition of Tau as neurofibrillary tangles is common, but variable in patients with FAD-SP [3,4,13,15,17]. In the proband with the Y154N mutation, the coexistence of Aβ and tau pathology was predicted by the results of CSF analysis, showing a decrement of Aβ42 and elevation of total and phosphorylated tau protein.

In cellular models bearing *PSEN1* mutations for FAD-SP (particularly in the Delta 9 mutation), a marked increase of Aβ42 production has been observed [2,10,16]. However, it has been reported that overproduction of Aβ42 in cellular models does not necessarily correspond to the clinical phenotype. Further, clinical manifestations (age of onset, initial symptom (e.g. dementia or paraparesis) and degree of dementia and spastic paraparesis) have been reported to be variable with the affected family members bearing the identical *PSEN1* mutation [1,3,13]. Assuming that the mother of the proband had the Y154N mutation, the age at onset of gait disturbance and cognitive decline, and the clinical course in this pedigree show generational differences. Based on this evidence, alternative cofactor(s) that can influence the clinical manifestations and/or the formation of "cotton wool" plaques in pedigrees of FAD-SP remain to be clarified.

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A haplotype of the methylenetetrahydrofolate reductase gene is protective against late-onset Alzheimer's disease

Yosuke Wakutani [a,1], Hisanori Kowa [a,1], Masayoshi Kusumi [a], Kazuhiro Nakaso [a], Ken-ichi Yasui [a], Kenji Isoe-Wada [a], Hidetaka Yano [a], Katsuya Urakami [b], Takao Takeshima [a], Kenji Nakashima [a,*]

[a] *Department of Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, 36-1 Nishimachi, Yonago 683-8504, Japan*
[b] *Department of Biological Regulation, School of Health Science, Faculty of Medicine, Tottori University, 86 Nishimachi, Yonago 683-8503, Japan*

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Abstract

Epidemiological studies have shown that elevated plasma homocysteine (Hcy) levels play an important role in the pathogenesis of Alzheimer's disease (AD). In spite of the evidence that a C677T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene elevates plasma Hcy levels, the impact of the C677T polymorphism on the development of AD is controversial. Here, we performed a genetic case-control study in a Japanese population to investigate whether three polymorphisms of the MTHFR gene, C677T (Ala222Val), A1298C (Glu429Ala), and A1793G (Arg594Gln), are associated with the development of late-onset AD (LOAD). In our study, the MTHFR gene had four major regional haplotypes: Haplotype A (677C-1298A-1793G), Haplotype B (677T-1298A-1793G), Haplotype C (677C-1298C-1793G), and Haplotype D (677C-1298C-1793A). The frequency of Haplotype C in LOAD was significantly lower than that in control group. Furthermore, the benefit conferred by the presence of at least one Haplotype C was stronger in LOAD patients who lacked the ApoE ε4 allele (OR = 0.293; 95% CI = 0.115–0.744; $P = 0.010$). The results indicate that Haplotype C of the MTHFR gene is protective against the development of LOAD.

Keywords: MTHFR gene; Haplotype; C677T; A1298C; A1793G; Association; Protective; Alzheimer

1. Introduction

Alzheimer's disease (AD) is one of the major neurodegenerative diseases in elderly population. Recent epidemiological studies have demonstrated that elevated levels of plasma homocysteine (Hcy) may play an important role in the pathogenesis of AD [3,19]. However, the detailed pathomechanism by which elevated plasma Hcy levels finally lead to AD is still uncertain.

Methylenetetrahydrofolate reductase (MTHFR; MIM *607093, EC 1.5.1.20) is one of the central enzymes for DNA synthesis and Hcy metabolism. In spite of the evidence that the C677T TT genotype (or the T allele) increases Hcy levels (particular in folate deficiency state) [8], its impact on the development of AD has been controversial [2,13,15]. No association studies of a second common polymorphism, A1298C (Glu429Ala) with AD were conducted.

Recent full genome scan studies have demonstrated the multiple candidate loci for late-onset AD (LOAD) including chromosome 1 [9,12,14]. The MTHFR gene locates chromosome 1p36.3 [5] and is predicted to be susceptible to LOAD. Our study was designed to evaluate whether the polymorphisms or the combined haplotypes of the MTHFR gene have an impact on the development of LOAD. We examined three MTHFR gene polymorphisms, C677T (Ala222Val), A1298C (Glu429Ala), and A1793G (Arg594Gln) (NCBI dbSNP cluster ID: rs2066462, rs1801131, and rs2274976, respectively), and the regional haplotypes derived from the three polymorphisms in a LOAD group and a control group.

2. Materials and methods

The study enrolled subjects from a western region of Japan. The diagnosis of AD was determined clinically according to the DSM-III-R and NINCDS-ADRDA criteria. Age at onset was defined by the appearance of the first clinical symptoms.

* Corresponding author. Tel.: +81-859-34-8032; fax: +81-859-34-8083.
E-mail address: kenaka@grape.med.tottori-u.ac.jp (K. Nakashima).
[1] These authors contributed equally to this work.

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Table 1
Primer sequence, annealing temperature, PCR product size, restriction endonuclease, and digestion pattern for the MTHFR C677T, A1298C, and A1793G polymorphisms

Position	Exon	Sequence 5′–3′	Annealing temperature (°C)	Size (bp)	Nuclease	Digestion pattern (bp)
C677T	Ex4-F	AGTCCCTGTGGTCTCTTCATC	58	387	Gain of *Hin*fI site	152/235
	Ex4-R	GGAGATCTGGGAAGAACTCAG				
A1298C	Ex7-MF	AGATGTGGGGGGAGGAGCTGACCAGTGC*AG	62	175	Gain of *Fnu*4HI site	28/147
	Ex7-MR	GCCCCA**CAGCCTGGCCTA**CAGCT				
A1793G	Ex11-F	TTGGAGAGCCCTGTTAATCTTG	58	390	Loss of *Bsr*BI site	125/264
	Ex11-R	AGAGACACGAAGGAGAGTGGAG				

* Mismatch position (A–C) for creation of an artificial *Fnu*4HI site.
** Mismatch positions (both of C–A) for abolishment of *Fnu*4HI sites.

After informed consent was given, blood leukocyte DNA was isolated using the standard phenol–chloroform method. We generated the three primer sets according to a genomic sequence in the NCBI database (GenBank accession No: AF257484). Table 1 depicts each of the primer sequences, the annealing temperature, the PCR product length, the restriction enzymes for PCR-restriction fragment length polymorphism (RFLP) analysis, and the digestion pattern. "Hot start" PCR reactions for each primer set were performed using a supplied kit (TaKaRa, Japan) in standard PCR reaction conditions. The amplified PCR products for each primer set were subjected to RFLP analysis by agarose electrophoresis. ApoE genotypes were determined by a standard *Hha*I RFLP analysis [6,7].

The Hardy–Weinberg equilibrium was confirmed for both populations. The allele frequencies and the number of positive individuals having at least one polymorphism or haplotype were compared using the chi-square test. The possible effects of each haplotype on LOAD were determined using logistic regression analysis with age, sex, and the presence of at least one ApoE ε4 allele as covariates. We also stratified the data sets into two types of subjects: subjects possessing at least one ApoE ε4 allele and subjects without an ApoE ε4 allele. Odds ratios (ORs) were calculated with exact 95% confidence intervals (CIs). *P* values and significance considerations are two-sided and subject to a significance level of 5%. Analyses were performed with the SPSS statistical package (Japanese version 11).

3. Results

A total of 307 Japanese subjects from a western region of Japan were enrolled, including 129 individuals with a clinical diagnosis of LOAD and 178 cognitively normal controls (CTLs). The mean age at onset of patients with LOAD was 74.4 years (65–85, S.D. = 5.4), and 76.0% were women. The corresponding values of the CTL group were 74.4 (65–85, S.D. = 4.5), and 73.0% were women.

Concurrences of the genotype distributions of C677T with A1298C indicated complete linkage disequilibrium between the polymorphisms at nucleotide 677 and nucleotide 1298, as previously reported [10,22]. Correspondingly, we did not detect the combinations of 677CT and 1793AA, 677TT and 1793AA, or 677TT and 1793AG, indicating complete linkage disequilibrium between all of these polymorphisms. Nor did we detect combinations of 1298AA and 1793AG, 1298AA and 1793AA, or 1298AC and 1793AA, indicating that the 1793A polymorphism is a concomitant allele to the 1298A allele. Thus, the 1298A and 1793A allele are always in the *cis* configuration. From these results, we divided the regional haplotypes of the MTHFR gene into four haplotypes, which we named Haplotype A (wild type 677C-1298A-1793G), Haplotype B (677T-1298A-1793G), Haplotype C (677C-1298C-1793G), and Haplotype D (677C-1298C-1793A) (Fig. 1); this results in eight diplotypes (genotypes). Further data analysis was performed using these haplotypes. The diplotype distributions between AD and CTL did not statistically differ because of a small number of cases for each diplotype (Table 2). Logistic regression analysis adjusted by age, gender, and the presence of at least one ApoE ε4 allele demonstrated a significant protective effect of Haplotype C (OR = 0.426; 95% CI = 0.220–0.827; $P = 0.012$, presence of at least one Haplotype C versus absence of Haplotype C), whereas the presence

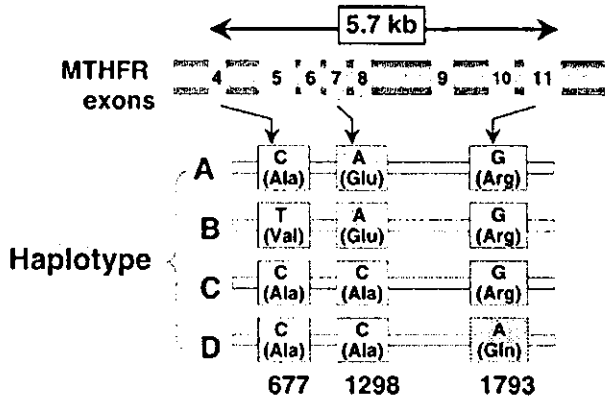

Fig. 1. A schematic representation of the estimated regional haplotypes of the MTHFR gene.

Table 2
Diplotype distribution and haplotype frequency for exon 4 to exon 11 of the MTHFR gene

	Diplotype distribution (%)									
	AA	AB	AC	AD	BB	BC	BD	CC	CD	DD
AD	25 (19.4)	49 (38.0)	5 (3.9)	9 (7.0)	17 (13.2)	6 (4.7)	10 (7.8)	3 (2.3)	3 (2.3)	2 (1.6)
CTL	31 (17.4)	49 (27.5)	17 (9.6)	10 (5.6)	25 (14.0)	18 (10.1)	18 (10.1)	3 (1.7)	6 (3.4)	1 (0.6)

Table 3
Logistic regression analysis of at least one of each haplotype adjusted by age, gender, and at last one ApoE ε4 allele

	d.f.	Odds ratio	95.0% CI	*P* value
Haplotype B	1	0.921	0.553–1.532	0.750
Haplotype C	1	0.426	0.220–0.827	0.012*
Haplotype D	1	1.023	0.549–1.906	0.943

* Statistically significant.

Table 4
Logistic regression analysis classified by ApoE ε4 status of at least of one each haplotype adjusted by age and gender

	d.f.	Odds ratio	95.0% CI	*P* value
Haplotype B				
ε4 (−)	1	1.180	0.638–2.183	0.598
ε4 (+)	1	0.502	0.176–1.427	0.196
Haplotype C				
ε4 (−)	1	0.293	0.115–0.744	0.010*
ε4 (+)	1	0.592	0.198–1.771	0.349
Haplotype D				
ε4 (−)	1	0.885	0.416–1.884	0.752
ε4 (+)	1	0.977	0.299–3.190	0.969

* Statistically significant.

Haplotype B and Haplotype D conferred no significant advantage (OR = 0.921; 95% CI = 0.553–1.532; $P = 0.750$, presence of at least one Haplotype B versus absence of Haplotype B, and OR = 1.023; 95% CI = 0.549–1.906; $P = 0.943$, presence of at least one Haplotype D versus absence of Haplotype D) (Table 3). Expectedly, the estimated risk of AD in the presence of ApoE ε4 was highly significant (OR = 5.318; 95% CI = 3.153–8.972; $P < 0.001$, presence of at least one ApoE ε4 allele versus absence of ApoE ε4 allele). Subsequent analysis stratified according to the ApoE ε4 status revealed that the protective effect of Haplotype C of the MTHFR gene against LOAD was more prominent in the group lacking the ApoE ε4 allele (OR = 0.293; 95% CI = 0.115–0.744; $P = 0.010$, presence of at least one Haplotype C versus absence of Haplotype C) (Table 4).

4. Discussion

In the present study, we used regional haplotypes to assess the association between polymorphisms (C677T, A1298C, and A1793G) in the gene encoding the MTHFR enzyme and susceptibility to LOAD. We evaluated complete linkage disequilibrium between the 677C and 1298A alleles in our samples as previous reported results [10,22]. Importantly, we found that the 1793G allele always appeared in *trans* with 677T and in *cis* with 1298C. Therefore, we allocated the regional haplotypes of the MTHFR gene into four haplotypes (Fig. 1).

We found that presence of at least one Haplotype C was significantly protective against LOAD (Table 3). Furthermore, the protective effect of Haplotype C was more predominant in ApoE ε4-negative individuals as compared to ApoE ε4-positive individuals (Table 4).

The marked impact of the MTHFR 677T allele in reducing enzyme activity and thermolability and increasing plasma Hcy levels has been well characterized. Although the influence of the 1298C allele (equivalent to Haplotype C) on MTHFR enzyme thermolability has been shown to be negligible, the effects on reducing the enzyme activity in vitro are controversial in independent studies [23,24]. The effects of the 1298C-1793A haplotype (Haplotype D) on the metabolism or activity of MTHFR enzyme have also yet to be discovered.

Biological studies have demonstrated the allele-specific antioxidant potential of ApoE (ε2 > ε3 > ε4) [4,11]. In addition, recent studies using ApoE-deficient transgenic mice have proposed that folate, a major regulatory factor for MTHFR activity and levels of the non-protein Hcy, quenches oxidative damage [20,21]. Therefore, MTHFR dismetabolism and/or inappropriate folate intake may impair the capacity against oxidative stress. We found the enhanced protective effect of Haplotype C of the MTHFR gene in ApoE ε4 lacking individuals, indicating that Haplotype C may have synergic beneficial effects with the negativity of ApoE ε4 against oxidative stress.

In conclusion, we propose that the extended genotypes and haplotypes of the MTHFR gene have important implications for the pathogenesis of LOAD. A negative correlation between the 1298C allele and plasma Hcy levels and an inverse association between Vitamin B-12 status and plasma Hcy have been reported for the 1298C allele [1,10]. In addition, it has been reported that allele or haplotype construction of the MTHFR gene differs according to ethnicity [18] and the polymorphisms of the MTHFR gene have implications for human fertility and dietary folate consumption [16,17]. However, because Haplotype C of the MTHFR gene is a genetic factor that provides protection

against the development of LOAD in the Japanese population, we suggest further analysis of samples from different ethnicities or communities to avoid type 1 (false positive) error. Studies to clarify the effects of the estimated haplotypes on MTHFR metabolism will also be required.

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ACTA NEUROLOGICA SCANDINAVICA

Serotonin 2C receptor gene Cys23Ser polymorphism: a candidate genetic risk factor of migraine with aura in Japanese population

Kusumi M, Araki H, Ijiri T, Kowa H, Adachi Y, Takeshima T, Sakai F, Nakashima K. Serotonin 2C receptor gene Cys23Ser polymorphism: a candidate genetic risk factor of migraine with aura in Japanese population.
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Objectives – The goal of this study is to clarify the association between migraine and Serotonin 2C receptor Cys23Ser polymorphism in Japanese population. *Materials and method* – This study included 37 individuals with migraine with aura (MWA), 80 with migraine without aura, 43 with tension type headache (TH) and 360 with controls. The genotypes of Cys23Ser polymorphism were confirmed by polymerase chain reaction-restriction fragment length polymorphism techniques. *Results* – The Ser allele frequency in control subjects is much less than that in Caucasian population. The Ser allele frequency in patients with MWA was higher than that in control subjects. *Conclusion* – The present study provides that 5HTR2c Cys23Ser polymorphism may be associated with MWA in Japanese population.

M. Kusumi[1], H. Araki[1], T. Ijiri[1], H. Kowa[1], Y. Adachi[1], T. Takeshima[1], F. Sakai[2], K. Nakashima[1]

[1]Department of Neurology, Institute of Neurological Sciences, Tottori University Faculty of Medicine, Yonago, Japan; [2]Department of Medicine, Kitasato University, Sagamihara, Japan

Abbreviations: 5HTR2c, 5-hydroxytriptamine 2c receptors; MWA, migraine with aura; MWOA, migraine without aura; TH, tension type headache.

Key words: Serotonin 2c receptor; migraine; polymorphism; genetic association study

Dr M. Kusumi, Department of Neurology, Institute of Neurological Sciences, Tottori University Faculty of Medicine, Nishi-machi 36-1,Yonago 683-8504, Japan
Tel.: +81 859 34 8032
Fax: +81 859 34 8083
e-mail: mkusumi@grape.med.tottori-u.ac.jp

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Serotonin (5-hydroxytriptamine, 5HT) and the receptors play the important roles in the mechanism of migraine (1). There are many subtypes of 5HT-receptors and 5HT2c-receptor (5HTR2c) is one of 5HT2 families. The antagonists at the receptors such as ergotamine, amitriptyline and methysergyde improve on the symptoms of migraine. In addition, the agonist at 5HTR2c such as m-chlorophenilpiperazine can induce migraine. Therefore it is considered that 5HTR2c receptors play the important roles in the pathogenesis of migraine (2, 3).

Recently, the genetic analysis of headache has evolved to include the identification of genes, a calcium channel gene or 1q21-q23, as one of the single responsible gene of familial hemiplegic migraine (4, 5). There were several positive genetic association studies such as gene polymorphisms of dopamine receptor type 2 (6), methylene tetrahydrofolate reductase (7), angiotensin-converting enzyme (8) or the gene polymorphism of glutathione S-transferase (9).

There is a common polymorphism of the 5HT2c-receptor gene at codon 23 in a Cys23-Ser23 substitution (10). There is a previous report about no association between 5HTR2c and migraine in Danish population (11). The prevalence of migraine is significantly different between Japanese and Caucasians (12–14). Therefore the genetic risk factor is also thought to be different between both them. We investigated whether the 5HTR2c Cys23Ser polymorphism has an association with migraine and tension type headache in Japanese population.

Materials and methods

Subjects

There were 35 individuals of migraine with aura (MWA), 81 of migraine without aura (MWOA),