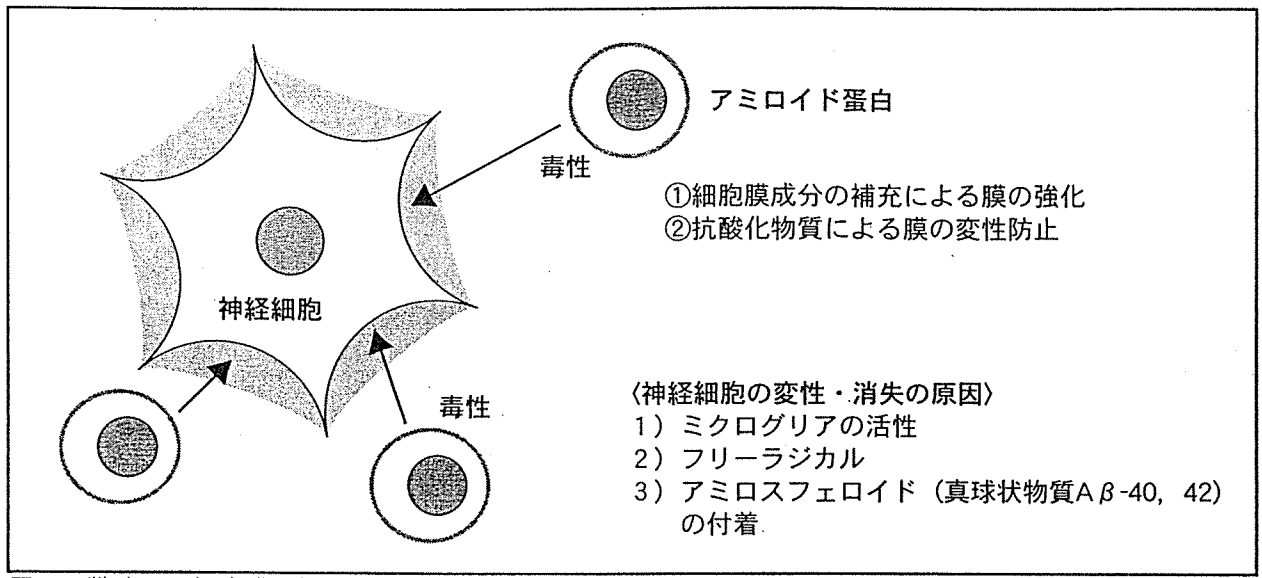

図4　戦略3：細胞膜の補修・強化により，外的影響を軽減する

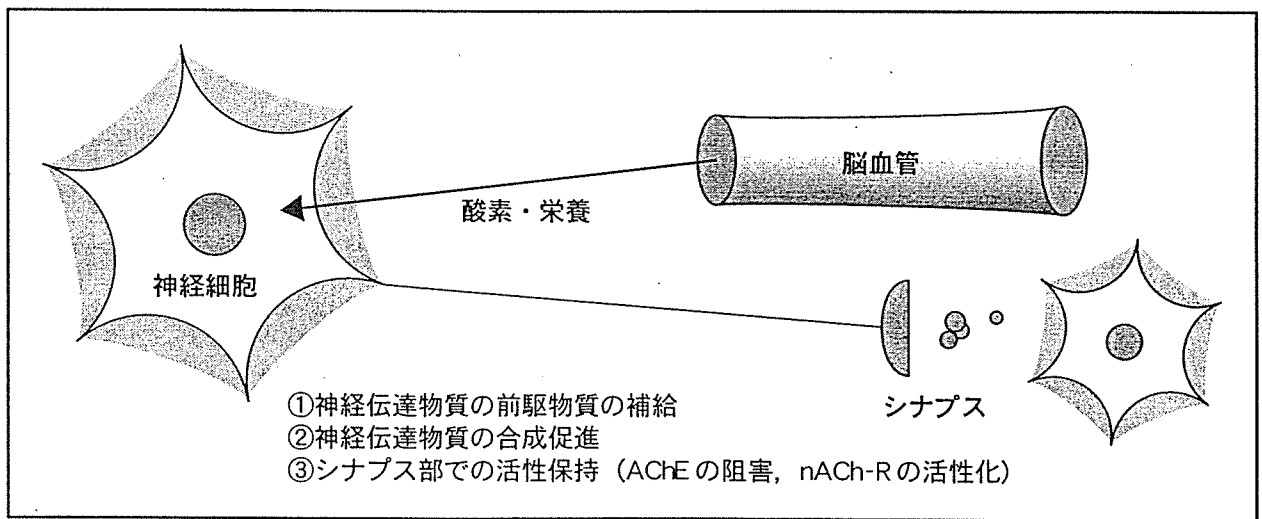

図5　戦略4：神経伝達物質を増加させる

（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。

引用・参考文献
1）秋山治彦：抗炎症薬による治療の試みの現況，老年精神医学雑誌，Vol.14，No.5，P.539～544，2003.
2）Herzig SJ et al. ：Novel dual inhibitors of AChE and MAO derived from hydroxyaminoindan and phenethylamine as potential treatment for Alzheimer's disease. J Med Chem 2000，45 (24)：5260-5279.
3）岩佐弘一他：女性ホルモンによる治療の試みの現況，老年精神医学雑誌，Vol.14，No.5，P.545～555，2003.
4）瓦林毅，東海林幹夫：Aβ生成の抑制を目的とした治療法開発の現況，老年精神医学雑誌，Vol.14，No.5，P.562～569，2003.
5）道川誠，柳澤勝彦：Aβとコレステロール代謝からみたスタチン系薬物による治療，老年精神医学雑誌，Vol.14，No.5，P.531～538，2003.
6）宮永和夫：アルツハイマー病の薬物療法，痴呆介護，Vol.2，No.3，P.50～57，2001.
7）宮永和夫：血管性痴呆の薬物療法，痴呆介護，Vol.2，No.4，P.46～50，2001.
8）中村重信：アセチルコリン系賦活療法のその後の進歩，老年精神医学雑誌，Vol.14，No.5，P.523～530，2003.
9）Rahman S, et al. ：Drug interventions in dementia：a multidisciplinary approach. In Early-Onset Dementia (ed Hodges JR)，pp422-448. New York，Oxford University Press，2001.
10）浅井昌弘編，田川皓一著：薬物療法，臨床精神医学講座，S2巻，記憶の臨床，P.415～424，中山書店，1999.
11）田中稔久，武田雅俊：Aβワクチンによる治療の現況，老年精神医学雑誌，Vol.14，No.5，P.556～561，2003.
12）浦上克哉，谷口美也子：アルツハイマー病に対するその他の治療の試みの現況，老年精神医学雑誌，Vol.14，No.5，P.567～569，2003.

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

宮永　和夫

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．

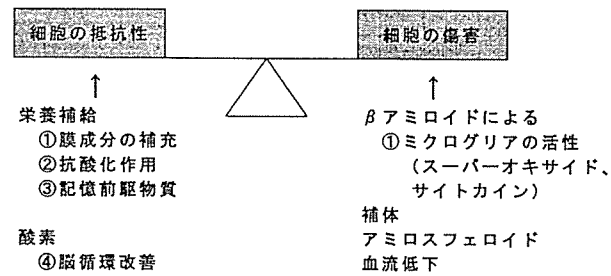

図1　補助療法の考え方

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，

K. Miyanaga：群馬県こころの健康センター

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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Gunma Mental Health Center

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A novel presenilin 1 mutation (Y154N) in a patient with early onset Alzheimer's disease with spastic paraparesis

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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

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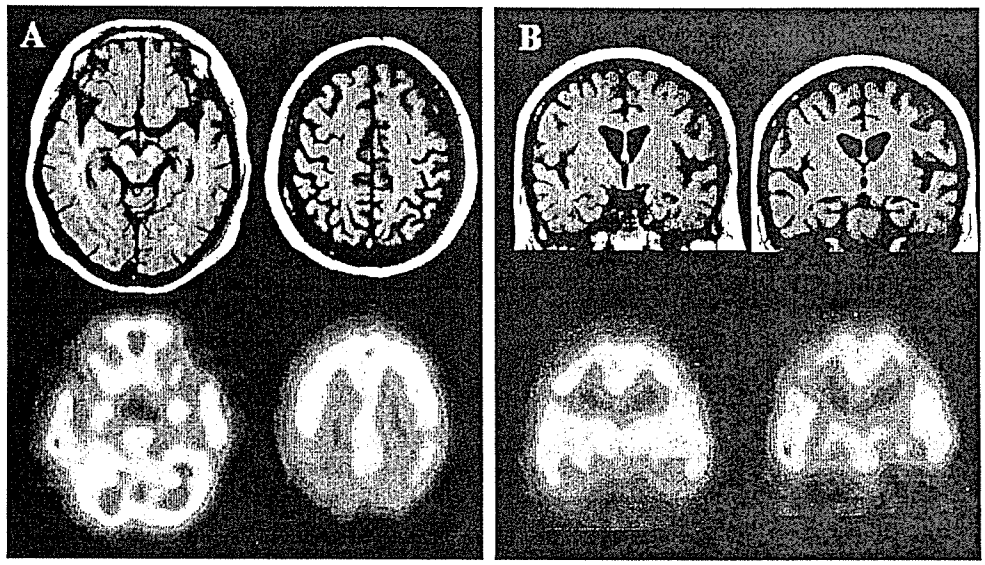

Fig. 1. MRI (T1 weighted image) and SPECT (^{99m}Tc-ECD) findings. (A) Axial T1 weighted images show apparent occipito-parietal atrophy and mild atrophy in internal portion of temporal lobes. Axial SPECT images show marked hypoperfusion in bilateral occipito-parietal areas and internal portion of temporal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

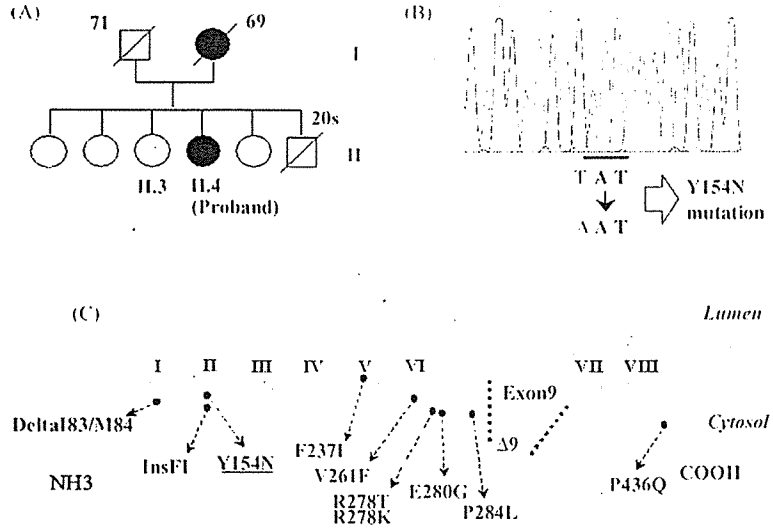

Fig. 2. (A) Pedigree of the family with Y154N *PSEN1* mutation. (B) Sequence diagram of *PSEN1* exon 5. Underlining indicates the heterozygous mutation from the T to A transition, resulting in a change from tyrosine (T) (TAT) to asparagine (N) (AAT) at codon 154 of *PSEN1*. (C) Schematic representation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

References

[1] A. Assini, L. Terreni, R. Borghi, L. Giliberto, A. Piccini, D. Loqui, S. Fogliarino, G. Forloni, M. Tabaton, Pure spastic paraparesis associated with a novel presenilin 1 R278K mutation, Neurology 60 (2003) 150.

[2] D.R. Borchelt, G. Thinakaran, C.B. Eckman, M.K. Lee, F. Davenport, T. Ratovitsky, C.M. Prada, G. Kim, S. Seekins, D. Yager, H.H. Slunt, R. Wang, M. Seeger, A.I. Levey, S.E. Gandy, N.G. Copeland, N.A. Jenkins, D.L. Price, S.G. Younkin, S.S. Sisodia, Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1–42/1–40 ratio in vitro and in vivo, Neuron 17 (1996) 1005–1013.

[3] W.S. Brooks, J.B. Kwok, J.J. Kril, G.A. Broe, P.C. Blumbergs, A.E. Tannenberg, P.J. Lamont, P. Hedges, P.R. Schofield, Alzheimer's disease with spastic paraparesis and 'cotton wool' plaques: two pedigrees with PS-1 exon 9 deletions, Brain 126 (2003) 783–791.

[4] R. Crook, A. Verkkoniemi, J. Perez-Tur, N. Mehta, M. Baker, H. Houlden, M. Farrer, M. Hutton, S. Lincoln, J. Hardy, K. Gwinn, M. Somer, A. Paetau, H. Kalimo, R. Ylikoski, M. Poyhonen, S. Kucera, M. Haltia, A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1, Nat. Med. 4 (1998) 452–455.

[5] H. Houlden, M. Baker, E. McGowan, P. Lewis, M. Hutton, R. Crook, N.W. Wood, S. Kumar-Singh, J. Geddes, M. Swash, F. Scaravilli, J.L. Holton, T. Lashley, T. Tomita, T. Hashimoto, A. Verkkoniemi, H. Kalimo, M. Somer, A. Paetau, J.J. Martin, C. Van Broeckhoven,

T. Golde, J. Hardy, M. Haltia, T. Revesz, Variant Alzheimer's disease with spastic paraparesis and cotton wool plaques is caused by PS-1 mutations that lead to exceptionally high amyloid-β concentrations, Ann. Neurol. 48 (2000) 806–808.

[6] F. Hulstaert, K. Blennow, A. Ivanoiu, H.C. Schoonderwaldt, M. Riemenschneider, P.P. De Deyn, C. Bancher, P. Cras, J. Wiltfang, P.D. Mehta, K. Iqbal, H. Pottel, E. Vanmechelen, H. Vanderstichele, Improved discrimination of AD patients using [beta]-amyloid$_{(1-42)}$ and tau levels in CSF, Neurology 52 (1999) 1555–1562.

[7] J.C. Janssen, J.A. Beck, T.A. Campbell, A. Dickinson, N.C. Fox, R.J. Harvey, H. Houlden, M.N. Rossor, J. Collinge, Early onset familial Alzheimer's disease: mutation frequency in 31 families, Neurology 60 (2003) 235–239.

[8] J.B.J. Kwok, K. Taddei, M. Hallupp, C. Fisher, W.S. Brooks, G.A. Broe, J. Hardy, M.J. Fulham, G.A. Nicholson, R. Stell, P.H. St. George Hyslop, P.E. Fraser, B. Kakulas, R. Clarnette, N. Relkin, S.E. Gandy, P.R. Schofield, R.N. Martins, Two novel (M233T and [rho]278T) presenilin-1 mutations in early-onset Alzheimer's disease pedigrees and preliminary evidence for association of presenilin-1 mutations with a novel phenotype, Neuroreport 8 (1997) 1537–1542.

[9] A. Maddalena, A. Papassotiropoulos, B. Muller-Tillmanns, H.H. Jung, T. Hegi, R.M. Nitsch, C. Hock, Biochemical diagnosis of Alzheimer disease by measuring the cerebrospinal fluid ratio of phosphorylated tau protein to β-amyloid peptide$_{42}$, Arch. Neurol. 60 (2003) 1202–1206.

[10] O. Murayama, T. Tomita, N. Nihonmatsu, M. Murayama, X. Sun, T. Honda, T. Iwatsubo, A. Takashima, Enhancement of amyloid β 42 secretion by 28 different presenilin 1 mutations of familial Alzheimer's disease, Neurosci. Lett. 265 (1999) 61–63.

[11] S. O'Riordan, P. McMonagle, J.C. Janssen, N.C. Fox, M. Farrell, J. Collinge, M.N. Rossor, M. Hutchinson, Presenilin-1 mutation (E280G), spastic paraparesis, and cranial MRI white-matter abnormalities, Neurology 59 (2002) 1108–1110.

[12] E.A. Rogaeva, K.C. Fafel, Y.Q. Song, H. Medeiros, C. Sato, Y. Liang, E. Richard, E.I. Rogaev, P. Frommelt, A.D. Sadovnick, W. Meschino, K. Rockwood, M.A. Boss, R. Mayeux, P. St. George-Hyslop, Screening for PS1 mutations in a referral-based series of AD cases: 21 novel mutations, Neurology 57 (2001) 621–625.

[13] M.J. Smith, J.B.J. Kwok, C.A. McLean, J.J. Kril, G.A. Broe, G.A. Nicholson, R. Cappai, M. Hallupp, R.G.H. Cotton, C.L. Masters, P.R. Shofield, W.S. Brooks, Variable phenotype of Alzheimer's disease with spastic paraparesis, Ann. Neurol. 49 (2001) 125–129.

[14] N. Sodeyama, T. Iwata, K. Ishikawa, H. Mizusawa, M. Yamada, Y. Itoh, E. Otomo, M. Matsushita, Y. Komatsuzaki, Very early onset Alzheimer's disease with spastic paraparesis associated with a novel presenilin 1 mutation (Phe237Ile), J. Neurol. Neurosurg. Psychiatry 71 (2001) 556–557.

[15] H. Steiner, T. Revesz, M. Neumann, H. Romig, M.G. Grim, B. Pesold, H.A. Kretzschmar, J. Hardy, J.L. Holton, R. Baumeister, H. Houlden, C. Haass, A pathogenic presenilin-1 deletion causes abberrant Aβ42 production in the absence of congophilic amyloid plaques, J. Biol. Chem. 276 (2001) 7233–7239.

[16] S. Sudoh, Y. Kawamura, S. Sato, R. Wang, T.C. Saido, F. Oyama, Y. Sakaki, H. Komano, K. Yanagisawa, *Presenilin 1* mutations linked to familial Alzheimer's disease increase the intracellular levels of amyloid β-protein 1–42 and its N-terminally truncated variant(s) which are generated at distinct sites, J. Neurochem. 71 (1998) 1535–1543.

[17] T. Tabira, H. Chui de, H. Nakayama, S. Kuroda, M. Shibuya, Alzheimer's disease with spastic paresis and cotton wool type plaques, J. Neurosci. Res. 70 (2002) 367–372.

[18] Y. Wakutani, Y. Adachi, K. Wada-Isoe, K. Yamagata, K. Urakami, K. Nakashima, Genetic analysis of familial Alzheimer's disease in a Japanese population, in: M. Takeda, T. Tanaka, R. Cacabelos (Eds.), Molecular Neurobiology of Alzheimer Disease and Related Disorders, KARGER, Basel, 2004, pp. 157–163.

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

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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 db-SNP 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.

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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

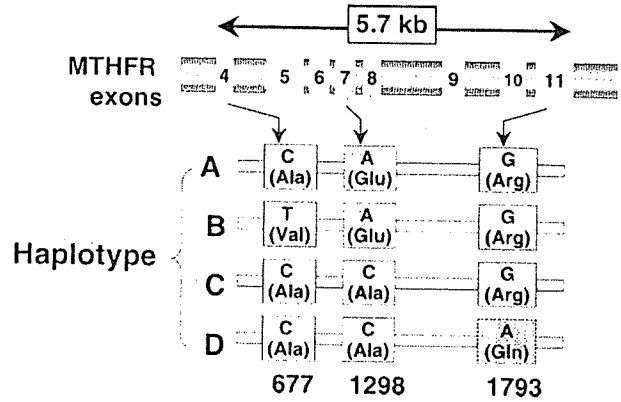

Fig. 1. A schematic representation of the estimated regional haplotypes of the MTHFR gene.

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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References

[1] Bailey LB, Duhaney RL, Maneval DR, Kauwell GP, Quinlivan EP, Davis SR, et al. Vitamin B-12 status is inversely associated with plasma homocysteine in young women with C677T and/or A1298C methylenetetrahydrofolate reductase polymorphisms. J Nutr 2002;132:1872–8.

[2] Brunelli T, Bagnoli S, Giusti B, Nacmias B, Pepe G, Sorbi S, et al. The C677T methylenetetrahydrofolate reductase mutation is not associated with Alzheimer's disease. Neurosci Lett 2001;315:103–5.

[3] Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449–55.

[4] Colton CA, Brown CM, Cook D, Needham LK, Xu Q, Czapiga M, et al. APOE and the regulation of microglial nitric oxide production: a link between genetic risk and oxidative stress. Neurobiol Aging 2002;23:777–85.

[5] Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome 1998;9:652–6.

[6] Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha*I. J Lipid Res 1990;31:545–8.

[7] Ji Y, Urakami K, Adachi Y, Maeda M, Isoe K, Nakashima K. Apolipoprotein E polymorphism in patients with Alzheimer's disease, vascular dementia and ischemic cerebrovascular disease. Dement Geriatr Cogn Disord 1998;9:243–5.

[8] Kang SS, Passen EL, Ruggie N, Wong PW, Sora H. Thermolabile defect of methylenetetrahydrofolate reductase in coronary artery disease. Circulation 1993;88:1463–9.

[9] Kehoe P, Wavrant-De Vrieze F, Crook R, Wu WS, Holmans P, Fenton I, et al. A full genome scan for late onset Alzheimer's disease. Hum Mol Genet 1999;8:237–45.

[10] Meisel C, Cascorbi I, Gerloff T, Stangl V, Laule M, Muller JM, et al. Identification of six methylenetetrahydrofolate reductase (MTHFR) genotypes resulting from common polymorphisms: impact on plasma homocysteine levels and development of coronary artery disease. Atherosclerosis 2001;154:651–8.

[11] Miyata M, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat Genet 1996;14:55–61.

[12] Myers A, Wavrant De-Vrieze F, Holmans P, Hamshere M, Crook R, Compton D, et al. Full genome screen for Alzheimer disease: stage II analysis. Am J Med Genet 2002;114:235–44.

[13] Nishiyama M, Kato Y, Hashimoto M, Yukawa S, Omori K. Apolipoprotein E methylenetetrahydrofolate reductase (MTHFR) mutation and the risk of senile dementia—an epidemiological study using the polymerase chain reaction (PCR) method. J Epidemiol 2000;10:163–72.

[14] Pericak-Vance MA, Bass MP, Yamaoka LH, Gaskell PC, Scott WK, Terwedow HA, et al. Complete genomic screen in late-onset familial Alzheimer disease. Evidence for a new locus on chromosome 12. JAMA 1997;278:1237–41.

[15] Prince JA, Feuk L, Sawyer SL, Gottfries J, Ricksten A, Nagga K, et al. Lack of replication of association findings in complex disease: an analysis of 15 polymorphisms in prior candidate genes for sporadic Alzheimer's disease. Eur J Hum Genet 2001;9:437–44.

[16] Rady PL, Szucs S, Grady J, Hudnall SD, Kellner LH, Nitowsky H, et al. Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) in ethnic populations in Texas; a report of a novel MTHFR polymorphic site, G1793A. Am J Med Genet 2002;107:162–8.

[17] Reyes-Engel A, Munoz E, Gaitan MJ, Fabre E, Gallo M, Dieguez JL, et al. Implications on human fertility of the 677C → T and 1298A → C polymorphisms of the MTHFR gene: consequences of a possible genetic selection. Mol Hum Reprod 2002;8:952–7.

[18] Rosenberg N, Murata M, Ikeda Y, Opare-Sem O, Zivelin A, Geffen E, et al. The frequent 5,10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in whites, Japanese, and Africans. Am J Hum Genet 2002;70:758–62.

[19] Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New Engl J Med 2002;346:476–83.

[20] Shea TB, Rogers E. Folate quenches oxidative damage in brains of apolipoprotein E-deficient mice: augmentation by vitamin E. Brain Res Mol Brain Res 2002;108(1/2):1–6.

[21] Shea TB, Rogers E, Ashline D, Ortiz D, Sheu MS. Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue. Free Radic Biol Med 2002;33:1115–20.

[22] Stegmann K, Ziegler A, Ngo ET, Kohlschmidt N, Schroter B, Ermert A, et al. Linkage disequilibrium of MTHFR genotypes 677C/T-1298A/C in the German population and association studies in probands with neural tube defects (NTD). Am J Med Genet 1999;87:23–9.

[23] Weisberg IS, Jacques PF, Selhub J, Bostom AG, Chen Z, Curtis Ellison R, et al. The 1298A → C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis 2001;156:409–15.

[24] Yamada K, Chen Z, Rozen R, Matthew RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci USA 2001;98:14853–8.

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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.

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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

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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),

Table 1 The number and mean age of subjects

	No. of cases	Male:female	Age in years (average ± SD)
MWA	35	10:25	34.1 ± 12.9
MWOA	81	15:66	37.9 ± 15.3
MWA + MWOA	116	25:91	36.8 ± 14.6
TH	42	09:33	48.4 ± 15.4
CTL	279	121:158	47.8 ± 10.9

42 of tension type headache (TH), according to the diagnostic criteria of International Headache Society (15) and 279 of healthy controls (CTL) (Table 1).

Genotyping

Genomic DNA was extracted from venous blood samples from total individuals. The genotypes of the Cys23Ser were confirmed by polymerase chain reaction (PCR)amplification of DNA and digestion with *Hinf*1 according to the method described previously (10). Briefly, the PCR primers were REPA1 (5′-TTG GCC TAT TGG TTT GGG AAT-3) and ARTIFACT2 (5′-GTC TGG GAA TTT GAA GCG TCC AC-3′). The PCR reaction mix of 25 μl contained: 100 ng of DNA, 0.25 μM each primer, 250 μM dNTP, 10 mM Tris HCL (pH8.3), 50 mM KCL, 1.5 mM $MgCl_2$ and one unit of Taq DNA Polymerase (Takara, Otsu, Japan). After initial 5 min denaturation at 95°C, samples were then amplified for eight cycles of 95°C for 1 min, 48°C for 1 min, 72°C for 1 min and eight cycles of 95°C for 1 min, 52°C for 1 min, 72°C for 1 min and 19 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 1 min. Ten units of *Hinf*1 (Nippon gene, Tokyo, Japan) and recommended buffer solution were then added and incubated for 10 h at 37°C. Restriction fragments were visualized by 12% polyacrylamide gel electrophoresis and stained with ethidiumbromide. Genotyping was carried out blind to clinical details.

Statistics

The genotype frequency with 5HTR2c-Cys23Ser polymorphism was compared between each case and controls by using a chi-squared test. The odds ratios (ORs) associated with the genotype and its 95% confidence interval (CI) were determined for adjusting age and gender differences by using unconditional multivariate logistic regression. Statistical analyses were performed using SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The distribution of 5HTR2c Ser or Ser/Ser genotype in MWA (8.3%) is significantly higher than that in CTL (1.1%). The Ser23 allele is more frequent in MWA (6.6%) than that in CTL (1.4%) ($P < 0.05$, OR = 5.04, 95%CI 1.62 ± 1.30) (Table 2). In males group, the 5HT2c Ser23 allele frequencies are 40% in MWA, 0% in MWOA, 12% in MWA and MWOA, 0% in TH and 3% in CTL.

Discussion

We investigated the 5HTR2c Cys23Ser polymorphism in Japanese patients with migraine and tension type headache. The Ser allele frequency in patients with MWA was higher than that in control subjects. However, there were no differences among MWOA, TH and control. Burnet and colleagues described that there were no differences with the 5HTR2c polymorphism and migraine in Danish population (11). Compared with this previous report, the Ser allele frequency of control group in Japanese population is much fewer than that in Caucasian population. It is considered that the ethnical difference exists between Caucasian and Japanese population with 5HTR2c polymorphism and this polymorphism is associated with MWA in Japanese population.

Nyholt et al. reported the linkage studies of two families with migraine and excluded the locus on chromosome Xq24-28 as a familial migraine (16). The 5HTR2c gene is also located on Xq24. The allele frequency of Ser in males is larger than that in females in MWA. This suggests that the hypersensitivity of males in migraine may arise with 5HTR2c Cys23Ser polymorphism.

Table 2 The 5HT2c genotype and odds ratios for headache sufferer

				Not adjusted			Adjusted for age and gender		
	N	Ser− [*n* (%)]	Ser+ [*n* (%)]	OR	95% CI	*P*-trend	OR	95% CI	*P*-trend
CTL	360	353 (98.16)	7 (1.94)	1			1		
MWA	37	33 (89.19)	4 (10.81)	**6.12**	**1.70–21.97**	**<0.01**	**5.12**	**1.13–23.17**	**0.03**
MWOA	80	78 (97.50)	2 (2.50)	1.30	0.26–6.34	0.75	1.63	0.32–8.31	0.56
TH	43	41 (95.35)	2 (4.65)	2.46	0.49–12.24	0.27	2.85	0.56–14.65	0.20

Significant differences of OR among the subjects are bold typed.

There are several reports about the localization of 5HTR2c mRNA and protein on the occipital cortex (17–19). The activation of 5HTR2c inhibits the visual cortex long-term potentiation, that observed in the visual cortex may underline an experience dependent modulation of visual functions (20). 5HTR2c mRNA is neither expressed in human endothelial cells nor in smooth muscle cells (21). 5HTR2c in migraine is not associated with vasoactivity but neural function such as the cortical spreading depression.

Cys-Ser substitution may affect the receptor folding caused by the ability of disulfide bonds in the presence of cysteine (11). However the functional differences between 5HT2c-Cys and 5HT2c-Ser allele are unknown. Further functional studies between Cys-Ser substitution and MWA are required for this association study.

In conclusion, the present study provides that the Ser allele frequency in Japanese population is much less than that in Caucasian and that 5HTR2c Cys23Ser polymorphism may be associated with MWA in Japanese population. However the number of subjects of this study is very small. Further investigation is needed to reveal the association between 5HTR2c Cys23Ser polymorphism and MA in larger subjects.

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References

1. Ferrari MD, Saxena PR. On serotonin and migraine: a clinical and pharmacological review. Cephalalgia 1993; 13:151–65.
2. Fozard JR, Gray JA. 5-HT1C receptor activation: a key step in the initiation of migraine? Trends Pharmacol Sci 1989;10:307–9.
3. Panconesi A, Sicuteri R. Headache induced by serotonergic agonists – a key to the interpretation of migraine pathogenesis? Cephalalgia 1996;17:3–14.
4. Ophoff RA, Terwindt GM, Vergouwe MN et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca^{2+} channel gene CACNL1A4. Cell 1996;87:543–52.
5. Ferrari MD. Migraine. Lancet 1998; 351: 1043–51.
6. Peroutka SJ, Wilhoit T, Jones K. Clinical susceptibility to migraine with aura is modified by dopamine D_2 receptor (DRD2) NcoI alleles. Neurology 1997;49:201–6.
7. Kowa H, Yasui K, Takeshima T, Urakami K, Sakai F, Nakashima K. The homozygous C677T mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for migraine. Am J Med Genet 2000;96:762–4.
8. Paterna S, di Pasquale P, D'Angelo A et al. Angiotensin-converting enzyme gene deletion polymorphism determines an increase in frequency of migraine attacks in patients suffering from migraine without aura. Eur Neurol 2000; 43:133–6.
9. Kusumi M, Adachi Y, Ishizaki K et al. Glutathione S-transferase polymorphisms: susceptibility to migraine without aura. Eur Neurol 2003;49:218–22.
10. Lappalainen J, Zhang L, Dean M et al. Identification, expression, and pharmacology of a Cys23-Ser23 substitution in the human 5HT2c receptor gene (HTR2C). Genomics 1995;27:274–9.
11. Burnet PW, Harrison PJ, Goodwin GM et al. Allelic variation in the serotonin 5HT2c receptor gene and migraine. Neuroreport 1997;8:2651–3.
12. Sakai F, Igarashi H. Prevalence of migraine in Japan: a nationwide survey. Cephalalgia 1997;17:15–22.
13. Launer LJ, Terwindt GM, Ferrari MD. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology 1999;53:537–42.
14. Russell MB, Rasmussen BK, Thorvaldsen P, Olesen J. Prevalence and sex-ratio of the subtypes of migraine. Int J Epidemiol 1995;24:612–8.
15. Headache Classification Committee of the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. Cephalalgia 1988;8(Supp. 7):1–96.
16. Nyholt DR, Curtain RP, Griths LR. Familial typical migraine: significant linkage and localization of a gene to Xq24–28. Hum Genet 2000;107:18–23.
17. Pasqualetti M, Ori M, Castagna M, Marazziti D, Cassano GB, Nardi I. Distribution and cellular localization of the serotonin type 2C receptor messenger RNA in human brain. Neuroscience 1999;92:601–11.
18. Dyck RH, Cynader MS. Autoradiographic localization of serotonin receptor subtypes in cat visual cortex: transient regional, laminar, and columnar distributions during postnatal development. J Neurosci 1993;13:4316–38.
19. Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KC. Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. Neuropharmacology 2000;39: 123–32.
20. Wang Y, Gu Q, Cynader MS. Blockade of serotonin-2C receptors by mesulergine reduces ocular dominance plasticity in kitten visual cortex. Exp Brain Res 1997; 114:321–8.
21. Ullmer C, Schmuck K, Kalkman HO, Lubbert H. Expression of serotonin receptor mRNAs in blood vessels. FEBS Lett 1995;370:215–21.

特集 痴呆高齢者の転倒・転落事故は防げるか？

施設における痴呆高齢者の転倒・転落事故の発生状況と対策

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介護事故の大部分は転倒・転落事故という事実

高齢者の医療現場や高齢者施設で生じている事故は多種多様である．医療施設で起こり得るさまざまな医療過誤のほかに，介護行為に伴う事故や日常生活行動のなかでの大小の事故が毎日のように起こっている．いわゆる「ヒヤリ，ハット」する事故だ．しかし，実際に高齢者施設の現場で事故調査をしてみると，その実態にいくつかの特徴が浮かび上がってくる．

図1は，高齢者医療を専門とする浴風会病院全体で最近1年間に起きた医療事故のまとめである．医療過誤に相当する注射間違え，誤投薬などは報告された事故全体の42%であるのに対して，転倒・転落事故が58%と最も多いことがわかる．これを痴呆高齢者の多い介護病棟に限ってみると，事故の大半は転倒・転落事故に集中していることがわかる(図2)．リスクマネジメントの観点からすると，痴呆高齢者の事故対策の力点は，まず転倒・転落対策に置かねばならないということになろう．

高齢者施設でみられる転倒・転落事故に関しては，医療施設でみられる医療過誤とはまた違った側面があることもみてとらねばならない．

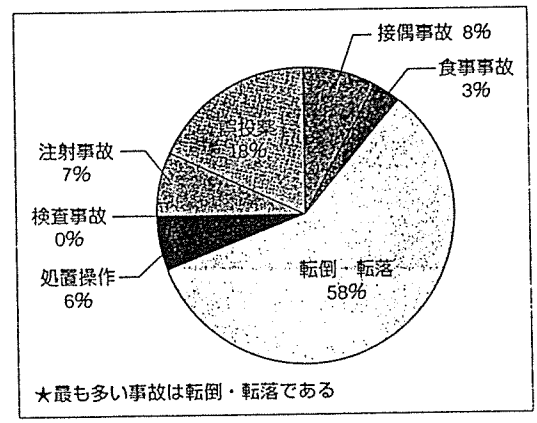

図1　老人病院(300床)における医療事故の内訳

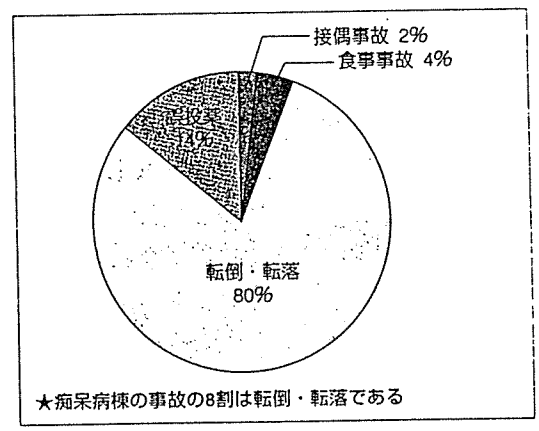

図2　老人病院(300床)の痴呆病棟における医療事故の内訳

すなわち，医療過誤は医療の提供者側に過失があり，医療を受ける高齢者にはなんら瑕疵も責任もない場合がほとんどである．一方，施設入

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所中の高齢者にみられる転倒・転落事故は日常生活行動のなかで生じる．精神障害や身体障害を持った高齢者が，自力歩行中や何らかの日常動作の際に転び，転落するのである．生活環境に潜むリスクと事故の被害者側にも内在するリスクを抱えていることになる．その意味で，高齢者施設での転倒・転落事故防止においては，医療者側・介護サービス提供者側のリスクマネージとともに，入所高齢者のリスクマネージも同時に進めていかねばならないという難しい課題を負うことになる．痴呆高齢者における転倒・転落事故防止の対策は，一般的な医療事故防止のマニュアルの形式では役立たない．本稿では，痴呆高齢者の転倒・転落事故の実態をふまえて，その対策の道筋について検討してみたい．

転倒・転落事故調査の進め方

筆者らは転倒・転落事故防止のための対策を最優先に進める目的で，痴呆高齢者の介護施設や療養病棟で日常的に生じている転倒・転落事故について，多数例の調査から事故の特徴，事故発生に関連する諸要因を明らかにしようと試みた．これまで報告されている転倒・転落事故調査の多くは，1つの施設内で発生した事故の集計と分析結果のまとめである．そのために施設特有の事情や入所者の特性が事故の様態を左右している可能性があった．

筆者らの調査では，より普遍的な事故要因を検出したいということから，特別養護老人ホーム，老人保健施設，介護療養型老人病院計10施設を横断的に調べるという方法をとった．このために表に示したような内容を盛り込んだ各施設共通の転倒・転落事故報告調査用紙を用いた．調査は各施設が使っている事故調査用紙やヒヤリハット報告書を従来どおり使用し，上がってきた事故報告書から共通調査用紙に転記する，あるいは不足した情報は補充して記載するというやり方で行なわれた．調査の対象となった人数は1493人である．期間は2002(平成14)年11月から2003(平成15)年1月の3か月間とした．

事故率からみた事故の施設間格差

調査期間中の転倒・転落件数は10施設で計398件だった．それを施設別にみた図を図3に掲げた．1か月間の事故率は，その施設の入所者の数を母数として1か月間の事故件数の割合を算出したものである．約4%から18%までであり，施設間で明らかに差があった．月ごとの変動を考慮してもその差は明瞭だった．調査対象の10施設(特養7施設，病院2施設，老健1施

表　事故の調査項目(各施設共通)

1）各施設の転倒事故総数
2）転倒者の特性(痴呆診断の有無，併存疾患，多重転倒，服薬，移動方法，日常生活援助状況，痴呆の重症度)
3）転倒時の障害部位と重症度
4）転倒場所(廊下，自室，トイレなど)
5）転倒発生(発見)時刻
6）発見の状況(転倒を目撃，転倒後を発見)
7）発生の状況(車椅子からの移動，歩行中など)
8）すでに行なわれていた予防策の状況(ケアプラン作成，予防具など)
9）事故後の処置

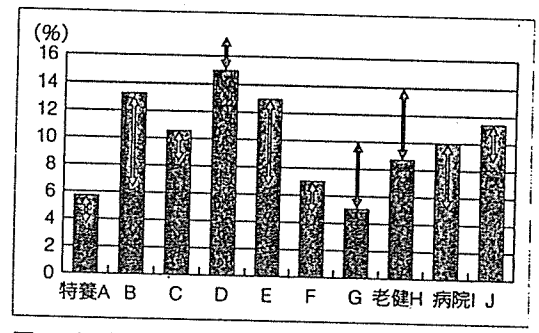

図3　調査参加10施設の事故率(12月分集計)
矢印は3か月間の変動

設)はそれぞれ，開設の時期，施設規模と入居空間の構造，入所者の特性などで違いがある．こうした施設間の何らかの要因が事故の多寡に関係しているであろうことは容易に想像がつくが，今回の調査からはそれが何であるかは読み取れなかった．

ただ，調査参加施設のなかで時期をずらして行なったJ病院での病棟別転倒・転落事故の事故率の多寡は，入所者の特性が大きく左右していることを示唆する興味深い結果となっている(図4)．J病院は病棟機能と入院患者の特性により病棟が区分けされている．すなわち，1か月間で最も事故率の高かった病棟は回復期リハビリテーション病棟で12%であった．ついで医療保険適用の痴呆病棟と主に短期間で入退院をしている医療保険適用の療養病棟が8%と目立つ．これに対して，2つの特殊疾患難病指定病棟(注：おもに重度のパーキンソン病患者や脊髄小脳変性症などの難病高齢者をみている)の事故率は1%に満たない．療養病棟でも入院期間が1年以上という長期入院者の多い介護保険適用の病棟では，転倒・転落事故は2.3%からせいぜい3.8%どまりという結果だった．転倒・転落事故の多寡は，入所者のADLや身体，精神状態が関与していることをうかがわせる結果だった．

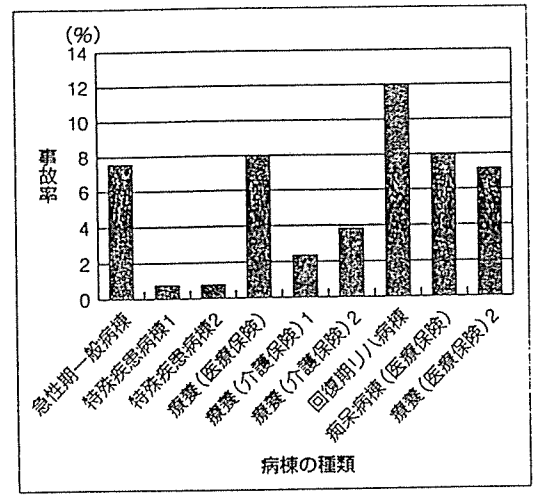

図4　調査対象J病院の病棟別転倒・転落事故発生率(1か月平均)

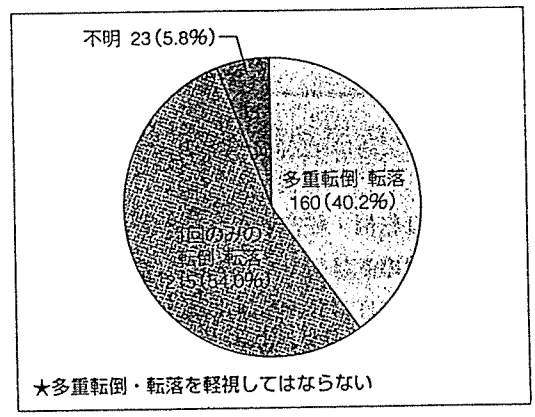

図5　転倒・転落件数(3か月間・総数398件)の内訳

転倒・転落常習者の存在と事故の発生状況

転倒・転落事故件数の内訳をみると，398件のうち160件は同じ人が何度も転倒・転落を繰り返している．多重転倒者とも呼べる一群で，事故件数の40.2%を占める(図5)．多重転倒・転落事故件数が全体の事故件数を押し上げている実態がわかる．わずか3か月間の集計でこの割合だから，はじめて転んだ人もそのうちにまた転ぶ可能性は高い．一度は転んだことがあるという既往は，転倒・転落事故のハイリスク群であることをこの数字は物語っている．その意味で新規に入院あるいは入所してくる痴呆高齢者にこれまでに転倒・転落の既往がないかどうかを把握しておくことは，事故を予測するうえで重要なことである．

報告された事故のうち，転倒・転落を目撃されているものは15%程度で80%以上は事故が起こったあとの状態を発見されたものであった．転倒・転落事故の8割以上が職員のみていないところで起こっているということだ(図6)．詳細をみると，音がしたのでかけつけてみると床に倒れていた，尻餅をついていた，しゃがみこ

んでいた，などの状態で発見されている．痴呆高齢者の場合，事故者本人が事故の起きた状況を説明できない場合がほとんどで，なぜ事故に至ったかを究明する手がかりが乏しく，原因を推測することが難しいという問題がつきまとう．

目撃された事故は歩行中の事故が最も多く，ついで，ベッドや車いす・いすからの移動中に起きているものが多かった．これらのうち目撃されたケースでは，介護者が「危ないな」と思いながらもみていたら転んだという状況が多く，見守りや介護者のとっさの機転で転倒を食い止められたという事例はわずかである．

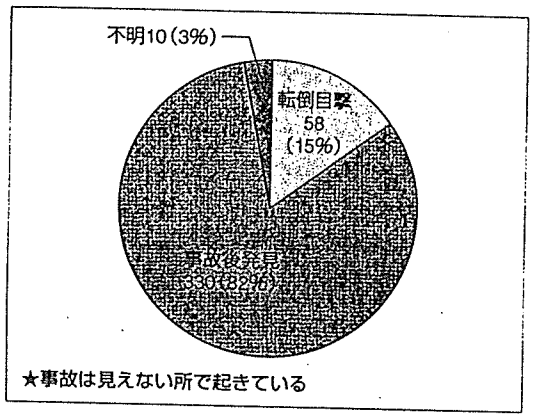

図6　事故発生時の状況

事故の発生時刻は施設によってまちまちで，共通する特定の傾向は見出せなかった．それぞれの施設の日課や人員配置，ケアの動きに左右されている可能性があり，各施設で個別に要因を追及すべき課題かもしれない．

それとは対照的に，事故発生場所をみるとどこの施設でも共通して利用者自室での事故が最も多く(図7)，事故全体の56.8%を占めていた．ついでデイルームであり，この2つで発生場所の4分の3以上を占めている．利用者自室での事故のほとんどはベッド周りでの転倒・転落である．このことは，ベッド周りの事故を集中的に減らすことができれば，転倒・転落事故を半減させることが可能なことを意味する．

転倒による傷害の特徴

転倒・転落によって実際に負傷した人の割合は，擦過傷程度の軽いものも含めると129件，

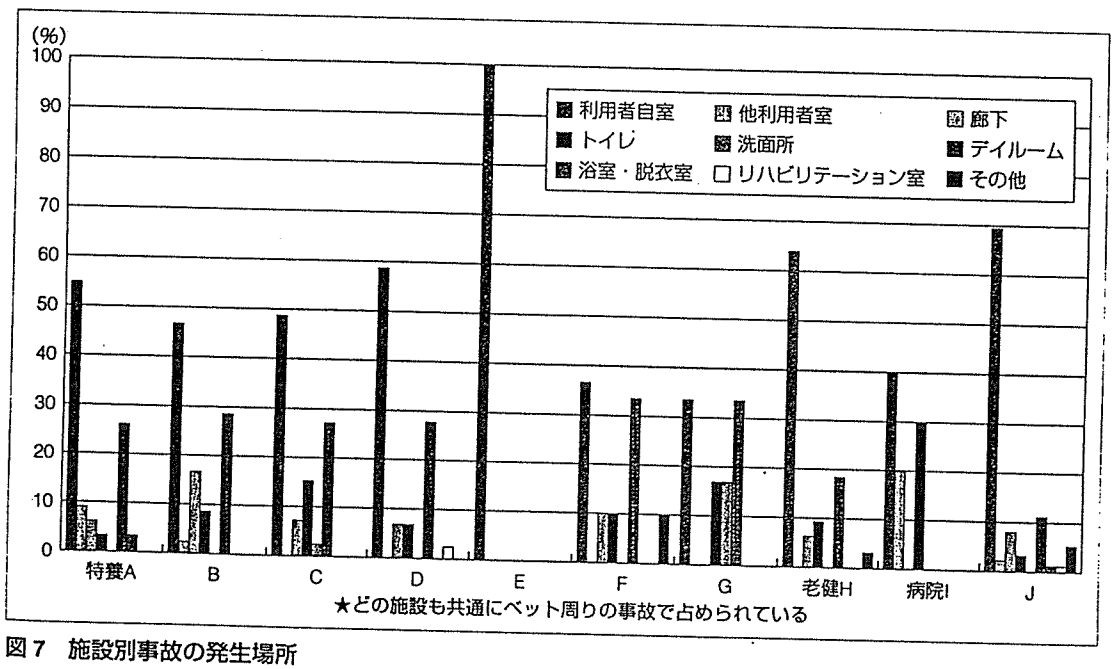

図7　施設別事故の発生場所