(97,324,281 bp), rs496641 (97,347,389 bp) and rs749049 (97,366,086 bp), were genotyped, but no association was detected for our exploratory sample set. Thus, none of the SNPs in the above-described candidate genes was significantly associated with LOAD with the *APOE-ε3*3* genotype in the Japanese population examined here. However, it is still possible that this finding is due to the ethnic difference.

The novel association locus found in this study contains five genes, *ENTPD7*, *COX15*, *CUTC*, *ABCC2* and *DNMBP* (Fig. 1). SNP rs911541 occurs in intron 3 of *ENTPD7*, also known as *LAPL1*, which encodes apylase with an intracellular catalytic domain (35). ENTPD7 exhibits 71% similarity to LALP70 (36), a lysosomal/autophagolysosomal membrane protein, suggesting that ENTPD7 is also located in a lysosomal/autophagic compartment, but its physiological function is unclear. The SNP rs3740066 (ATC → ATT, Ile1324Ile) is in exon 28 of *ABCC2*, which is a member of the ATP-binding cassette transporter superfamily that transports various molecules across extra- and intracellular membranes. *ABCC2* is expressed predominantly in the liver but was undetectable in human brain on immunocytochemistry (37). The SNP rs11190302 is located in the intergenic region between *ABCC2* and *DNMBP*. Three SNPs, rs11190305, C_11214959_10 and rs3740058, are present at a high density in the 3′ region of *DNMBP*: rs11190305 causes a non-synonymous exchange (TGT → TGG, Cys1413Trp). Taking these data together, we focussed on *DNMBP*, although *ENTPD7* and *ABCC2* may also influence the pathogenesis of AD. This is the first description of a significant association between a *DNMBP* polymorphism and the risk of LOAD with the *APOE-ε3*3* genotype or lacking the *APOE-ε4* allele.

DNMBP binds to dynamin selectively through four N-terminal Src homology-3 (SH3) domains. GTPase dynamin is an essential component for vesicle formation in receptor-mediated endocytosis, synaptic vesicle recycling, caveolae internalization and possibly vesicle trafficking in and out of the Golgi complex (38,39). DNMBP also binds to several actin regulatory proteins including direct binding partners, i.e. N-WASP (neuronal Wiskott–Aldrich syndrome protein) and Ena (Enabled)/VASP (vasodilator-stimulated phosphoprotein), via two SH3 domains at the C-terminus. The DH domain in the middle of DNMBP is involved in the activation of Cdc42. The molecule promotes F-actin nucleation and/or recruitment within cells (28). N-WASP, which acts as a key molecule for fillopodium formation through Cdc42 activation (40), is increased in the AD brain and may be involved in aberrant neuronal sprouting (41). DNMBP is co-localized with synapse-enriched proteins, amphiphysin-1 and dynamin-1 (28). The BAR domain of amphiphysin-1 is required for the triggering of dynamin GTPase activity and fission of the endocytic pit (42). Amphiphysin-knockout mice have defects in synaptic vesicle recycling and major learning deficits (43). The polymorphism of T/G in rs11190305 corresponds to an amino acid change of cysteine to tryptophan (Cys1413Trp). The Cys1413Trp mutation occurs at a position between the two SH3 domains at the C-terminus of DNMBP. It is possible that this amino acid change leads to a conformational alteration, and subsequently affects the interactions with binding partners, although further experiments are necessary to confirm this. To find a new variation, we sequenced all exons, exon–intron boundaries and an about 200 bp 5′ upstream region of *DNMBP* in 92 LOAD patients, but found no polymorphism in these sequenced regions (data not shown).

Thus far, there has been no information about the expression level of *DNMBP* in the AD brain. In this study, we demonstrated a significant reduction of *DNMBP* transcripts in the cerebral cortex of autopsy-confirmed AD patients using quantitative real-time RT–PCR (Fig. 2A–C). Although risk alleles of three SNPs, rs11190305 (allele G), C_11214959_10 (allele C) and rs3740058 (allele A), obviously decreased the *DNMBP* expression level in a dominant model, there were no significant interactions between *DNMBP* gene expression, genotype variation and diagnosis (Fig. 2D—F). *APOE-ε4*-carrying AD subjects also tended to exhibit decreased levels of *DNMBP* expression (data not shown). An alternative and attractive interpretation would be that reduced *DNMBP* expression is caused not only by the SNPs identified here but also by altered expression of other genes. Thus, it is possible that several pathways lead to the reduced *DNMBP* expression that acts as a risk factor for LOAD.

Recently, Yao *et al.* (29) described the reduced expression of a group of genes including those of dynamin I and amphiphysin-1, all of which are involved in synaptic vesicle trafficking, in the frontal cortex of AD brains. AD begins with subtle alterations of hippocampal synaptic function prior to Aβ deposition followed by frank neuronal degeneration (31). Dynamin I is an important mediator of clathrin-dependent endocytosis and synaptic vesicle recycling. These facts may be consistent with our observation that DNMBP is a genetic risk factor for LOAD. The decrease in the level of *DNMBP* mRNA might be related to the pathogenesis traced to synapses in the brain of LOAD patients and is probably caused by multiple environmental and genetic factors and their combination.

MATERIALS AND METHODS

Subjects

To search for susceptibility genes for LOAD by means of genome-wide screening, the Japanese Genetic Study Consortium for AD (JGSCAD) was organized in 2000, and blood samples were collected. The subject information is summarized in Table 1. Expectedly, the *APOE-ε4* allele was found to be a highly significant risk factor for LOAD (OR 4.96, 95% CIs 4.22–5.84; p of chi-square test <0.0001). All individuals included in this study were Japanese. Probable AD cases met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders. Controls who had no signs of dementia and lived in an unassisted manner in the local community were also recruited. AAO is here defined as the age at which the family and/or individuals first noted cognitive problems while working or in daily activities. For evaluation of cognitive impairment, the mini-mental state examination (MMSE) was used.

A total of 41 post mortem brains from LOAD patients and control subjects were obtained from the Bioresource Center, Brain Research Institute, Niigata University (Supplementary Material, Table S3). The distribution of the *APOE-ε4* allele was significantly different between LOAD and control

subjects, as expected (OR 5.70, 95% CIs 1.25–25.93, Fisher's exact $P = 0.0367$). Autopsies were performed after a mean post mortem interval of 4.2 h (range 1–22 h). LOAD patients with dementia were neuropathologically characterized based on consensus criteria that included physiologically age-matched densities of senile plaques and neurofibrillary tangles as distinguished from other neurodegenerative disorders, i.e. dementia with Lewy body disease, frontotemporal dementia and Parkinson's disease according to published criteria. Autopsied controls were confirmed to have no diagnosable brain disease.

The present study was approved by the Institutional Review Board of the University of Niigata and by all participating institutes. Informed consent was obtained from all controls and appropriate proxies for patients, and all samples were anonymously analyzed for genotyping.

Marker selection and genotyping

SNP information was obtained from four open databases: NCBI dbSNP (Build 125, http://www.ncbi.nlm.nih.gov/SNP/), International HapMap Project (Rel#20/phaseII on NCBI Build 35 assembly and dbSNP Build 125, http://www.hapmap.org/index.html), Ensemble Human (Version 37 on NCBI Build 35, http://www.ensembl.org/Homo_sapiens/) and Celera myScience (Version R27 g on NCBI Build 35, http://myscience.appliedbiosystems.com/). We selected 1322 SNPs in the region from 60 to 107 Mb on chromosome 10q (Supplementary Material, Tables S1 and S2); mean inter-marker distance ±SD, 34.9 ± 87.4 kb; 95% CIs, 30.2–39.6 kb. To examine the genotyping quality of the 1322 SNPs, the HWE test was performed with 337 control subjects (carrying *APOE-ε3*3* in the exploratory sample set, as shown in Table 1). These SNPs consisted of 29 missense mutations, 27 silent mutations, six SNPs in the 5′-UTR, 29 SNPs in the 3′-UTR, 921 SNPs in introns, 282 SNPs in intergenic regions and 28 SNPs in four loci shared by two different genes (*CTNNA3/LRRTM3*, *CDH23/C10orf54*, *C10orf55/PLAU* and *PGAM1/EXOSC1*). We used 1206 SNPs that were shown to be actually polymorphic in the Japanese population and showed $P > 0.05$ in the HWE test; mean inter-marker distance ± SD, 38.3 ± 93.3 kb; 95% CIs, 33.0–43.6 kb.

Genomic DNA was extracted from peripheral blood with a QIAamp® DNA Blood Maxi Kit (Qiagen, Duseldorf, Germany) and examined fluorometrically with a PicoGreen® dsDNA quantification kit (Molecular Probes, CA, USA). SNP genotyping for individual samples was performed with an ABI PRISM® 7900HT instrument using TaqMan technology, and TaqMan SNP Genotyping Assays were purchased from Applied Biosystems (CA, USA).

Sequencing

APOE genotyping of all samples was performed by direct cycle sequencing with an ABI 3100 sequencer and a BigDye® Terminator v3.1 kit (Applied Biosystems) using the following primers: C19APOE001-F (sense 5′-GCCTACAAATCGGAACTGGA-3′) and C19APOE001-R (antisense 5′-ACCTGCTCCTTCACCTCGT-3′). All exons and their exon–intron boundaries and the 5′ upstream region of *DNMBP* were sequenced with 20 primer pairs (Supplementary Material, Table S5).

Case–control study

To identify candidate loci in a broad region of chromosome 10q (60–107 Mb on NCBI build 35), two independent sample sets comprising case–control subjects with *APOE-ε3*3* were constructed (Table 1). The exploratory sample set comprising 363 LOAD patients and 337 control subjects was genotyped, and SNPs showing significant association (allelic $P < 0.01$) were used for further examination using the validation sample set comprising 336 LOAD patients and 372 control subjects. Subsequently, we increased the samples and stratified them as the *APOE-ε4* carrier status: Negative-ε4, *APOE-ε 2*2*, *2*3* and *3*3*; Positive-ε4, *APOE-ε 2*4*, *3*4* and *4*4*; All, all genotypes of *APOE*. The sample numbers for LOAD patients and controls in All, Negative-ε4, ε3*3 and Positive-ε4 were 1526 and 1666, 749 and 1378, 699 and 1243, and 777 and 288, respectively. To examine the genetic association of multiple SNP combinations, case–control haplotype analysis with significant SNPs was performed using the following sample sets: All, Negative–ε4, ε3*3 and Positive-ε4.

Statistical analysis

Using SNPAlyze ver. 3.2.3 software (DYNACOM, Chiba, Japan; http://www.dynacom.co.jp/index.html.en), we performed the HWE test, single SNP case–control analysis, haplotype estimation based on the EM algorithm, case–control haplotype analysis with 10 000 iterated permutations and calculation of LD measures ($|D'|$) to elucidate the LD block structure. The Mantel–Haenszel test was performed using Statcel 2 software (OMS, Tokyo, Japan). Evidence of replication, rather than multiple testing corrections, was used to evaluate the significance of associated SNPs (32–34). We carried out the two-sided Student's *t*-test for comparison of the mean *DNMBP* expression levels between the AD and control brain tissues, using Prism 4.0 b (GraphPad Software, CA, USA). The effects of variation on gene expression were examined using the two-way ANOVA (Prism 4.0 b) with the genotype and case–controls as independent variables.

Quantitative real-time PCR

Frozen materials (Supplementary Material, Table S3) were prepared on dry ice blocks from 1 cm thick slices of the cerebral cortices. RNA was extracted directly from the frozen preparations with an ISOGEN solution (Nippongene, Tokyo, Japan). The first strand cDNA was synthesized from total RNA (2.0 μg) with SuperScriptsIII™ (Invitrogen, CA, USA) and random hexamers in a total volume of 20 μl according to manufacturer's protocol. The synthesized cDNA solution was diluted 1:20 and then used for quantitative real-time PCR amplification with TaqMan Gene Expression Assays (Applied Biosystems) and an ABI PRISM 7900HT instrument in a total volume of 10 μl according to manufacturer's instructions. Briefly, 2.5 μl of a diluted cDNA solution (corresponding to 12.5 ng of total RNA) was mixed with 5.0 μl of 2 × TaqMan Universal PCR Master Mix (Applied

Biosystems), 0.5 μl of 20 × TaqMan Gene Expression Assay and 2.0 μl of distilled water on a 384-well optical PCR plate. The PCR conditions were: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All measurements were performed in quadruplicate. The threshold cycle was determined in the linear range and relative gene expression was calculated as the cycle difference. Each measurement of *DNMBP* mRNA (Celera assay ID, Hs00324375_m1) was normalized to the expression levels of *GUSB* (Celera assay ID, Hs99999908_m1) and 18S rRNA (Celera assay ID, Hs99999901_s1), which were selected from among the 11 housekeeping genes [acidic ribosomal protein, beta-actin, cyclophilin, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerokinase, beta-2-microglobulin, GUSB, hypoxanthine ribosyl transferase, transcription factor IID (TATA binding protein), transferring receptor genes and 18S rRNA] on a TaqMan Human Endogenous Control Plate (Applied Biosystems) as internal standards.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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Conflict of Interest statement. None declared.

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APPENDIX

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特集

遺伝環境相互作用

認知症と遺伝環境相互作用

浦上克哉*

KEY WORDS

・アポリポ蛋白E（ApoE）
・栄養
・運動
・モデル動物
・双生児研究

SUMMARY

アルツハイマー病（AD）や血管性認知症をはじめとする認知症は，高齢化社会になるに伴いその頻度が増加してきており，その発症には遺伝因子と環境要因の相互作用が重要であると推定されている．しかし，疫学的に得られる危険因子・防御因子と遺伝学的に得られる危険因子・防御因子は相互に研究対象となってきたものの，統合的な研究はまだ少数にとどまっている．本稿では認知症，特にADにおける遺伝環境相互作用の理解を進めるうえで重要と考えられる知見について概観する．

はじめに

アルツハイマー病（Alzheimer disease：AD）や血管性認知症をはじめとする認知症は，高齢化社会になるに伴いその頻度が増加してきており，その発症には遺伝因子と環境要因の相互作用が重要であると推定されている．一方，ADの分子遺伝学的研究の飛躍的な進歩によって，AD発症の分子メカニズムが明確になりつつある．特にアミロイドβ蛋白（Aβ）を中心としたアミロイドカスケードに焦点が当てられ，Aβの生合成，分解，重合・線維形成，神経活動，細胞機能への影響，およびそれにかかわる分子の研究が重点的・多面的に研究されている（図1）．それらの分子のなかには，食事，精神・身体活動，ライフスタイル，ストレスなどの環境因子の影響を受ける可能性があるものが数多くあると想定されており，危険因子・防御因子としておもに疫学的手法を用いて解析されている．しかし，環境因子が遺伝子発現や機能にどのような影響を与えることがADの発症につながるのかは，いまだ明確になってはいない．

本稿ではそれらに焦点を当てた，疫学研究，遺伝学的研究・遺伝疫学的調査およびモデル動物を用いた研究を紹介する．

1．遺伝子―アポリポ蛋白E遺伝子（*ApoE*）

*ApoE*遺伝子多型は，ADの発症に最も強力に影響を与える遺伝子多型として非常に重要である[1]．ApoEε4アレルを一つもつと約2～4倍，2つもつと10～30倍のAD発症への危険度をもつといわれている．ApoEε4アレルの遺伝子頻度は，若干人種によって異なるものの，基本的に人種を超えた遺伝的危険因子と考えられており，今のところ*ApoE*遺伝子多型と同等あるいはそれを凌駕する因子は報告されていない．

しかし，最近の研究では同じ遺伝的背景（人種）をもつ場合でも，環境（広い意味での）が異なることで*ApoE*遺伝子多型のAD発症に対する危険度は異なることが報告されている．たとえばアフリカ人では，ApoEε4のアレル頻度は非常に高いにもかかわらずADの発症率は非常に低いが，米国に在住する同じ遺伝

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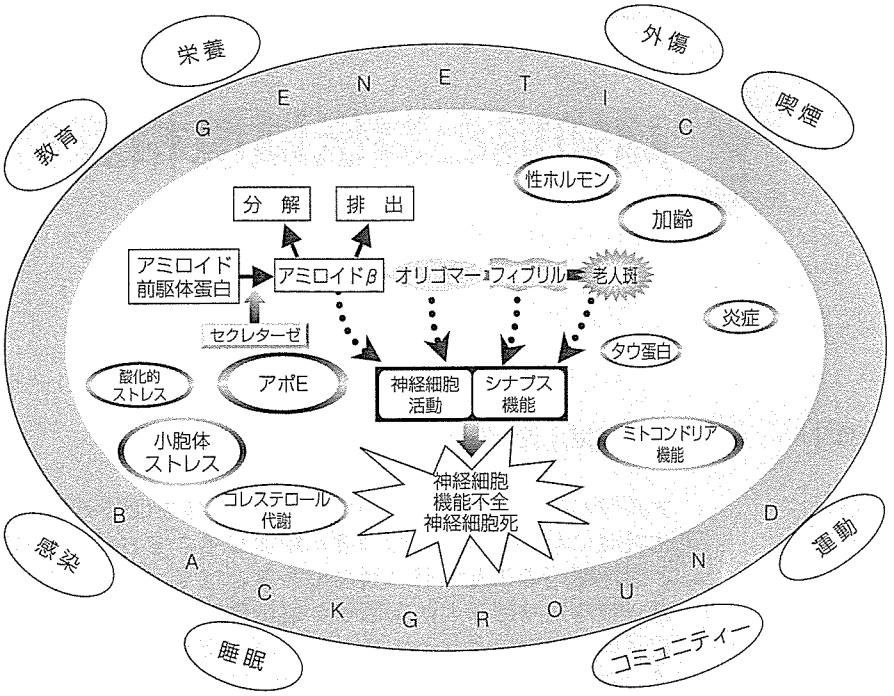

図 1. アルツハイマー病の発症機序

(筆者作成)

的な背景を共有するコミュニティー（アフリカ系アメリカ人）においては，ADの発症率は高く，ApoEε4がADの発症に寄与している[2]．この現象には生活習慣が深く関連していると考えられており，特に後述する食事因子・栄養因子が深く関連していると想定されている．

2．その他の遺伝子・遺伝子多型

ADにおける危険因子・防御因子としての遺伝子多型の研究は，世界中で行われている．たとえば，Alzheimer Research ForumのAlzGene（http://www.alzforum.org/res/com/gen/alzgene/default.asp）には，これまでに報告されているAD関連遺伝子の研究が網羅されており，登録されている遺伝子は250を超えている．これらのADとの関連が検討された遺伝子のなかには，ADと種々の環境要因を研究した疫学研究に端を発したものも数多くみられる．特に，最大の遺伝的危険因子である*ApoE*と同様に，コレステロール代謝に深く関与している遺伝子や酸化的ストレスに関連する遺伝子研究が多い．しかし，前述のとおり*ApoE*のようにはっきりとしたコンセンサスが得られた遺伝的危険因子・防御因子は，明らかとなっていない．もし，環境要因がAD，特に遅発性ADの発症に深く関与していると考えるならば，遺伝子多型の解析のみでは不十分で，前向き研究によって長期間にわたり種々の環境要因をモニターしながら遺伝子解析を行っていく必要があるであろう．

3．食事因子・栄養因子とAD

前述のアフリカ人とアフリカ系アメリカ人の食事因子の比較では，アフリカ人では脂質の摂取は少なく，抗酸化物の摂取は反対に多く，アフリカ系アメリカ人では反対に高脂肪・高カロリーであり，肥満も非常に多い．糖尿病，脳梗塞，高血圧，心筋梗塞などのいわゆる生活習慣病の頻度も非常に高くなるが，ADの発症にもこれらの食生活の違いが寄与していると考えられている[3]．

また，ADの有病率と総エネルギー・総脂質摂取の増加は高い相関をもつといわれており，糖尿病や耐糖能障害もAD発症の独立した危険因子であるとされている．総カロリー摂取の増加や糖尿病・耐糖能障害のAD発症に対する分子メカニズムとしては，インスリン代謝やフリーラジカルの関与が研究されている．高インスリン血症やいわゆるインスリン抵抗性が，Aβの合成・放出・分解の調節機構や海馬神経細胞におけるインスリン受容体

を介したシグナル伝達を障害することが報告され，総カロリー摂取の増加は脳内のフリーラジカルの増加，サイトカインの活性化・炎症反応の促進を引き起こす．

食事因子に焦点を当てたロッテルダムやシカゴなどにおける欧米の疫学研究では，魚の摂取がADの発症に予防的であるとされ，特にエイコサペンタエン酸(eicosapentaenoic acid：EPA）やドコサヘキサエン酸(docosahexaenoic acid：DHA）といったn-3系（ω-3系）の不飽和多価脂肪酸（polyunsaturated fatty acid：PUFA）の摂取が重要であるとされている．ADに対する予防的な分子メカニズムとしては，脳内のアラキドン酸代謝やサイトカイン活性化抑制を介した抗炎症作用や，抗動脈硬化作用などの血管系を介した作用が主として報告されている．

最近では，n-3系PUFAの作用はモデル動物を用いた研究も行われている．AD病理を再現するトランスジェニックマウス（TGM）にDHAを大量に与えて飼育すると，脳内のAβ量が著しく減少し，老人斑の出現も著明に抑制される[4]．また，Hashimotoら[5]の研究によると，脳内にAβペプチドを人工的に投与して認知機能障害を引き起こすモデルラットにおいて，あらかじめDHAを豊富に含む食事を投与しておくと，明らかに認知機能の低下が抑制できることを報告している．脳内のDHAの増加に従い，Aβペプチドの投与によって引き起こされる過酸化脂質や反応性活性酸素の上昇も抑制が可能であったという．

脂質摂取以外の研究では，おもにビタミンC，Eといった抗酸化作用をもつビタミンの摂取や，ビタミンB_{12}，葉酸といったホモシステイン代謝に関連するビタミンの研究も数多く行われており，これらのビタミン群の摂取はADの発症に対して防御的に働いていることが推定されているが，今のところ一定のコンセンサスは得られていない．最近の，葉酸・ホモシステインと認知症の発症に関する縦断的な疫学調査では，高ホモシステイン血症および低葉酸血症はいずれも2倍のAD発症に対する危険率があると報告されている[6]．実験的な研究では，これらのビタミン群はADに関与しているAβ代謝，炎症，酸化的ストレス応答に対して防御的に働くことが，ほぼ確実視されていると思われる．

4．運動・身体活動・社会活動

AD発症に対する運動の予防的効果を検討したいくつかの報告では，歩行や定期的な運動などの身体活動レベルの高い群では，低い群にくらべて加齢に伴う認知機能の低下ならびに認知症発症の危険度が低下することが示されている[7][8]．脳機能画像を用いた検討でも，運動の継続により脳血流および脳代謝の改善が特に前頭葉においてみられることが報告されている．その他の身体活動や社会活動も，活動度の高いほうが認知機能に対して防御的であることが疫学的なコンセンサスとなっていると思われる．運動・身体活動・社会活動の活発度は，精神状態，特にうつ的気分・うつ状態に対しても同時に防御的である．

これらの活動の認知機能に対する防御的な効果は，基礎研究ではおもにノルアドレナリン，セロトニン，ドパミンなど上行性脳幹網様体賦活系にかかわる因子やシナプスの可塑性にかかわる因子がターゲットとなって研究されてきたが，実際の脳内での分子メカニズムやそれらにかかわる遺伝子の相互作用や発現の変化は明らかとなっていなかった．

ごく最近では，運動・身体活動・社会活動の認知機能や認知症発症・進行に対する影響を実験的に証明するため，TGMも用いられている．Lazarovら[9]は，身体活動の活発化が保証されている“environment enrichment”の状態に置かれたTGM（APPswe X PS1 ΔE9）と通常の飼育状態に置かれたTGMとを比較しているが，“environment enrichment”の状態に置かれたTGMは脳内のAβの量および沈着が著しく抑制されることを示した．さらにDNAマイクロアレイ法を用いて，脳内（特に海馬領域）において学習・記憶，血管・神経形成および神経細胞の生存にかかわる遺伝子の発現が実際に変化していることを示した．ヒトにおいても経験的あるいは疫学的に推察されていた身体活動の活発さとAD発症の関係を，はじめて動物モデルにおいて証明した重要な知見である．さらに，ADモデル動物と同様にハンチントン病のモデル動物においても“environment enrichment”の状態が，発症および進行を遅らせることが示されている[10]．

マウスよりも大型の動物においては，Milgramら[11]はイヌを用いて“behavioral enrichment”（トレーニングを十分に行ったりする）とビタミンC，Eおよび果物や野菜からの抽出物を用いた栄養因子の長期にわたる複合的な効果を検討しており，それらはイヌにおける加齢による認知機能の低下を抑制し，さらに，病理学的にはイヌにおいてもみられる脳内のアミロイドの沈着を抑制していた．

これら栄養因子や身体活動の，アミロイド沈着や神経活動低下に対する防御的な効果の分子メカニズムの解明は今後の重要な課題ではあるが，むしろ，それらの効果はAβ代謝やシナプス・神経活動などの特定のステップに効果を発現しているというよりは，各種の代謝過程に対して全般的に良好な“場”を提供しているのかもしれない．

さらに，各種の疫学研究によると，前述の食事・栄養，運動のバランスを改善させることは，心筋梗塞，脳卒中などの血管性，動脈硬化性疾患の予防につながることが示されており，認知症予防の観点からもこれらの環境要因への医学的な介入を積極的に行うことが，今後の医学的な課題になると考えられる．

5．双生児研究

双生児研究は，同一の遺伝子をもつ一卵性双生児と，出生は同時であるものの兄弟と同程度の遺伝子の類似度をもつ二卵性双生児を比較することによって，各種の疾患に対する遺伝と環境の影響度を明らかにしていこうとする方法論であるが，認知機能の加齢による変化や認知症の発症に関しても各種の検討が行われている．スウェーデンで1960年代初頭に設立されたSwedish Twins Registryでの研究では，一卵性双生児のAD発症の一致率は約6割に上り，二卵性双生児では3割であったことから，遺伝的背景はAD発症に大きく関与していると報告されている．また，認知症に対する環境要因の研究も行われており，社会的活動・娯楽活動の参加，定期的な運動，複雑な知的作業を要する就労，教育歴の高さなどが，認知症ならびにAD発症の防御因子になっていると考えられている[12][13]．

おわりに

Nun Study（http://www.mc.uky.edu/nunnet/）は，米国の修道院の修道女の協力を得て，食事，精神・身体活動など種々の環境因子や遺伝的因子の影響を長期間追跡し，最終的な病理学的検討も加えて，認知症を含めた精神・神経疾患について詳細に検討している研究である．そのなかで，剖検脳ではADとしか考えられないのに，臨床的には認知症の兆候を示さなかった症例が報告されている．その修道女は，終生自身の生活を律し，何事にも興味を示し，バランスの良い食事をとり，日課を欠かさなかったという．そのような個別の症例の詳細な検討からも，食事・身体活動を中心とした環境因子の重要性が推察される．

一方，ヒトゲノム計画や国際ハプマップ計画の推進によってヒトの遺伝子多型の詳細が明らかにされ，さらに，一塩基変異多型（single nucleotide polymorphisms：SNP）チップなどの遺伝子多型を迅速・網羅的にタイピングする技術が開発されたことにより，近い将来，種々の疾患につながるヒトのいわゆる“体質”にかかわる“遺伝子”あるいは“遺伝子型”の“組み合わせ”が明らかにされるかもしれない．また，前述のADモデル動物を用いて，DNAマイクロアレイやプロテオミクス，あるいはこれから開発されると期待される細胞機能の変化を網羅的に一望できる手法を使うことにより，環境因子の遺伝子・蛋白質の発現や相互作用に対する総合的・網羅的な解析が行われるものと考えられる．

疫学的には，個人個人から得られる網羅的なデータを集めた前向き調査に高度な統計学的手法を応用することにより，ADの発症にかかわる明確な「遺伝環境相互作用」が解明されるかもしれない．わが国でも，環境因子・遺伝的因子を総合的に観察可能な長期縦断前向き研究がいくつか行われており，認知症に対する遺伝環境相互作用の影響が日本人において明らかにされるものと期待される．

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ORIGINAL ARTICLE

Mild cognitive impairment: biological diagnostic markers for early stages of Alzheimer's disease

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Key words: CSF, dementia with Lewy body, phosphorylated tau, tauopathy, total tau, WGA binding glycoprotein.

ABSTRACT

Background: With the aging population in Japan, the inevitable increase in the prevalence of Alzheimer's disease (AD) and the difficulty in diagnosing the condition. Reliable early diagnostic biomarkers for early stage of AD are needed.

Method: We examined a total 570 CSF samples from a variety of diseases, including AD, other types of dementia and controls to quantitate levels of tau protein phosphorylated at serine 199 by sandwich ELISA. WGA-binding glycoproteins were measured by western blot analysis.

Results: The CSF phosphorylated tau protein levels in the AD group were significantly elevated compared to those in all the other non-AD groups. CSF phosphorylated tau protein levels in the AD progressed from mild cognitive impairment (MCI) group were significantly elevated compared to those in no progression AD from MCI groups and controls. A comparison of the phosphorylated tau protein: WGA-glycoprotein fragment A ratio for AD and other tauopathies showed that this ratio has potential for differentiating between AD and other tauopathies, particularly dementia with Lewy bodies.

Conclusion: Our data showed that measuring cerebrospinal fluid (CSF) levels of phosphorylated tau protein may be useful as an early diagnostic marker in AD and MCI. Furthermore, the measurement of WGA-binding glycoprotein in CSF may provide a useful tool for differentiating AD from dementia of Lewy bodies and other tauopathies.

With the aging population in Japan, the inevitable increase in the prevalence of Alzheimer's disease and the difficulty in diagnosing the condition, reliable biological diagnostic biomarkers for both mild cognitive impairment (MCI) and Alzheimer's disease are needed.

PHOSPHORYLATED TAU IN ALZHEIMER'S DISEASE

The measurement of cerebrospinal fluid (CSF) levels of tau, a microtubule-associated protein that is an important component of neurofibrillary tangles, is helpful for diagnosing Alzheimer's disease when used in conjunction with other investigations. However, some overlap has been found between levels of total tau in patients and healthy controls.[1] To further improve and extend the diagnostic value of tau, a new enzyme immunoassay (EIA) system has been developed to measure phosphorylated tau (p-tau). While total tau is thought to reflect neuronal degeneration, p-tau levels are considered a marker for hyperphosphorylation of tau and, possibly, for the formation of neurofibrillary tangles.

Phosphorylated tau 199 was discovered by our group and an EIA was developed in collaboration with Mitsubishi Chemical Company (Tokyo, Japan). The selectivity and specificity of the p-tau assay was compared with the existing total tau assay in a study of CSF samples from over 550 patients (Table 1) .[2]

Total tau levels were significantly higher in CSF samples from Alzheimer's disease than normal con-

Table 1 Patient demographic data

Diagnosis	No. patients	Mean age (years)	Gender (no. patients, M/F)
Alzheimer's disease	235†	71 ±9	66/172
Normal controls	95	57 ±16	51/44
Neurological disease control	122	59 ±13	70/52
Frontotemporal dementia	16†	63 ±12	9/7
Progressive supranuclear palsy	21	63 ±7	10/11
Corticobasal degeneration	15	64 ±4	8/7
Dementia with Lewy bodies	13†	63 ±10	8/5
Vascular dementia	23	71 ±6	16/7
Meningoencephalitis	18	51 ±21	7/11
Creutzfeldt-Jakob disease	11†	71 ±6	6/5

†Two patients with Alzheimer's disease, one patient with frontotemporal dementia, one patient with dementia with Lewy bodies and four patients with Creutzfeldt-Jakob disease were confirmed by autopsy.

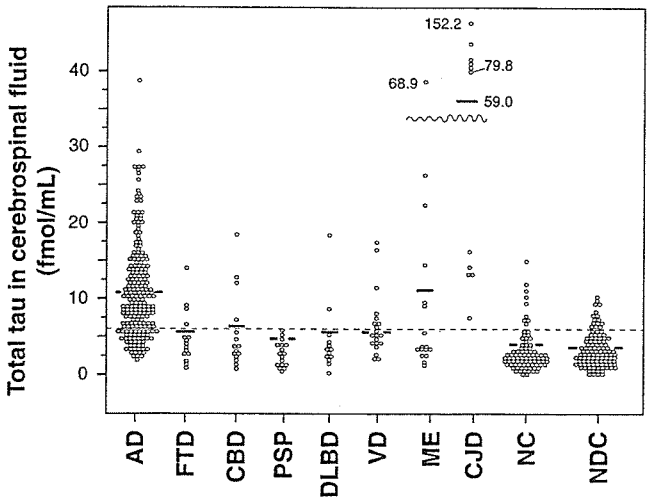

Figure 1 Distribution of cerebrospinal fluid (CSF) total tau concentrations in Alzheimer's disease, other tauopathies and controls. AD, Alzheimer's disease; FTD, frontotemporal dementia; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; DLBD, dementia with Lewy bodies; VD; vascular dementia; ME, meningoencephalitis; CJD, Creutzfeldt-Jakob disease; NC, normal controls; NDC, neurological disease controls.

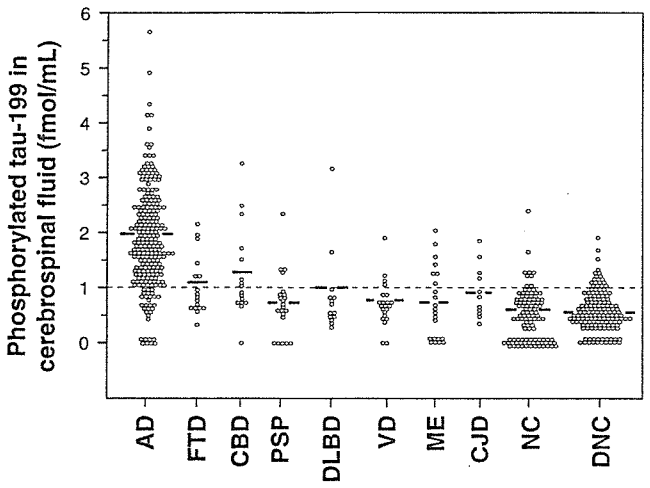

Figure 2 Distribution of cerebrospinal fluid (CSF) phosphorylated tau concentrations in Alzheimer's disease, other tauopathies and controls. AD, Alzheimer's disease; FTD, frontotemporal dementia; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; DLBD, dementia with Lewy bodies; VD; vascular dementia; ME, meningoencephalitis; CJD, Creutzfeldt-Jakob disease; NC, normal controls; NDC, neurological disease controls.

trols or neurological disease controls (Fig. 1). However, total tau levels displayed some overlap across the tauopathy diagnoses and, in particular, very high levels of total tau were evident in some patients with meningoencephalitis and Creutzfeldt-Jakob disease. All tauopathies had some cases with high levels of total tau.

In comparison, p-tau 199 levels were also significantly higher in Alzheimer's disease than in normal and disease controls but, importantly, there was less overlap between the levels found in other tauopathies (Fig. 2). Meningoencephalitis and Creutzfeldt-Jakob disease did not show the same outlying p-tau 199 values as had been found for total-tau. However, high levels also existed in some patients with other tauopathies and, in this respect, using p-tau 199 has not solved this problem.

However, the p-tau 199 assay provides a substantial improvement over total tau in sensitivity and specificity for differentiation of Alzheimer's disease from other causes of dementia. ROC analysis showed that the sensitivity and specificity of the assay for total tau were 77.1% and 77.6%, respectively. The p-tau 199 assay had a sensitivity of 85.2% and a specificity of 85.0%. This is the first report of an assay that exceeds 85% for both sensitivity and specificity. The cut-off level of p-tau 199 for diagnosis of Alzheimer's disease was 1.05 fmol/mL.

PHOSPHORYLATED TAU AND MCI

MCI is often, though not always, the transitional state between normal aging and Alzheimer's disease. Much interest has focused on diagnosing patients with MCI and, possibly, preventing progression to Alzheimer's disease. In addition, being able to identify MCI patients at highest risk of progressing to Alzheimer's disease could help to target treatment for those most in need.

A study of 17 individuals was conducted to determine whether p-tau levels differed between MCI patients who progressed to Alzheimer's disease and those who did not. CSF p-tau levels were measured in 13 cases with MCI and four control cases. MCI was diagnosed according to the following criteria:[3] (i) memory complaints; (ii) impaired memory function; (iii) preserved general cognitive function; (iv) intact activities of daily living; and (v) not demented.

The study cohort was divided into three groups: seven cases with MCI that had not progressed to Alzheimer's disease (MCI→NPMCI), six cases MCI that later progressed to Alzheimer's disease (MCI→PMCI) and four controls (complainer→complainer). Subjects in the control group presented to the hospital with complaints of memory disturbance, but no abnormalities were found after further examination. p-tau 199 levels in Alzheimer's disease converters were significantly higher than non-converters and controls (Fig. 3). These results suggest that early identification of patients who may develop Alzheimer's disease is possible, using p-tau 199 levels when the patients are suffering from MCI.

MCI displays considerable clinical and etiological heterogeneity, and the challenge for future research will be to investigate whether CSF p-tau and other candidate diagnostic markers vary in different situations.

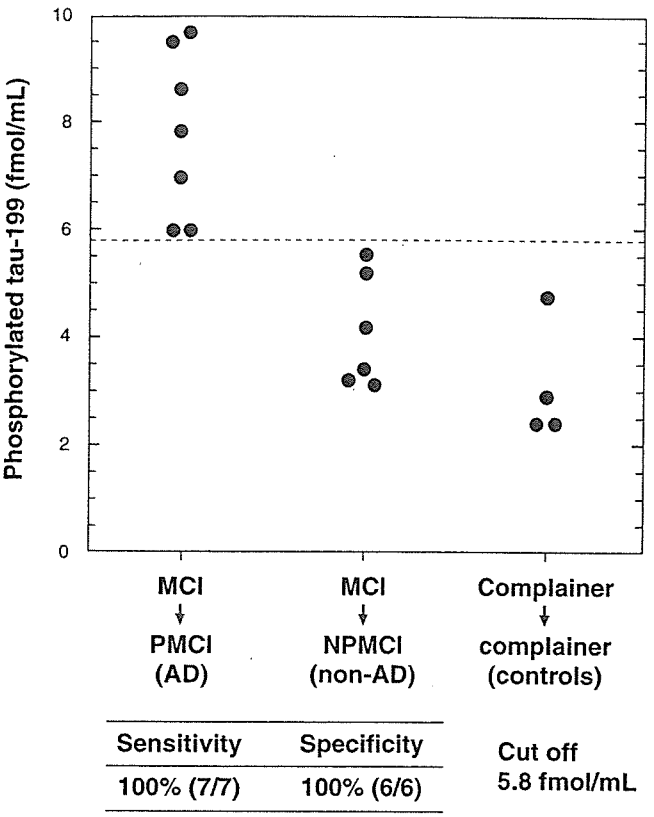

Figure 3 Comparison of p-tau 199 concentrations in cerebrospinal fluid samples from patients with mild cognitive impairment and controls. MCI, mild cognitive impairment; PMCI, progression from mild cognitive impairment; NPMCI, no progression from mild cognitive impairment; AD, Alzheimer's disease.

WGA BINDING GLYCOPROTEIN

Wheat germ agglutinin (WGA)-binding glycoprotein is a new candidate biomarker in Alzheimer's disease. High concentrations of WGA-binding glycoprotein exist in the cerebral cortex of the normal brain, but levels are markedly reduced in the temporal–parietal cortex of patients with Alzheimer's disease.[4,5] WGA-binding glycoprotein concentrations are also decreased in the CSF of patients with Alzheimer's disease.[5] WGA binds specifically to sialic acid, and detects O-type and N-type glucose chains, especially mixed and complex N-type glucose chains.

Western blot analysis of WGA-binding glycoprotein from CSF samples showed three bands of high molecular weight: fragments A (75 kDa), B (30 kDa) and C (25 kDa). The levels of fragment C from patients with Alzheimer's disease and a non-Alzheimer's control group were not significantly different. However, relative to the control group, significantly greater amounts of fragments A and B were found in the Alzheimer's disease group ($P < 0.01$ and $P < 0.05$, respectively).

Calculating the ratio of p-tau 199 to WGA-binding glycoprotein fragment A revealed that the ratio was significantly lower in patients with Alzheimer's disease ($n = 40$) than in non-Alzheimer's controls ($n = 36$; $P < 0.001$). A second comparison of the p-tau : WGA-binding glycoprotein fragment A ratio for Alzheimer's disease and other tauopathies (corticobasal degeneration, progressive supranuclear palsy and dementia with Lewy bodies) showed that this ratio has potential for differentiating between Alzheimer's disease and

other tauopathies, particularly dementia with Lewy bodies. Further studies are warranted to confirm this initial finding, as differentiating Alzheimer's disease from other tauopathies is difficult with currently available methods.

CONCLUSION

Measuring CSF levels of p-tau may be useful as an early diagnostic marker in Alzheimer's disease and MCI. Furthermore, the measurement of WGA-binding glycoprotein in CSF may provide a useful tool for differentiating Alzheimer's disease from dementia with Lewy bodies and other tauopathies.

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Alzheimer病の早期診断と軽度認知障害(MCI)

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☞ *Point*

- Alzheimer病(AD)は早期から治療できれば、より多くの効果が期待できる。このような観点から、認知症の前段階の概念として、軽度認知障害(MCI)が提唱された。
- MCIの概念はいまのところ一致した見解が得られていないが、Petersenらにより提唱された基準は、①自覚的な記憶障害の訴えがある、②客観的な記憶検査の異常がある、③全般的な認知機能は正常、④日常生活に差し支えない、⑤認知症の診断基準を満たさない、というものである。
- MCIの診断には、バイオマーカーでは、髄液中リン酸化tau蛋白やアミロイドβ蛋白(Aβ)、画像診断では、脳血流シンチ(SPECT)画像、その三次元定位脳表投射法(3D-SSP)解析が有用である。
- わが国ではMCIに対する塩酸ドネペジルの適応はないが、欧米では塩酸ドネペジル投与群がADAS-cogスコアを有意に改善することが示されている。
- 現在、新しいMCIの基準が示されているので、今後この基準に従って分類した群に分けて、進行率、バイオマーカーの有用性、その他を検討していく必要がある。

認知症は現在65歳以上の10人に1人にみられる“ありふれた疾患”であり、その認知症の約半数を占めるのがAlzheimer病(AD)である[1,2]。ADは現在塩酸ドネペジル(商品名アリセプト)がわが国でも市販され、治療可能となっている[3]。ただし、この薬剤ではADを根本的に治すことはできず、症状の進行を遅らせるだけである。このため、より早期から治療ができれば、より多くの効果が期待できる。このような観点から、認知症の前段階の概念として、軽度認知障害(mild cognitive impairment；MCI)が提唱された[4]。

本稿では、ADの早期診断を考えるうえで重要なMCIを取り上げて、AD早期診断について考察したい。

MCIの概念

Petersenらにより提唱された基準は、①自覚的な記憶障害の訴えがあ

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る、②客観的な記憶検査の異常がある、③全般的な認知機能は正常、④日常生活に差し支えない、⑤認知症の診断基準を満たさない、というものである[4]。この基準によりMCIと診断された者のうち、毎年12.5％がADに移行することが示されている。またこのような症例の剖検では、大部分がADの変化を伴っており、ADの初期状態と捉えることができる。

バイオマーカーからみたMCI

ADでは髄液中リン酸化tau蛋白やアミロイドβ蛋白（Aβ）の測定が診断マーカーとして有用であることが報告されている[5-9]。これらをMCIで測定したデータをみると、髄液中Aβ蛋白は有意な変化を示していないが、髄液中リン酸化tau蛋白はMCIの段階からすでに高値を示している。髄液中リン酸化tau蛋白はMCIの診断に有用と考えられる。

画像検査からみたMCI

画像検査においては、ADでは脳血流シンチ（SPECT）で側頭、頭頂葉の血流低下がみられ、鑑別診断に有用である（図1）。最近では、三次元定位脳表投射法（3D-SSP）、eZISなどの統計学的画像解析法を用いると、個々の症例が正常データベースから求めたピクセルごとの平均値と標準偏差値からどれだけかけ離れているか（Zスコア）を示すことができる。この方法により、ADにおいては、側頭、頭頂葉の機能低下よりも、後部帯状回における代謝の低下がごく初期の段階からみられることが明らかとなった（図2）[10]。現在これらの解析ソフトウエアはインターネットで簡単に入手でき、ルーチンで検査を行っている施設も多くなっている。

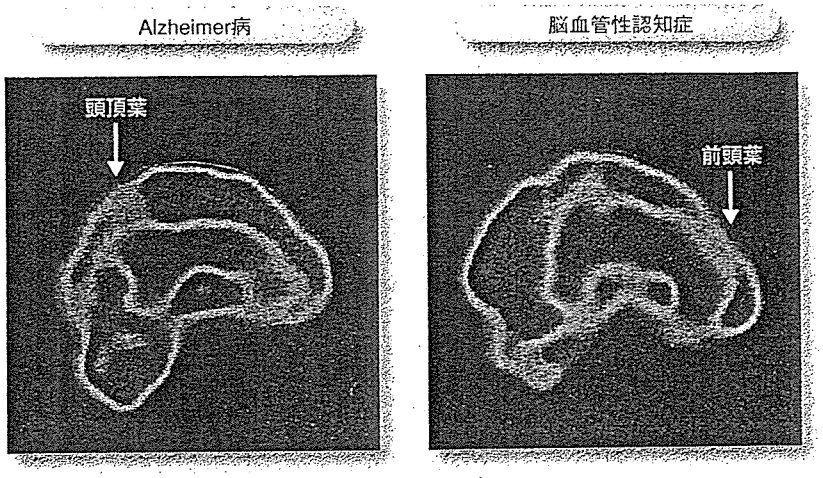

図1　認知症のSPECT（脳血流シンチ）画像

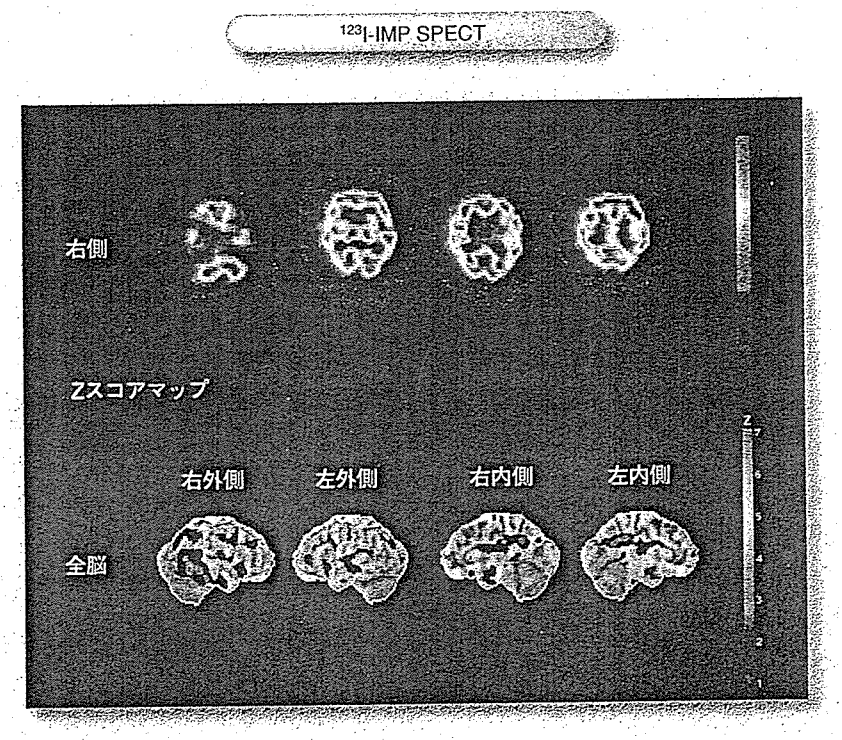

図2　Alzheimer病の^{123}I-IMP SPECTイメージを3D-DDPで解析したZスコアマップ
（日本医科大学内科 北村 伸 博士のご好意による）

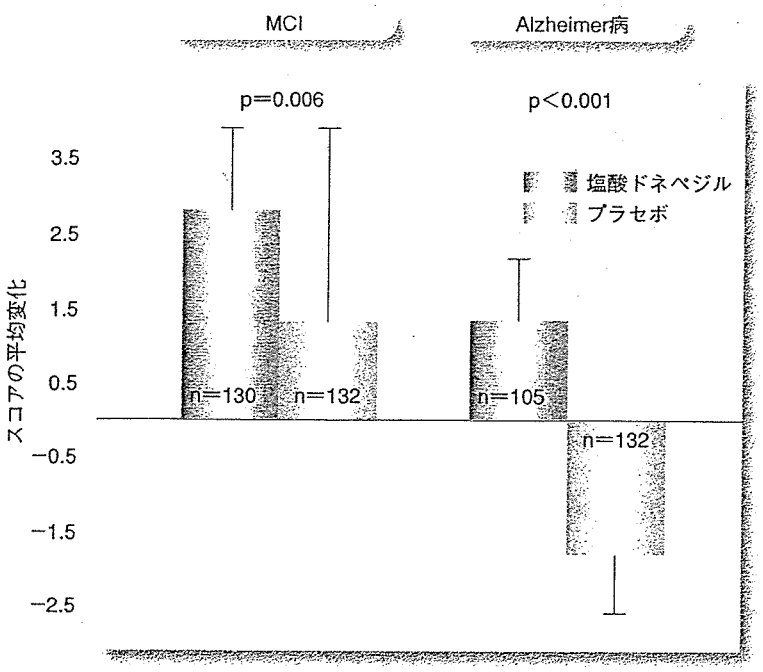

図3　塩酸ドネペジルの認知障害に対する効果：ADAS-cogスコアの変化（文献11より引用）
投与24週後のADAS-cogスコアの変化。

- Amnestic form of MCI（健忘症MCI）
  記憶＜平均−1.5SD
  その他の領域＞平均−0.5SD
- Multiple-domain MCI（複数の高次機能領域にまたがったMCI）
  いくつかの領域
  平均−0.5SD≫平均−1.0SD
  AACDに類似の概念
- Single non-memory MCI

表1　改定されたMCIの基準
AACD：aging-associated cognitive decline（年齢関連認知的低下）。

MCIの治療

前述したMCIの基準には現在のところ一致した見解が得られていないが、少なくとも正常とADの間に移行期のような状態が存在することは確かであり、認知症の前段階あるいはきわめて早期のADを捉えている可能性がある。

わが国ではMCIに対する塩酸ドネペジルの適応はないが、自験例では、「物忘れが改善した」あるいは「頭がスッキリした」という自覚が得られ、長谷川式簡易知的機能検査-改訂版（HDS-R）あるいはMini-mental state examination（MMSE）などのスコアの改善もみられた症例を経験した。

欧米では塩酸ドネペジルをはじめ各種薬剤のMCIに対する臨床試験が行われている。米国でのMCI患者270例を対象とした多施設共同二重盲検プラセボ対照比較試験では、プラセボ投与群に比し塩酸ドネペジル投与群で24週後のADAS-cogスコアが有意に改善することが示された。また、患者の全般評価においても悪化例はプラセボ群に多く、ドネペジル投与群では改善例が多いという結果が得られている（図3）[11]。

新しいMCIの基準とその問題点と今後の展望

新しく提唱されたMCIの基準を示す（表1）。発症機序も考慮され、より科学的な内容であるが、一方でたいへん煩雑でわかりにくい印象がある。今後この基準に従って分類した群に分けて、進行率、バイオマーカーの有用性、その他を検討していく必要がある。

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特集　認知症のケア

認知症の予防と早期発見

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認知症の予防と早期発見

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◇認知症は早期発見・診療が重要であるが、現状では困難である。そこで初期症状早期発見の手助けとなるタッチパネル式コンピューターを用いたスクリーニング機器を開発した。
◇この機器を用いてスクリーニングを実施したところ、感度96％、特異度97％と高い信頼性を示した。定期的に行うことで、確実に認知症早期発見に役立つ。
◇市町村で実施されている認知症予防教室の対象者選定にこの機器を用いた。ここで選定された軽度認知障害のある人に予防教室を実施したところ、3か月で改善傾向が見られた。さらに1年経過追跡したところ、有意な改善が見られた。

KeyWords
アルツハイマー型認知症
軽度認知障害
認知症
診断マーカー
塩酸ドネペジル

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はじめに

現在65歳以上の10人に1人が認知症と言われ、なかでもアルツハイマー型認知症は約半数を占める[1,2)]。しかし、物忘れなどの初期症状は「年だから仕方がない」と見過ごされがちである。徘徊、暴力行為などの問題行動などが出て家族が困ってから病院へ行くケースは多いが、これは症状がすでに進行しているもので早期発見にはなっていない。このように早期発見が難しくてできていないことが、認知症診療の大きな問題点である。この早期の気付きを手助けできる簡単な機器があれば、この問題点を解決できる。そこで、われわれはタッチパネル式コンピューターを用いた認知症のスクリーニング機器（図1）[3)]を開発したので、その有用性と意義を報告する。

タッチパネル式コンピューターを用いた認知症のスクリーニング機器の開発と意義

タッチパネル式コンピューターは音声と映像による対話形式で、質問に答えながらゲーム感覚で検査を受けることができる。言葉や日時に関する質問、立方体を識別する質問など合計5問で構成し、所要時間は結果の印刷まで含めて合計5分以内である。

このコンピューターを用いて、アルツハイマー型認知症49例、健常対照群30例を対象にスクリーニング検査を実施した。15点満点で、アルツハイマー型認知症ではほとんどの例が12点以下であり（図2）、専門医への受診が望まれる。感度（疾患がある場合、検査が陽性になる割合）96％、特異度