

図3 全例での手指名称記憶の点数推移
 クロスオーバー法によって、手指名称記憶の点数推移を検討した。* : $p < 0.05$

しかし、何れの場合も post hoc テストでは有意な差はみられなかった。

HDS-R の点数変化に統計的有意差はなかった。

FAST スコアの推移について、有意な差がみられなかった。

4 考 察

アロマセラピーの作用は、におい分子がそれぞれに固有の受容体に結合することからはじまる。におい分子は鼻腔を通り、嗅上皮へ付着する。そして、嗅上皮に集中している嗅覚神経系を介し、海馬や扁桃体といった大脳辺縁系に刺激が伝達される。海馬や扁桃体は認知症に深い関わりを持ち、特に海馬はADで顕著に萎縮する。大脳辺縁系に投射された匂い刺激は、自律神経系や内分泌系を調整している視床下部へ情報を送り、神経伝達物質の放出等の一連の生体反応を誘導する。ローズマリー・レモンオイルは集中力を高め、記憶力を強化する刺激的な作用がある。人間の基本的な生体調節機構として、サーカディアンリズムが存在する。その為、朝はローズマリー・レモンオイルによる刺激作用により交感神経を優位に働かせ、夜はラベンダー・オレンジオイルによる鎮静作用により副交感神経を優位に働かせると考えられている。よって、我々は、集中力を高めるローズマリーオイル（カンファー）及びレモンオイル混合液による交感神経系の活性化と鬱状態などの改善に役立つ真性ラベンダーオイル及びスイートオレンジオイル混合液による副交感神経系の活性化を組み合わせることで、ADにおける脳を活性化するためのアプローチとなるだろうと考えた。

アロマセラピーを認知症患者に実施し、TDASにおい

て、認知症の好発年齢である65歳以上の群において、認知機能の改善を示す総点の改善と、手指名称記憶の点数改善とが見られた。これにより、アロマセラピーは高度ADにおいても、認知機能の改善に効果的であると示唆できた。この傾向は、これまで到我々が行ってきた前調査と同様であると言える。

今回の結果においては、TDASでは認知機能全体の改善が示唆された。しかしGBSでは有意な改善は見られなかった。このことは、GBSは観察式評価スケールであり、詳細な検討を行うにあたって、質問式評価スケールであるTDASの方がより鋭敏に改善を捕らえられたものと考えられる。また、我々が過去行ってきた前調査において、アロマオイルの量が効果に影響を及ぼすことが示唆されていた。このことから、今回は、オイル使用量を多くして検討したが、予想していたように、高度なADに対しても改善が示唆された。今後は、ADの進行に伴い、最適なオイルの量などを検討したいと考えている。また、TDAS検査は、高度すぎるAD患者に対しての適応が薄いと言え、実際、認知機能障害が進行し、質問に全く答えられない状態の患者に対しては、その認知機能を測定することは難しい。そのため、今回も高度ADの内、質問に答えられる患者を対象とした。

前述したように、嗅覚機能は、大脳辺縁系に刺激を投射する。大脳辺縁系には、認知症の中核症状である認知障害に深く関係する海馬や扁桃体といった非常に重要な領域を含んでいるが、ADでは、早期に内嗅皮質、海馬、扁桃、視床、大脳辺縁系から刺激を伝達される視床下部に神経原線維化の蓄積が観察される²⁶⁾。これらから、ADにおいて嗅覚野が密接に関わることが推察されている。

ADの早期で嗅覚機能障害が進行することはこの仮説に

合致する³⁾。さらに、ヒト海馬歯状回と側脳室脳室下帯では、生涯、神経細胞新生が続いているが、この神経細胞発生は、様々な環境要因により左右され²⁾、実際に、快適な環境によって、神経変性の進行が緩和されるという報告もある²⁾。また、リナロールや、リモネンなどの精油の成分が、集中力などの増強をもたらしているのではないとも言われている。これを踏まえて、交感神経系の賦活や、副交感神経系の賦活についても、未だ経験的に用いられることが多い。そこで、今後、こうした成分ごとの効果についても検討することが必要だと考えられる。

これらふまえて、我々はアロマセラピーがADの中核症状である認知機能障害を改善することを示唆してきた。今回、アロマセラピーが軽度から中等度のADのみならず、高度なADの認知機能にも改善作用をもたらすだろうという報告は、世界で初めて行うものである。Post hocの結果では、群間での明確な差はなかった。しかし、これまで我々が行ってきた検討でも、アロマセラピーがADを主とする認知症における認知機能障害に効果があると示唆されてきている。よって、今後、より例数を増やし、検討を進めることが必要だと考えられる。

我々の行った方法は、誰でも簡易に治療に取り入れ、予防法としても、治療法としても、非常に有益であると考えられる。今後は、さらに多数例での検討や、精油の違いによってその効果は変化しうることといった検討を含めた、より最適な香りの開発などを行うと共に、そのメカニズムについても検討を進めていきたいと考えている。

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Sugar Chains of Cerebrospinal Fluid Transferrin as a New Biological Marker of Alzheimer's Disease

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Key Words

Alzheimer's disease · Biological marker · Tauopathy · Wheat germ agglutinin · Transferrin · Glycosylation

Abstract

Background/Aims: Alzheimer's disease (AD) is a well-known type of dementia. However, it remains difficult to identify AD in the early stage and to distinguish it from other dementing disorders. We examined glycoproteins in cerebrospinal fluid (CSF) as potential biological markers of AD. **Methods:** CSF samples were collected from AD, other dementia and non-demented patients. Glycoproteins in CSF were detected by lectin blotting using wheat germ agglutinin (WGA), and sugar chain analysis was performed by isoelectric focusing. **Results:** In Alzheimer's CSF, several glycoproteins had lower WGA-binding activities, one of which was sufficiently sensitive and specific to distinguish AD from nondemented controls and other dementias. Further analysis identified this glycosylated protein as transferrin, and altered sugar chain composition of transferrin isoforms was observed despite normal protein levels in CSF. **Conclusion:** The decreased WGA-binding activity of transferrin in AD is probably due to altered glycosylation of transferrin molecules. Transferrin

glycosylation is thus a potential biological marker for AD diagnosis, and changes in this glycosylation may play an important role in the pathophysiology of AD.

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Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders in the elderly. Familial early-onset AD is known to be caused by mutations in the amyloid β protein (A β) precursor (APP) [1], and presenilin 1 or 2 [2, 3]; however, this type of AD is relatively rare, with genetic risk factors accounting for less than 2% of cases. Although nonfamilial AD can be diagnosed only by pathologic examination, diagnosis at the earliest stage possible will contribute to its treatment. However, symptom overlap between AD and other neurodegenerative dementing disorders complicates the differential diagnosis. Identifying AD in its earliest stage is very difficult because memory loss, an initial symptom of AD, is common in normal aging. It is thus necessary to identify biological markers with high sensitivity for AD to distinguish it from other types of dementia and normal individuals.

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Table 1. Patient profiles

	Patients (M/F)	Mean age \pm SD, years (M/F)	Mean MMSE score \pm SD
AD	50 (19/31)	73 \pm 8 (74 \pm 8/72 \pm 8)	20.4 \pm 4.4 (range: 11–28)
Other dementias	14 (10/4)	72 \pm 7 (73 \pm 3/68 \pm 4)	NA
Dementia with Lewy bodies	4 (3/1)	75 \pm 5	
Progressive supranuclear palsy	5 (3/2)	67 \pm 9	
Corticobasal degeneration	5 (5/0)	71 \pm 7	
Nondemented controls	38 (21/17)	71 \pm 10 (71 \pm 8/72 \pm 12)	NA
(including normal controls)	5 (1/4)	64 \pm 1	

A large number of trials have attempted to identify diagnostic markers for AD. Current approaches focus on 2 major protein aggregates in the AD brain, A β and phosphorylated microtubule-associated tau protein (phospho-tau), as reflected by senile plaques and neurofibrillary tangles, respectively. In cerebrospinal fluid (CSF), the levels of A β ₄₂, which ends at amino acid 42, are significantly decreased in AD patients [4, 5], while excess levels of phospho-tau have been observed [6–10]. The levels of these proteins in CSF reflect their metabolism in the central nervous system. Sensitive enzyme-linked immunosorbent assay (ELISA) systems have been established, and findings on phospho-tau and A β ₄₂ levels have been reported and clinically applied to AD diagnosis. However, diagnosis in the early stage of AD is critically important for treatment based on drugs that delay neurodegenerative dysfunction.

Protein glycosylation, particularly O-glycosylation of tau protein by O-GlcNAc, has been studied in AD, and it has been found that the phosphorylation and O-GlcNAc glycosylation of tau proteins maintain a co-dependent balance [11, 12]. Fodero et al. [13] reported that the levels of some wheat germ agglutinin (WGA)-reactive glycoproteins in CSF from patients with AD were lower than in controls. This suggests that protein glycosylation is altered in AD. In order to identify potential diagnostic biomarkers of AD, we used WGA, which exhibits specific binding to terminal sialic acid moieties and N-acetylglucosamine, to identify glycoproteins in CSF, and confirmed the presence of glycoproteins with lower WGA-binding activity in AD. Further analysis of the least stained (most significant) glycoprotein indicated altered glycosylation of CSF transferrin, suggesting that glycosylation of transferrin is a biological marker for the diagnosis of AD and that alteration of glycosylation plays a role in the pathophysiology of AD.

Materials and Methods

Patients and CSF Collection

CSF samples were collected from 102 patients at the Department of Neurology of the Tottori University Hospital after informed consent had been obtained from the patients and/or their relatives. The collected CSF samples were stored immediately at -80°C until use. The study was performed in accordance with the Helsinki Declaration.

The profiles of all subjects are shown in table 1. A total of 50 patients (19 males/31 females; mean age = 75 \pm 8/72 \pm 6 years) with probable AD were included. The clinical diagnosis of AD was established according to the DSM-IV [14] and NINCDS-ADRDA criteria [15], with magnetic resonance imaging, single-photon emission computed tomography and electroencephalography, and measurement of A β ₄₂ and phospho-tau in CSF also performed. The group with other dementias included 4 patients with dementia with Lewy bodies (DLB), 5 with progressive supranuclear palsy and 5 with corticobasal degeneration (CBD). The diagnosis of DLB and tauopathies was made based on consensus criteria for DLB [16], NINDS-SPSP for progressive supranuclear palsy [17] and the original descriptions by Rinne et al. [18] for CBD. The nondemented control group consisted of patients with motor neuron disease, Parkinson's disease, multiple system atrophy, amyotrophic lateral sclerosis and cerebrovascular disease without dementia, as well as 5 normal (healthy) controls without neurological disease.

The study was approved by the ethics committee of Tottori University.

Lectin Blotting

CSF mixed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer was subjected to 5–20% gradient SDS-PAGE (Bio-Rad, Hercules, Calif., USA) and then electrophoretically transferred onto polyvinylidene difluoride membranes. Membranes were blocked with Block Ace (Dainippon Sumitomo Pharma, Osaka, Japan) and incubated with 20 $\mu\text{g}/\text{ml}$ of biotinylated WGA (J-Oil Mills, Tokyo, Japan), followed by incubation with streptavidin conjugated to alkaline phosphatase (Promega, Madison, Wisc., USA). Blots were visualized with BCIP/NBT development substrate. The stained blots were scanned and the WGA-binding activity of the bands was quantified by densitometry using Multi-Gauge V2.1 in Science Lab (Fuji Photo Film, Kanagawa, Japan). The densitometric intensity was record-

ed after subtracting the background value obtained from a blank region of the blot.

ELISA for CSF/Phospho-Tau199 and $A\beta_{1-42}$

The phospho-tau199 and $A\beta_{1-42}$ levels in CSF were determined using a commercially available human tau (pS199) sandwich ELISA kit (Invitrogen, Carlsbad, Calif., USA) and $A\beta_{1-42}$ sandwich ELISA kit (Innogenetics, Belgium), respectively, according to the manufacturers' instructions.

Mass Spectrometry Analysis of WGA-Binding Protein-a

Analysis was performed by ProPhoenix (Hiroshima, Japan). Briefly, the gel piece containing WGA-binding protein-a was destained and digested with trypsin, and the extracted peptides were then subjected to mass spectrometry (MS). Matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) MS was performed using an Ultraflex MALDI-TOF mass spectrometer (Bruker-Daltonics, Billerica, Mass., USA). The acquired MS and MS/MS data were analyzed applying the peptide mass fingerprint method and MS/MS ion search with the database search program MASCOT (<http://www.matrixscience.com>) in order to compare the monoisotopic m/z values and amino acid sequences of the tryptic fragments to those of known proteins in the NCBI database.

Transferrin Quantification

We measured the transferrin levels using the sandwich ELISA method (Bethyl Laboratories, Montgomery, Tex., USA).

Isoelectric Focusing of Transferrin Isoforms

Isoelectric focusing (IEF) of human CSF transferrin was performed by Multiphor II (GE Healthcare Bio-Sciences, Piscataway, N.J., USA), according to previously described methods [19]. Iron-saturated CSF transferrin was separated on homogeneous polyacrylamide gels (5% T, 3% C) containing carrier ampholytes (pH = 4–6.5). After IEF, the gels were stained with Coomassie brilliant blue R-250.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney test and/or Student *t* test. Receiver operating characteristic analysis was carried out to assess the sensitivity, specificity and the appropriate cutoff levels for WGA-binding activity and phospho-tau199 in comparisons between AD and the other 2 groups.

Results

Detection of WGA-Binding Glycoproteins in CSF

The lectin blotting results for glycosylated CSF proteins are shown in figure 1. The levels of some glycoproteins stained by WGA were reduced in AD patients, and 3 such glycoproteins were evaluated and tentatively designated WGA-binding protein-a, -b and -c, with approximate molecular weights of 75,000, 30,000–35,000 and 25,000 Da, respectively. Preliminary examination of the intensities of each blot indicated significant decreases in the WGA-binding activities of protein-a and -b in AD patients com-

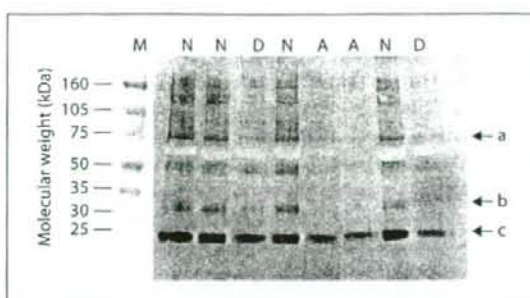


Fig. 1. Banding patterns of WGA-reactive glycoprotein from CSF after SDS 5–20% gradient polyacrylamide gel electrophoresis. M = Molecular markers (GE Healthcare Bio-Science); N = nondemented control group; D = other dementia group; A = AD group. Arrows indicate candidate glycoprotein markers with lower WGA-binding activity (designated a, b and c).

pared with the other groups ($p < 0.001$ and $p < 0.05$, respectively, data not shown). We therefore further examined protein-a as a candidate protein biomarker.

In nondemented controls, the WGA-binding activity of protein-a (densitometric intensity) was $25,997 \pm 13,465$, with no significant difference between male and female subjects. The WGA-binding activity of protein-a was also determined in the AD ($16,264 \pm 6,128$) and other dementia ($27,082 \pm 11,424$) groups and found to be significantly lower in the AD group than in nondemented controls ($p < 0.0001$).

However, as shown in figure 2a, although the quantification of WGA-binding activity indicated significant decreases in the AD group ($p < 0.0001$, fig. 2a), these were inadequate to discriminate AD from other conditions, with extensive overlap among the groups.

Measurement of CSF $A\beta_{1-42}$ Levels

There were no significant differences in CSF $A\beta_{1-42}$ levels between the AD (389.7 ± 240.9 pg/ml), nondemented control (440.8 ± 257.8 pg/ml) and other dementia groups (415.4 ± 189.5 pg/ml) due to scatter in values in each group.

Combined Measurement of Phospho-Tau199 and WGA-Binding Activity

In order to eliminate overlap in WGA-binding activity, we also measured phospho-tau199 in the same patients and devised an index, WGA-binding activity of protein-a divided by phospho-tau199 protein. In the AD group, the protein-a/phospho-tau199 index was significantly lower

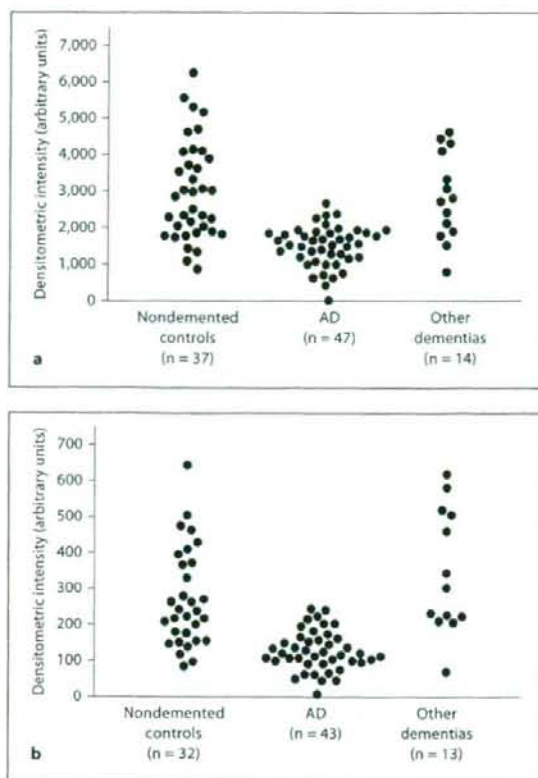


Fig. 2. Densitometric intensities of WGA-binding protein-a. The WGA-binding activities of the stained bands were determined by densitometry. Densitometric intensities of the bands of protein-a band (a) and plot of the index of WGA-binding activity, protein-a/phospho-tau199 (b).

than in the other groups (fig. 2b). The mean index was 125.7 ± 57.6 in the AD group, 268.7 ± 138.4 ($p < 0.0001$) in the nondemented control group and 353.3 ± 181.9 ($p < 0.0001$) in the other dementia group. The most valuable feature of this glycoprotein as a biomarker is that it enables differentiation between AD and other dementias ($p < 0.0001$). The sensitivity and specificity in distinguishing AD from other dementias as determined by receiver operating characteristic analysis with a cutoff point of 205 were 88.4 and 92.3%, respectively.

MS of WGA-Binding Protein-a

The findings of MS indicated that WGA-binding protein-a is transferrin, a metal-binding glycoprotein con-

Table 2. CSF transferrin levels

	Patients	Mean transferrin \pm SD μ g/ml
AD	40	24.6 \pm 10.6
Other dementias	10	22.8 \pm 8.2
Nondemented controls	29	25.0 \pm 12.5

sisting of 1 polypeptide chain with 679 amino acid residues folded into 2 domains. Transferrin is N-glycosylated at 2 asparagine residues with 2 forked carbohydrate chains containing N-acetylglucosamines and sialic acid residues and thus exhibits WGA-binding activity. The identity of the protein was confirmed using transferrin antibody (data not shown).

Measurement of CSF Transferrin Levels

The transferrin concentrations in CSF are shown in table 2. There were no significant differences in CSF transferrin levels among the AD, nondemented control and other dementia groups.

Sugar Chain Analysis of CSF Transferrin

Structural studies of the sugar chains performed by IEF confirmed that transferrin is present as several isoforms with different amounts of sialic acid, resulting in different isoelectric points. The CSF transferrins at isoelectric points 5.4, 5.6, 5.7, 5.8 and 5.9 contain 4, 3, 2, 1 and 0 sialic acid residues, respectively [20]. Figure 3 shows the banding patterns of CSF transferrin obtained on IEF. The patterns of separated transferrin isoforms were different in AD compared with control CSF. The controls had common banding patterns, consisting of tetra-, tri-, di-, mono- and asialotransferrin. In AD, increases in tetra- and trisialotransferrin were typically observed, while lower levels of di- and monosialotransferrin were noted.

Discussion

In the AD patients we examined, some CSF glycoproteins exhibited decreased WGA-binding activity compared with the controls. The most significantly decreased glycoprotein ($p < 0.01$), with a weight of 75 kDa, was more reliable when measurement of it was combined with phospho-tau199. Although we also determined CSF $A\beta_{1-42}$, there was wide dispersion in values in each group. $A\beta_{1-42}$ level was not an effective indicator in the subjects

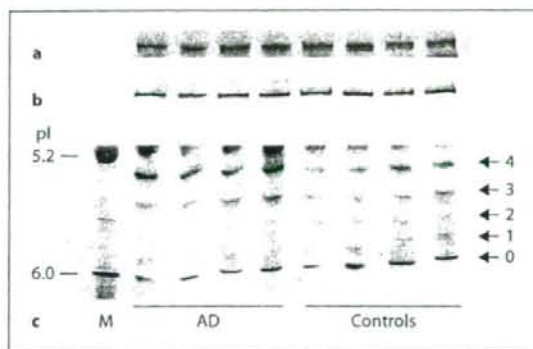


Fig. 3. CSF transferrin from 4 controls and 4 AD patients was used in each experiment, including 0.2 μ g of transferrin protein in SDS-PAGE (a) and WGA blotting (b) and 1 μ g in IEF (c). Arrows labeled with 4, 3, 2, 1 and 0 indicate tetra-, tri-, di-, mono- and asialotransferrin, respectively. Lane M, isoelectric point (pI) marker proteins (Bio-Rad): β -lactoglobulin B (5.1) and bovine carbonic anhydrase (6.0).

of this study. The observed abnormality in protein levels was specific to AD, making it a novel biological marker for the diagnosis of AD. The most promising aspect of this marker is its potential for distinguishing between AD and other dementias including tauopathies. The presently known biological markers in CSF are $A\beta$ [4, 5], tau protein and phospho-tau protein [6–10]. However, differential diagnosis between AD and non-AD dementias has been an important problem both clinically and biologically, as tau and phospho-tau are established markers. Elevated levels of CSF total tau protein have also been reported in nontauopathy and tauopathy patients [21, 22], as a result of cerebral injury. Furthermore, 3 types of phospho-tau (phosphorylated at threonine 231, threonine 181 and serine 199) have been evaluated and found to be more useful markers than total tau. In a recent study, these 3 types of phospho-tau were measured in the same set of patients, and the diagnostic performance in distinguishing AD from related diseases was assessed [10]. In the present study, glycoproteins were used as a new element in the process of distinguishing AD from other important cases of dementia, particularly DLB and tauopathies, while new trials have focused on more sensitive markers in CSF and serum. Thus, glycoproteins may also yield unique findings regarding pathophysiology, in addition to acting as biological markers. The next step in establishing glycoproteins as biological markers will be to quantify them with precision.

The 75-kDa glycoprotein was identified as transferrin, an iron-carrying glycoprotein with 2 forked carbohydrate chains capped by sialic acid at asparagine residues. The low levels of WGA staining for transferrin suggest 2 possibilities: decreased quantity in CSF or altered glycosylation of the sugar chain. In AD, DLB and tauopathies, the CSF transferrin levels were normal. We therefore separated transferrin isoforms using IEF and noted altered isoform patterns in AD. Although the isoelectric points of transferrin revealed that decreased amounts of terminal sialic acids decreased the WGA-binding activity of transferrin in AD, this reduction was probably due to variations in the glycosylation of transferrin molecules, particularly sialic acids.

Several genetic studies of AD have been performed, and the transferrin gene has been suggested as a candidate locus for AD. Among the many variants, the allelic frequencies of C1 and C2 variants, which account for the majority of the world population, have long been considered important [23–27], though some controversy remains concerning this. Because transferrin has been detected around senile plaques in the AD brain, suggesting disruption of brain iron homeostasis [28], it might play a critically important role in AD.

Further evidence supporting our findings was reported by Kitazume et al. [29, 30], who found that β -secretase (BACE1), a protease that cleaves APP, also cleaves β -galactosyl α 2,6-sialyltransferase (ST6Gal I). This finding indicates that $A\beta$ production following the cleavage of APP is associated with protein sialylation. Transferrin molecules possess N-linked glycans containing catalytic sites for ST6Gal I, which transfers terminal sialic acids. Our findings thus suggest a new pathophysiological feature of AD, dysfunction of sialylation.

Glycosylation and/or sialylation of transferrin can be developed as an effective biological marker for diagnosing AD, suggesting that aberrations in glycosylation play a role in the pathophysiology of AD.

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SHORT REPORT

GAB2 is not associated with late-onset Alzheimer's disease in Japanese

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The $\epsilon 4$ allele of the apolipoprotein E gene (*APOE*) is unequivocally recognized as a genetic risk factor for late-onset Alzheimer's disease (LOAD). Recently, single-nucleotide polymorphisms (SNPs) of the GRB2-associated binding protein 2 gene (*GAB2*) were shown to be associated with LOAD in Caucasians carrying the *APOE- $\epsilon 4$* allele through a genome-wide association study. Here, we attempted to replicate the finding by genotyping these SNPs in a large clinical cohort of Japanese. We observed no association of any of the SNPs with LOAD. *GAB2* may not be a disease susceptibility gene for LOAD in Japanese.

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Keywords: Alzheimer's disease; *GAB2*; *APOE*; SNP

Introduction

It is well known that the development of Alzheimer's disease (AD) is consequence of complex interactions between multiple genetic and environmental factors. To date, only the $\epsilon 4$ allele of the apolipoprotein E gene (*APOE*) is universally recognized as a genetic risk factor for late-onset AD (LOAD) in a variety of populations,^{1–3} but not in elderly Nigerians.⁴ As the presence of risk genes other than

APOE is speculated,⁵ many studies have been performed to identify them.

Genome-wide association studies (GWAS) involving high-density single-nucleotide polymorphism (SNP) genotyping technologies have led to great success in the identification of risk genes for various common diseases.^{6,7} With regard to LOAD, the GRB2-associated binding protein 2 gene (*GAB2*) on chromosome 11q was recently identified in Caucasians through GWAS: 10 SNPs of this gene have been shown to be associated with LOAD in *APOE- $\epsilon 4$* carriers.⁸ It is noteworthy that the most significant SNP, rs2373115, exhibits an odds ratio (OR) of 4.1 (95% confidence intervals (Cis), 2.8–14.7), which is almost equal to the strong risk effect exerted by the *APOE- $\epsilon 4$* allele ($\epsilon 3$ vs $\epsilon 4$, OR = 3.2–4.1).² Furthermore, the following

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findings with relation to AD neuropathology have been made:⁹ in LOAD brains *GAB2* is detected in highly dystrophic neurons, including neurofibrillary tangle (NFT)-bearing neurons, and interference with *GAB2* expression increases TAU phosphorylation, which leads to NFT formation. With this genetic and biological evidence, *GAB2* is considered to be a promising candidate for LOAD, although a recent replication study revealed a lack of association of this gene with LOAD in Caucasians.⁹ Therefore, we here assessed whether or not the genetic association of *GAB2* with LOAD can be reproduced in Japanese.

Subjects and methods

Subjects

Blood samples were collected by the Japanese Genetic Study Consortium for AD (JGSCAD): the members are listed in our recent publications.^{10,11} The LOAD patients were clinically validated, and satisfied the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association for a diagnosis of probable AD.¹² Non-demented controls living in an unassisted manner in the local community were recruited from among elderly subjects. The Mini-mental State Examination (MMSE) and Clinical Dementia Rating and/or the Function Assessment Staging were used to assess severity of the cognitive impairment. Basic information on the sample sets used is presented in Table 1. The total sample size is 1656 LOAD patients (female, 71.6%) and 1656 controls (female, 58.7%), which is large enough to detect risk alleles assuming $OR > 1.3$ (range of risk allele frequency = 0.1–0.9, $\alpha = 0.05$, power = 80%). This subject group is referred to as overall sample set *All* in this study (Table 1). A large proportion (79.4%) of the subjects are the same as in our previous overall sample set.^{10,11} To construct two sub-sample sets, the *All* set was stratified as to the *APOE-ε4*

carrier status: *Negative-ε4* (LOAD, 790; control, 1378) and *Positive-ε4* (LOAD, 866; control, 278) (Table 1).

This study was approved by the Institutional Review Board of Niigata University, and by all participating institutes. Informed consent was obtained from all controls and appropriate proxies for patients, and all subjects were anonymously subjected to SNP genotyping.

Genotyping

Genomic DNA preparation and genotyping were described previously.¹⁰ We did not genotype additional SNPs to the 10 ones reported by Reiman et al.⁸ as strong LD ($|D'| > 0.8$) was observed across the *GAB2* (see Supplementary Figure 1).

Statistical analysis

We carried out a Hardy–Weinberg equilibrium (HWE) test based on an exact test, single SNP and haplotype-based case–control studies, haplotype inference, and computation of LD measures (D'). As an estimate of the relative risk of disease, OR with 95% CIs of each marker or haplotype was calculated from a 2×2 contingency table. For all statistical analyses mentioned above, we used SNPalyze[®] software version 6.0.1 (DYNACOM): the analytical methods were described in detail elsewhere.¹¹ For evaluation of the LD block structure in and around *GAB2*, Haploview software version 3.32 was used. We considered $P < 0.05$ statistically significant.

Results

To determine whether the *GAB2* association can be replicated in Japanese or not, we analyzed the 10 SNPs using a total of 3312 clinical subjects for genotyping (see Supplementary Figure 2). These SNPs are encompassed by *GAB2* (Table 2), which consists of 10 exons and spans about 202.4 kb on chromosome 11. To examine population

Table 1 Information on sample sets

Sample set	No. of subjects	AAO/AEE		MMSE		Genotype					APOE			Allele			
		Mean (SD)	Range	Mean (SD)	Range	2*2	2*3	2*4	3*3	3*4	4*4	ε2	ε3	ε4			
Overall set																	
<i>All</i>																	
LOAD	1656	73.1 (6.2)	60–85	16.5 (6.7)	0–30	1	53	21	736	686	159	76	2211	1025			
Control	1656	75.3 (6.1)	64–96	28.3 (1.7)	24–30	4	126	17	1248	248	13	151	2870	291			
Subsets																	
<i>Negative-ε4</i>																	
LOAD	790	73.7 (6.4)	60–85	16.2 (7.2)	0–30	1	53	0	736	0	0	55	1525	0			
Control	1378	75.4 (6.2)	64–96	28.3 (1.7)	24–30	4	126	0	1248	0	0	134	2622	0			
<i>Positive-ε4</i>																	
LOAD	866	72.5 (5.9)	60–85	16.9 (6.3)	0–30	0	0	21	0	686	159	21	686	1025			
Control	278	75.1 (5.8)	64–95	28.3 (1.7)	24–30	0	0	17	0	248	13	17	248	291			

AAO, age at onset; AEE, age at examination.

differences in the allele frequencies of the SNPs, we first assessed the HapMap genotype data (<http://www.hapmap.org/index.html>) for four populations: Japanese in Tokyo (JPT), US Utah residents with northern and western European ancestry (CEU), Han Chinese in Beijing (CHB) and Yoruba in Ibadan, Nigeria. These 10 SNPs for JPT exhibited similar allelic frequencies to CHB, but not to CEU: for example, the frequencies for allele G of SNP rs2373115 were 0.47 for JPT and 0.89 for CEU (see Supplementary Figure 3). HWE exact tests were performed to detect genotyping errors. SNP rs7101429 slightly deviated from the HWE in the *All* ($P=0.0497$) and *Negative-e4* ($P=0.0416$) sample sets in LOAD. Remaining nine SNPs were in HWE ($P \geq 0.05$) (Table 2).

A single SNP case-control study (χ^2 test) was then carried out. We did not observe any significant association of the SNPs with LOAD in not only the *All* set but also the two subsets (*Negative-e4* and *Positive-e4*) (Table 3). Multiple logistic regression analysis, with adjustment for the carrier status of the *APOE-e4* allele, age and gender as covariates, did not reveal any significant evidence of association (data not shown).

Pairwise LD measures, D' , of the SNPs are given in Supplementary Table 1. We found a strong correlation ($|D'| > 0.93$) between the 10 SNPs in each of the three sample sets. No difference in the LD block structure was observed between LOAD and control subjects. Using the HapMap genotype data for JPT and CEU, we further performed *in silico* LD mapping of a genomic region spanning about 500 kb. It was found that *GAB2* was completely encompassed by a highly structured single LD block in both JPT and CEU (see Supplementary Figure 1). However, there was an evident difference in the LD block boundary in the *S'* region of *GAB2*: in JPT, we observed a definitive break point in the block, but not in CEU (see Supplementary Figure 1).

In the LD block, including the whole *GAB2*, three common haplotypes (frequency > 1%), H1, H2 and H3, were inferred in all sample sets (see Supplementary Table 2). Haplotype H2 consisted of all major alleles of the 10 SNPs. In every sample set, no haplotypes exhibited significant differences between LOAD and controls (see Supplementary Table 2).

Discussion

Recently, it was shown that *GAB2*, encoding a scaffolding adaptor protein involved in several signal-transduction pathways, is associated with LOAD in Caucasians.⁸ At SNP rs2373115 located within this gene, a noticeable significance in allelic association ($P_{\text{allele}} = 9.7 \times 10^{-11}$) has been observed.⁸ Interestingly, the disease risk of this gene is increased by the *APOE-e4* allele: maximum OR of 24.6 (95% CIs, 7.4–116.8) was computed in carriers with both the

Table 2 SNP information on 10 GAB2 SNPs

dbSNP	Physical position (bp)	SNP position	Allele		GSR (%)	All			Negative-e4			Positive-e4							
			Maj	Min		Allele frequency	HWE	LOAD	Control	GSR (%)	HWE	LOAD	Control	Allele frequency	HWE	LOAD	Control		
rs901104	77608147	intron 9	G	A	99.1	0.58	0.42	0.1417	0.5789	99.3	0.57	0.43	0.1894	0.8252	98.6	0.58	0.42	0.4388	0.3866
rs1385600	77613814	exon 5	G	C	99.7	0.56	0.44	0.0792	0.6180	99.7	0.56	0.44	0.0962	0.7433	99.7	0.57	0.43	0.4042	0.6249
rs1007837	77618724	intron 3	T	A	98.4	0.56	0.44	0.0964	0.4818	98.3	0.56	0.44	0.1102	0.6206	98.7	0.57	0.43	0.4421	0.5383
rs2510038	77643682	intron 2	G	A	99.0	0.57	0.43	0.0695	0.3665	99.0	0.56	0.44	0.0944	0.5456	99.0	0.58	0.42	0.3633	0.3910
rs4945261	77667908	intron 2	G	A	98.5	0.57	0.43	0.0768	0.4817	98.6	0.56	0.44	0.0582	0.6203	98.2	0.58	0.42	0.5278	0.6205
rs7101429	77670615	intron 1	A	C	99.0	0.56	0.44	0.0497	0.5479	99.0	0.56	0.44	0.0416	0.7019	98.9	0.57	0.43	0.4857	0.5344
rs10793294	77674051	intron 1	C	A	98.0	0.80	0.20	0.3389	0.2567	98.3	0.80	0.20	0.7184	0.0780	97.5	0.79	0.21	0.0905	0.2930
rs4291702	77678896	intron 1	G	A	98.4	0.57	0.43	0.0681	0.3456	98.4	0.56	0.44	0.0794	0.6593	98.3	0.58	0.42	0.3992	0.7089
rs7115850	77722719	intron 1	C	G	99.7	0.56	0.44	0.1194	0.4842	99.8	0.56	0.44	0.0960	0.7017	99.4	0.58	0.42	0.5772	0.4600
rs2373115	77768798	intron 1	C	A	98.8	0.56	0.44	0.0770	0.5824	99.0	0.56	0.44	0.0930	0.7846	98.3	0.57	0.43	0.4019	0.4606

The genomic position of each SNP is according to NCBI build 36.2. The allele frequency for each sample set was calculated by combining LOAD and control subjects. Maj, major; Min, minor; GSR, genotyping success rate; HWE, Hardy-Weinberg equilibrium exact test (bold value indicates statistical significance at $P < 0.05$).

Table 3 Genotypic and allelic associations

Sample set	dbSNP	Genotype						Allele				OR (95% CIs)		
		LOAD			Control			LOAD		Control			P-value (d.f. = 1)	
		Maj	Ht	Min	Maj	Ht	Min	P-value (d.f. = 2)	Maj	Min	Maj	Min		
Overall set														
All	rs901104	559	768	306	554	793	301	0.8206	1886	1380	1901	1395	0.9539	1.00 (0.91–1.11)
	rs1385600	550	775	326	518	804	328	0.473	1875	1427	1840	1460	0.4007	1.04 (0.95–1.15)
	rs1007837	541	769	324	514	788	324	0.6366	1851	1417	1816	1436	0.5163	1.03 (0.94–1.14)
	rs2510038	550	766	321	531	789	321	0.7156	1866	1408	1851	1431	0.6263	1.02 (0.93–1.13)
	rs4945261	549	763	318	523	789	319	0.5864	1861	1399	1835	1427	0.4978	1.03 (0.94–1.14)
	rs7101429	541	764	329	524	797	323	0.6084	1846	1422	1845	1443	0.7602	1.02 (0.92–1.12)
	rs10793294	1044	518	54	1035	519	77	0.1347	2606	626	2589	673	0.2034	1.08 (0.96–1.22)
	rs4291702	555	760	314	525	789	315	0.5021	1870	1388	1839	1419	0.438	1.04 (0.94–1.15)
	rs7115850	551	777	321	530	799	323	0.6982	1879	1419	1859	1445	0.5612	1.03 (0.93–1.13)
	rs2373115	543	763	321	519	800	326	0.5071	1849	1405	1838	1452	0.4355	1.04 (0.94–1.15)
Subsets														
Negative- <i>e4</i>	rs901104	265	365	152	456	666	249	0.6473	895	669	1578	1164	0.8361	1.01 (0.89–1.15)
	rs1385600	262	365	162	425	672	275	0.4462	889	689	1522	1222	0.579	0.97 (0.85–1.09)
	rs1007837	255	362	162	421	659	272	0.5943	872	686	1501	1203	0.7715	0.98 (0.87–1.11)
	rs2510038	260	361	160	436	661	268	0.617	881	681	1533	1197	0.8747	0.99 (0.87–1.12)
	rs4945261	264	357	159	429	660	269	0.4285	885	675	1518	1198	0.5942	0.97 (0.85–1.10)
	rs7101429	258	355	165	427	669	273	0.3526	871	685	1523	1215	0.8232	0.99 (0.87–1.12)
	rs10793294	519	228	27	873	418	67	0.2019	1266	282	2164	552	0.0953	0.87 (0.74–1.02)
	rs4291702	264	357	156	432	660	265	0.4674	885	669	1524	1190	0.6136	0.97 (0.85–1.10)
	rs7115850	263	365	162	433	670	271	0.5133	891	689	1536	1212	0.751	0.98 (0.87–1.11)
	rs2373115	259	358	159	425	672	274	0.4068	876	676	1522	1220	0.5528	0.96 (0.85–1.09)
Positive- <i>e4</i>	rs901104	294	403	154	98	127	52	0.9071	991	711	323	231	0.9743	1.00 (0.82–1.21)
	rs1385600	288	410	164	93	132	53	0.9997	986	738	318	238	0.9994	1.00 (0.82–1.21)
	rs1007837	286	407	162	93	129	52	0.9866	979	731	315	233	0.9244	0.99 (0.82–1.20)
	rs2510038	290	405	161	95	128	53	0.9637	985	727	318	234	0.9757	1.00 (0.82–1.21)
	rs4945261	285	406	159	94	129	50	0.9616	976	724	317	229	0.7902	0.97 (0.80–1.18)
	rs7101429	283	409	164	97	128	50	0.7886	975	737	322	228	0.5107	0.94 (0.77–1.14)
	rs10793294	525	290	27	162	101	10	0.6649	1340	344	425	121	0.3862	1.11 (0.88–1.40)
	rs4291702	291	403	158	93	129	50	0.9981	985	719	315	229	0.9674	1.00 (0.82–1.21)
	rs7115850	288	412	159	97	129	52	0.8926	988	730	323	233	0.8083	0.98 (0.80–1.19)
	rs2373115	284	405	162	94	128	52	0.9571	973	729	316	232	0.8382	0.98 (0.81–1.19)

Maj, major; Ht, heterozygous; Min, minor; d.f., degree of freedom.

APOE-ε4 and *GAB2* SNP rs2373115 risk (G) alleles,⁸ suggesting a genetic interaction between these two genes. On the basis of these findings, we attempted here to replicate the genetic association of *GAB2* with LOAD in Japanese. In Reiman *et al*'s study,⁸ neuropathologically well-characterized brains of Caucasians were largely used (LOAD, 643; control, 404), whereas we utilized only clinically confirmed subjects (LOAD, 1656; control, 1656). However, no evidence of association of this gene was obtained in the *All*, *Negative-ε4* and *Positive-ε4* sets (Table 3). *GAB2* may not be a disease susceptibility gene for LOAD in Japanese.

As a possible explanation for the discrepancy between our results and the initial study,⁸ we consider an ethnic difference (Japanese vs Caucasian), genotyping technology (TaqMan[®] vs GeneChip[®] genotyping) and subject selection (clinically vs neuropathologically verified subjects) described above. With regard to the ethnic difference, Wright's F_{ST} statistic has been proposed for clarifying the level of between-population differentiation.¹³ F_{ST} is 0.145 (estimated from 3845 SNPs) among Asian (Japanese and

Chinese), African-American and European-American, and 0.013 (estimated from 8801 SNPs) between Japanese and Chinese.¹⁴ Across the 10 *GAB2* SNPs, we calculated F_{ST} using HapMap genotype data of JPT, CHB and CEU. The mean F_{ST} of these SNPs was 0.012 (standard deviation (SD), 0.006; range, 0.000–0.025) between JPT and CHB, and 0.219 (SD, 0.077; range, 0.164–0.435) between JPT and CEU. These data indicate that a higher level of genetic differentiation exists between JPT and CEU for *GAB2*. Recently, Chapuis *et al*⁹ could not replicate the initial finding⁸ even in European-Caucasian subjects ($N > 3000$), suggesting that *GAB2* is at best a minor disease susceptibility gene for LOAD. A meta-analysis is needed to confirm the association of *GAB2* with LOAD.

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3. アルツハイマー型認知症におけるアパシー(意欲障害)

鳥取大学医学部保健学科生体制御学 神保太樹・浦上克哉

日本が超高齢化社会となった現在において、認知症患者のいちじるしい増加は大きな社会問題となっている。おもなものとして、アルツハイマー病などの神経変性疾患による認知症が増加している。とくに、認知症全体の約半数を占めるアルツハイマー病に対しては薬物治療が可能となったが、いまだに根本治療薬は研究中の段階である。こうした現状で、認知症の周辺症状、いわゆる認知症の行動心理学的症候 (behavioral and psychological symptoms of dementia : BPSD) の理解と、それに対する対応が非常に重要となっている。本稿では、アルツハイマー病の BPSD の中でも、アパシーと呼ばれる症候について概説する。

認知症とは、脳や身体に生じた障害によって、正常に発達した知的機能が全般的かつ持続的に低下するために、日常生活に支障を生じる状態と定義されている。今日においては、腫瘍性疾患や、心臓脳血管性疾患などと共に、本邦での重要な課題として認識されている。

認知症には、アルツハイマー病 (Alzheimer's disease : AD) などに代表される神経変性疾患によるもの、脳血管性認知症 (Vascular Dementia : VaD)、アルコール脳症などの内分泌・代謝性中毒性疾患によるもの、クロイツフェルトヤコブ病な

どの感染性疾患によるもの、脳腫瘍などの腫瘍性疾患によるものなど、多様な原因がある。一般に、中核症状として認知機能の障害と、認知機能の障害に起因した周辺症状が観察される。

さらに、平均寿命の上昇によって、超高齢化社会となった本邦において、近年の認知症患者のいちじるしい増加は、社会の問題としても捉えられるが、多くは AD、あるいは VaD、ないしはその混合型であるとされる。かつ、本邦においては、VaD がかつて多くを占めていたが、現在は AD がその約半数を占めている。しかも、その割合は増加傾向にあるとされている¹⁾。2005 年の総務省統計局による統計結果では、本邦の 65 歳以上の高齢者は、男性は 1081 万人 (男性全体の 17.4%)、女性は 1475 万人 (女性全体の 22.5%) であり、その合計は 2556 万人である。これは本邦が世界的にも高齢者率が高いことを示しており、また、厚生省の試算結果によれば、その内、認知症有病率は、約 7.6% 程度になるだろうとされていた (図 1)。また、鳥取県大山町での疫学調査²⁾などを主として、いくつかの疫学調査が行われており、それによっても、現在までに認知症の有病率、とくに AD の有病率が顕著に増加していることが分かっている (図 2)。

このような現状にあつて、認知症の多くを占め

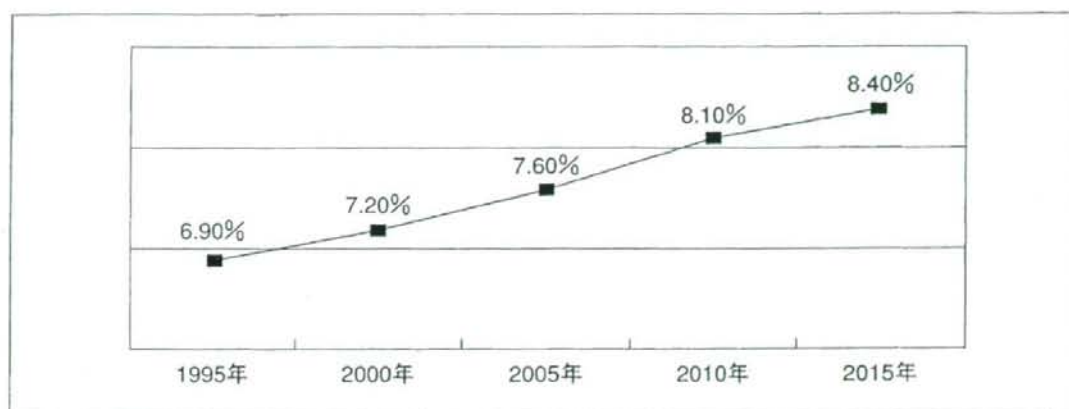


図1 認知症患者数の推計推移

(厚生省, 痴呆性老人対策に関する検討会報告, 1994年より引用して作成)

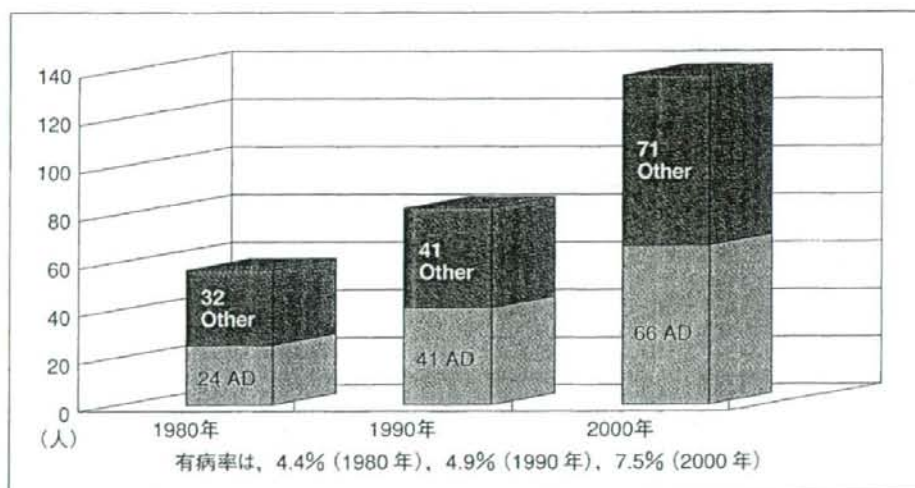


図2 鳥取県大山町における老年期認知症患者数の経年変化 (文献2より)

るADに対しては、塩酸ドネペジル（アリセプト®）などのアセチルコリン分解酵素阻害剤などによる薬物治療が可能となったことや、アミロイドβ蛋白の沈着を阻害するためのワクチン療法²⁹や、tauのリン酸化を減らし、神経原線維変化を抑制するGSK-3β阻害剤など、多様な治療法の研究が行われるようになってきていることなどから、すでに治療の時代に入ったといえるだろう。しかしながら、

本邦において認知症に対する根本治療薬は、いまだに開発中の段階であり、それ故に、認知症によって生じる多くの症候に対する理解と対応は、より重要になってきているといえる。認知症によってきたされる障害は、中核症状である認知機能の障害、すなわち記憶力の低下、見当識の低下、判断力の低下などを含む症状を軸として、その周辺症状であるBPSDへと発展する。

BPSDには、異常行動、過食、拒食などの摂食障害、攻撃性や、暴力行為などの障害、易刺激性、不安、脱抑制、多幸症状、抑うつ、睡眠障害、そしてアパシーといったさまざまな様相を呈するとされており、中でも、近年の報告では、アパシーがよく観察されるとされている。アパシーが出現しやすい疾患は、皮質下性認知症であるVaDであるとされてきた。このことは、CummingsらがVaDにおいてアパシーが普遍的に出現している病態であることを述べている³⁾ことなどからも明らかである。しかし、ADにおいても、アパシーが高頻度にみられるとする報告もあり、アパシーがおもに自発性の低下や、やる気の低下などによって日常生活動作 (Activities of Daily Living : ADL) 障害をきたすことを考慮すれば、アパシーに対する理解を深め、適切に対応することは非常に重要だと思われる。アパシーの出現率は、最高92%の患者に起こるとする報告もある⁴⁾が、概して動機づけの損失として定義されて、おもに情動の反応性が鈍くなるなどの挙動としても現れる。

A. アパシーとはどのような病態か?

今日、Apathyの訳語としては、発動性低下、意欲低下、発語低下、運動量低下、無関心、無感動などの多くの訳語が存在しているが、ADなどにおける、その本態としては、「やる気の低下」などが適切と考えられる。

ところで、アパシーにおいてやる気が低下する、という言葉を使うと、それは感情の障害としての印象を受けるが、実際には、アパシーはADL低下などをその本態とし、睡眠障害、異常行動、摂食障害などと関連の深い身体行動的因子として区分されている。Mirakhorらが、ADにおけるアパシーを初めとするBPSDについて、認知症の精神症

状評価法の1つであるNeuropsychiatric Inventory (NPI-D)⁵⁾によって得られた12項目の評価結果について、因子分析を行ったところ、4つのカテゴリに分けることができたとしている。それによれば、アパシーはいずれの場合であっても、異食・過食などの摂食障害、異常行動、睡眠障害、摂食障害などと同様に分類されると報告している⁶⁾(表1)。また、認知症の抑うつ症状の診断評価法として、Cornell Scale for depression in dementia (CSDD)がある。CSDDでは、面接を行うことによって、19項目を評価するが、ここでも意欲の低下は身体症状として分類されている。

さらに、上記の報告では、発生頻度からみると、アパシーの発生率は76%となっており、この報告が、全経過中での報告であることを加味して考えても、ADにおいてもアパシーが高度に発生するというを示唆している。また、アパシーと混同されやすい、抑うつ症状については、ここでは54%程度にとどまっている⁶⁾(表1)。また、他の報告においては、割合が多少変化していることもある。Starksteinらによれば、AD患者でアパシーをきたしたのは、354例中120例であり、これは約38%程度であった⁷⁾。さらに、Sergioらによれば、抑うつをきたさず、なおかつアパシーだけを呈するような群は、おおよそ、45%程度であった。ただし、抑うつと同時にアパシーをきたしているような群は、おおよそ、59%程度であったとしている。また、この時抑うつの指標となるハミルトンうつ病評価尺度においては、抑うつをきたしているような群と、アパシーのみの群では、スコアに有意な差があったとしている。さらに、この報告によれば、アパシーではADL障害がみられるとされていた⁸⁾。

また、Paulinoらの報告によれば、ADにおけるADLに対して、アパシーが関連する因子の1つで

表1 精神症候の発生率と、その因子分析結果(文献6より)

精神症候	因子負荷量 (VARIMAX rotation)				因子負荷量 (QUARTIMAX rotation)				因子負荷量 (EQUAMAX rotation)				発生数/対象数(%)
	1	2	3	4	1	2	3	4	1	2	3	4	
興奮	0.370	<u>0.639</u>	0.039	0.166	-0.406	<u>0.625</u>	-0.030	0.135	<u>0.632</u>	0.355	0.068	0.211	273/435 (62.8)
不安	0.139	<u>0.619</u>	0.289	-0.099	0.179	<u>0.627</u>	0.238	-0.117	<u>0.618</u>	0.125	0.305	-0.066	218/434 (50.2)
抑うつ	0.096	<u>0.610</u>	0.390	-0.003	-0.045	<u>0.638</u>	0.352	-0.004	<u>0.601</u>	-0.118	0.397	0.024	234/431 (54.3)
易刺激性	0.310	<u>0.699</u>	-0.072	0.125	0.339	<u>0.680</u>	0.141	0.093	<u>0.695</u>	0.300	-0.045	0.171	272/432 (63.0)
妄想	0.176	0.255	<u>0.686</u>	0.156	0.237	0.294	<u>0.653</u>	0.149	0.235	0.137	<u>0.699</u>	0.169	215/434 (49.5)
幻覚	0.259	0.059	<u>0.738</u>	-0.011	0.307	0.096	<u>0.716</u>	-0.016	0.048	0.227	<u>0.750</u>	-0.003	121/435 (27.8)
脱抑制	0.196	0.376	-0.188	<u>0.651</u>	0.229	0.366	-0.234	<u>0.631</u>	0.349	0.172	-0.168	<u>0.678</u>	128/434 (29.5)
多弁性	0.028	-0.119	0.241	<u>0.845</u>	0.076	-0.086	0.234	<u>0.848</u>	-0.163	-0.018	0.245	<u>0.837</u>	72/433 (16.6)
食行動の異常	<u>0.527</u>	0.141	0.176	0.143	<u>0.548</u>	0.130	0.131	0.118	0.134	<u>0.511</u>	0.202	0.171	268/421 (63.7)
アパシー	<u>0.725</u>	0.258	-0.068	-0.034	<u>0.727</u>	0.217	-0.128	-0.073	0.265	<u>0.725</u>	-0.032	0.011	330/434 (76.0)
異常行動	<u>0.653</u>	0.194	0.184	0.138	<u>0.677</u>	0.177	0.129	0.106	0.188	<u>0.636</u>	0.216	0.174	280/434 (64.5)
睡眠異常	<u>0.659</u>	-0.030	0.349	-0.006	<u>0.677</u>	-0.038	0.310	-0.032	-0.032	<u>0.644</u>	0.376	0.018	229/426 (53.8)

あることを報告している。それによれば、アパシーの関与は13.6%程度とされており⁹⁾、VaDにおいてZawackiらが報告している36%の関与¹⁰⁾に比べれば少ないながらも、ADLにアパシーが密接に関係していることを示唆しているといえる。

以上のように、アパシーとはおもにADLの低下をきたす症候であり、身体症状として観察されることが多く、また従来いわれていたVaDのみならず、ADであっても一般的にみられる疾患であろうと考えられる。

しかし、一般的には、アパシーの発生率は40~80%程度というように、幅をもって考えられている。このことからADにおいてアパシーが恐らく高頻度で発生するであろうことは予想できても、正確なアパシーの発生割合については、今後のさらなる報告が待たれていると考えられる。

B. アパシーにおける神経生理学的変化

アパシーは、前頭葉への損傷に伴う症候群と、それによる行動の幅広い変化によって特徴づけられる、自発的な実行の機能不全であるともいえる。これまで、前頭葉は物事を実行するためのもので

あり、思考、自発性、感情、性格、理性などの中心であるとされてきた。前頭葉を含んで、前頭連合野は頭頂連合野や側頭連合野などから入力を受けると同時に、運動系に出力がある。

ところで、前頭連合野は、おもにワーキングメモリと関連して語られるが、ワーキングメモリとは、行動や決断のための情報を統合して、行動を導くための脳内システムであるといつてよい¹¹⁾。前頭連合野がそのセンターとなっていることは、すでに確認されているが、ヒトの遅延反応の遂行に従って前頭連合野の背外側部が賦活するなど、脳イメージング法による研究によっても示されつつある。また、ワーキングメモリは、少なくとも情報の受容と、保持、そして行動としての出力という3つの過程からなることはよく知られているが、前頭連合野においては、これらはその過程それぞれに対して、コラム状に神経細胞が存在しており、ある情報処理において、前頭連合野に刺激が与えられると、コラム状に位置する神経細胞を活動が伝わっていくことが明らかにされている。こうした前頭前野でのデータの統合を前頭前野の総合コラム説¹²⁾というが、各コラム内でそれぞれ起こった過程が、層状に進行していくことによ

て、行動を導くとしている。今、AD やその他のアパシーの病態は、前頭葉前部で皮質下構造でのコリン作動性ニューロンの損失に関連がある可能性があるとされているが、この場合、こうしたワーキングメモリに影響があり、それによって感情を発露することも含めた、行動の実効性が阻害されているのかもしれない。

これを裏付けるように、Benoit らの報告によれば、AD におけるアパシーでは、内側眼窩正面の前帯、舌状回とその周辺でかなり活動の低下がみられる¹⁰⁾。さらに、アパシーをきたす群とアパシーのない群を比較したとき、アパシーをきたす群では、前頭回 (ブロードマン野 10, 8, 9 など) で活動量が顕著に低下している。また、前帯状皮質における血流の低下はアパシーにおいてよく現れるが、Lanctôt らによれば、それはおもに左前帯状皮質におけるものらしい¹⁰⁾。

さらに、Benoit らによれば、AD 患者における認知、情動および抑うつといったアパシーの症候が、前頭回などの脳の血流低下と相関していたと報告されている¹⁰⁾。

しかし、たとえば CERAD 基準¹⁰⁾では、確かに AD の組織学的な基準に前側頭回などが含まれるものの、AD において、前頭連合野はおもな障害部位とは言い難いかもしれない。AD におけるアパシーを考えた場合に、前頭連合野は、顕著に障害される部位とは言い難いが、Braak らによれば、アルツハイマー神経原線維変化 (neurofibrillary tangle: NFT) は、嗅内野から、大脳辺縁系、海馬と進展した後に、新皮質連合野に出現するとされている¹⁰⁾。確かに、初期の AD であっても、新皮質連合野にわずかながら NFT が出現するが、しかし、AD において、アパシーが障害部位と関連するとすれば、新皮質ステージ以前にも原因が見い出せるかもしれない。

表 2 アパシーと、脳の活動との相関 (文献 14 より)

関心領域 (ROI)	相関係数	p 値	
眼窩前頭野	右半球	-0.29	0.04*
	左半球	-0.05	0.72
前帯状回	右半球	-0.14	0.34
	左半球	-0.39	0.005*
視床/視床下部	右半球	0.08	0.58
	左半球	-0.12	0.42
上側頭回	右半球	-0.10	0.49
	左半球	0.27	0.06
中側頭回	右半球	0.26	0.07
	左半球	0.33	0.02*
海馬	右半球	0.43	0.002*
	左半球	0.27	0.06

*スピアマンの順位相関係数の有意性検定で有意差有り

前述した Lanctôt らの報告でも、眼窩前頭野や、前帯状回、中側頭回などに加えて、海馬についても、顕著な活動性の低下がみられており、正常な海馬の血流の維持は、アパシー症状の重症度の予測因子として現れるとしている (表 2)¹⁰⁾。

ところで、海馬とはおもにエピソード記憶や、陳述記憶に関わり、こうした記憶を記録、あるいは符号化して、一時的に保持、貯蔵を行ってから新皮質に定着させる部位であるとされている。また、海馬は動機付けをコントロールしているともいわれている。これらを踏まえれば、VaD のように、前頭前野におもに障害があるような認知症に対してアパシーが高率にみられることと比較して、AD においてもアパシーがある程度顕著にみられることを理解する助けとなるかもしれない。

C. アパシーと抑うつとの鑑別

前述したように、アパシーは抑うつと類似した捉えられ方をしている。実際、アパシーが抑うつと認知障害の双方の結果から起こるとすることもある。また、BPSD の中で、両者はいずれも感情障害と捉えることもできる。そして、やる気というものは、一般に精神症状として捉えられること

表3 アパシーと抑うつにおける特徴的な症候の比較（文献19より一部改変）

アパシーに特有の症候	アパシーと抑うついずれにもみられる症候	抑うつに特有の症候
反応が鈍くなる 無関心 社交性が低下する 自発性の低下 物事に対する持続性の低下	物事に関する関心が低下する 精神活動の低下 易疲労性/睡眠障害 洞察力の低下	不快を表す 自殺願望がある 自己批判的 罪悪感を有する 悲観的/絶望的

が多い。これらから、混同されがちな両者ではあるが、アパシーと抑うつとの発生頻度には隔たりがあり、臨床的にも別の病態であるにとらえた方が利にかなっていると考えられる。ADにおけるアパシーの頻度についてはすでに述べたとおりだが、ADにおいての抑うつについて述べると、その頻度はおおむね20%程度であろうと考えられている。さらに、Sergioらによれば、その両方をきたす場合もかなり多いと報告されている⁸⁾。ADにおいては、コリン作動性ニューロンの機能障害が顕著であるが、Emeryらによれば、抑うつはこの機能障害が顕著にみられる場合にきたされるといわれている¹⁰⁾。

アパシーと抑うつとの鑑別については、症状の重なりから、混同されて診断されることが多い。鑑別方法は、まだ十分確立されているとは言い難いが、観察によってある程度の鑑別を行うことも可能である。Allanらによれば、アパシーと抑うつとは、同様に現れる症状もかなり多いが、社交性の低下、自発性の低下、持続性の低下などのように、アパシーに特有とされる症候と、自殺願望、罪悪感、自己批判性など、抑うつに特有の症候とに分類することができるとしている（表3）。しかし、とくに、不快を表すかどうか（悲しみ、罪責感、自己批判、無力感や絶望感）などによって、抑うつとアパシーとはよく区別できると述べている。抑うつでみられる興味の喪失は絶望感、悲壮感、絶望感のような感情に関連がある可能性が高

いが、それとアパシーを区別するのは、こうした感情の問題に対する認識がどのように現れてくるかであるとしている。彼らはまた、アパシーと抑うつとの関連はより多くのものを意味する可能性があり、それは、脳の障害部位とも関連があるのではないかとしている¹⁰⁾。

以上のような点で、アパシーと抑うつとは症状からの鑑別がある程度可能であるが、アパシーには抗うつ薬は効果が無く、むしろアセチルコリン分解酵素阻害剤が有効と示唆されて²⁰⁾おり、薬物療法への反応からの鑑別も不可欠であるといえる。

まとめ

アパシーを含めて、BPSDに対して対応することは、臨床現場で非常に重要なことといえる。認知症にBPSDが出現する背景として、神経変性からなる中核症状、すなわち記憶障害などによるものがあるが、身体機能の低下や、身体疾患の合併、環境因子などが高度に関係する。アパシーを含んで、ADにおける精神症状には、介護者に対する誤認などはみられるものの、統合失調症などでみられるような精神症状はみられず、また対症療法によって寛解することが多い。

アパシーをきたす患者は、自発性の低下によって、より多くのサポート、管理と社会資源利用を必要としている。また、近年問題になっている、

高齢者の交通事故にも関連があると指摘されており²⁰⁾、介護者に高度な負担をかける可能性がある。

さらに、アパシーは他の問題行動に非常に深い関連があるとされており、とくにケアの現場においては、自発的に何もしないこと、できないことにどのように対処すべきか、ということは大きな課題である。BPSDをきたす認知症に対して、機能、すなわちできることが失われないようにするということや、失われたと思われる機能をどのように拾い上げ、患者に「できること」を提供するかというのは非常に重要な課題といえる。この時、アパシーが含む自発性の問題は、こうした機能のリカバリと密接に関係しているといっておく、軽度なアパシーに対しては、薬物療法のみならず、周囲から自発的にできるであろうことを提供することや、対症療法として何か楽しさを与えるようなケアも必要だと最後に書き添えたい。

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