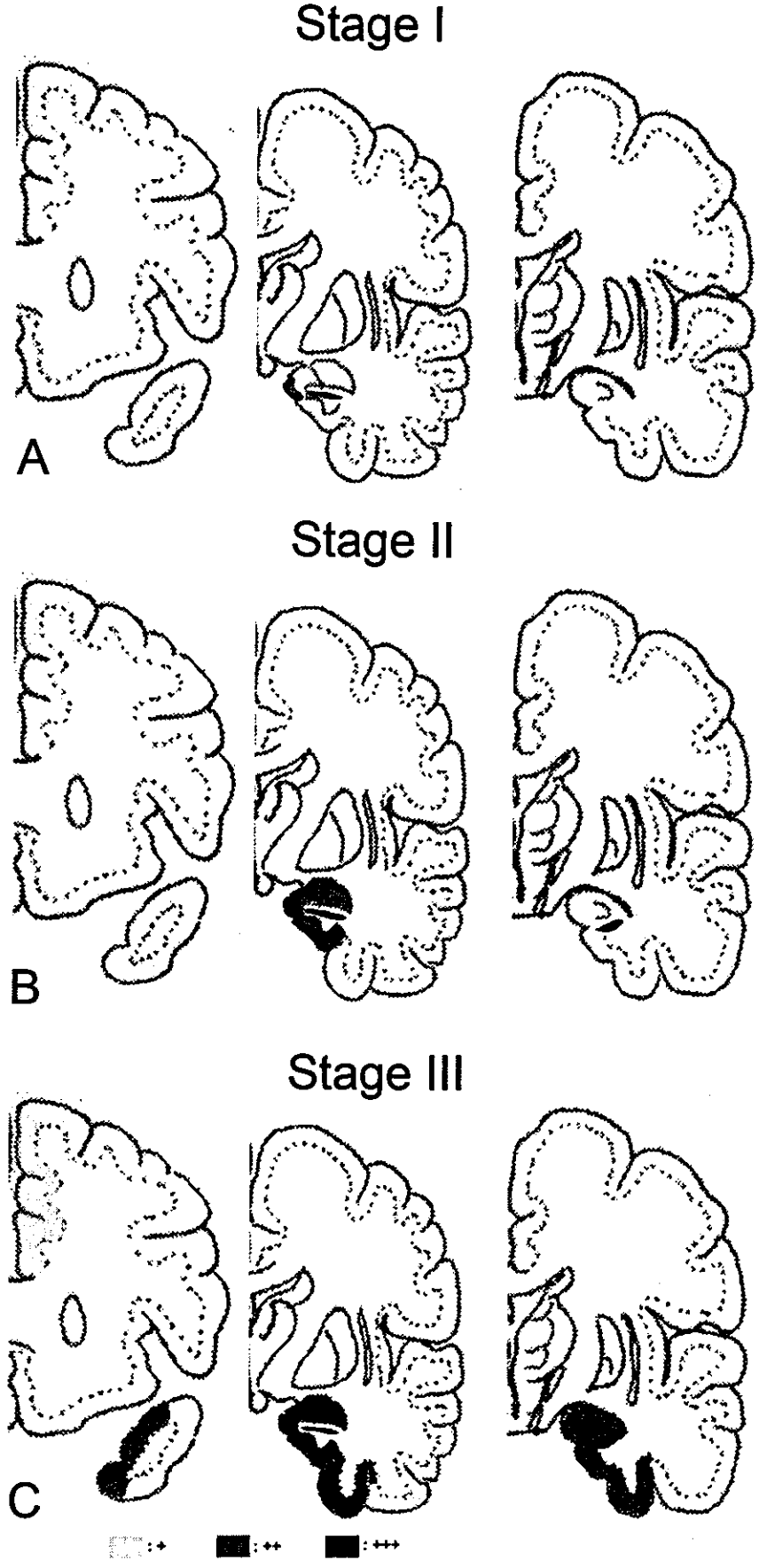
Stage I
A
Stage II
B
Stage III
C
: +
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methods, as well as immunohistochemically using anti-phosphorylated tau (AT8, Innogenetics, Themes, Belgium; PHF1, a gift from Dr. P. Davies), anti-tau (anti-human tau, a gift from Dr. Y. Ihara; Alz50, a gift from Dr. P. Davies), anti-4-repeat-specific (4R) tau antibody (a kind gift from Dr. H. Mori), anti-Aβ 11–28 (12B2, IBL, Maebashi, Japan), anti-α-synuclein (LB509, a gift from Dr. T. Iwatsubo), and anti-ubiquitin (Dako, Glostrup, Denmark) antibodies using a Ventana NX20 (Ventana, Tucson, AZ) autoimmunostainer (11).

For the staging of neurofibrillary tangles (NFTs) and senile plaques (SPs), Braak and Braak criterion (9) was applied. For the staging of Lewy bodies, our staging method (12) was employed. Neuropathological diagnosis of degenerative dementia followed the previously reported criteria (1). Briefly, a diagnosis of Alzheimer disease (AD) was based on Braak's tangle stage equal to or above IV combined with plaque stage C, diagnoses of neurofibrillary tangle-predominant form of dementia and dementia with grains on Jellinger's criteria (13, 14), and a diagnosis of dementia with Lewy bodies (DLB) on its consensus guidelines (15). Neuropathological diagnosis of vascular dementia followed clinical (16), radiological (17), or pathological (18) criteria.

ApoE Genotyping

Genomic DNA was extracted from the kidneys (which had been snap-frozen at autopsy) and apoE genotyping was performed by the PCR method (19), as previously reported (1, 2). The results of typing were available for 1,114 of 1,241 cases in Group A.

Statistical Analysis

Statistical analysis was performed using the chi-square test or the Fisher exact test for comparisons of categorical data, Student *t*-test for comparison of means for continuous outcomes, Mann-Whitney *U*-test and Kruskal-Wallis test for nonparametric analysis, and Spearman correlation coefficient by rank for correlation of discrete scores. Statistical significance was established at the $p < 0.05$ level.

RESULTS

Neuropathology

The cases of degenerative dementia in Group A were neuropathologically classified into 105 cases of AD, 50 cases of dementia with grains, 33 cases of DLB (14 cases of neocortical form and 19 cases of transitional form), 13 cases of AD plus DLB, 13 cases of neurofibrillary tangle-predominant form of dementia, 8 cases of progressive supranuclear palsy, 4 cases of corticobasal degeneration, 4 cases of dementia with grains plus neurofibrillary tangle-predominant form of dementia, and 2 cases of DLB plus dementia with grains. 103 cases were categorized as vascular dementia. Group B did not include either degenerative or vascular dementia, except for a 49-year-old man with Huntington disease and a 57-year-old man with myotonic dystrophy, both of whom may have presented with mild cognitive impairment.

Staging of AGs

The detection of AGs was done by the Gallyas-Braak method and confirmed by immunohistochemical analyses with AT8, PHF1, Alz50, anti-human tau, anti-4R-specific antibody, and anti-ubiquitin antibodies.

The youngest case with AGs was a 56-year-old male from Group B. The incidence of grain-positive cases definitely increased with age. The distribution of AGs followed a stereotypic regional pattern and could be classified into the following stages (Fig. 1):

Stage 0: No grains are detected.

Stage I: Argyrophilic grains are observed in the ambient gyrus, usually forming clusters and frequently affecting the most anterior area of the CA1 of the hippocampus. The cortical and basolateral nuclei of the amygdala may be mildly involved. Oligodendroglial coiled bodies are scattered in the affected gray matter as well as its subcortical white matter. Bush-like astrocytes (20), defined as astrocytes with many thin processes immunoreactive with anti-phosphorylated tau antibodies but not well visualized with the Gallyas-Braak silver staining method, may be seen in the affected areas of the amygdala, but are quite rare.

Stage II: Argyrophilic grains definitely involve the amygdala and accompany ballooned neurons and bush-like astrocytes. Argyrophilic grains are apparent in the more posterior transentorhinal cortex and subiculum and the more anterior medial temporal lobe. A few pretangles, defined as intracytoplasmic neuronal accumulation of the epitope of anti-phosphorylated tau antibody not recognized by authentic silver staining, appear in the dentate gyrus. Bush-like astrocytes appear in the ambient gyrus, but superficial spongy degeneration involving the ambient gyrus is not observed.

Stage III: Argyrophilic grains are more apparent now in the insular cortex, the anterior cingulate gyrus, the nucleus accumbens, the septal nuclei, the hypothalamus,

←

Fig. 1. The topographical distribution of argyrophilic grains (AGs) in each grain stage. Three coronal sections through the genu of corpus callosum, the mammillary body, and the lateral geniculate body. Scoring of the frequency of AGs in sections stained with the Gallyas-Braak silver impregnation method, based on the number of grains in a ×400 field was as follows: + = 20 to 50; ++ = 50 to 100; and +++ = >100, as reported (1). **A:** Stage I: AGs are localized to the ambient gyrus, the anterior CA1 of hippocampus, the anterior entorhinal area, and the amygdala. **B:** Stage II: AGs are more apparent in the medial temporal pole, in the posterior subiculum, and the entorhinal and transentorhinal cortex. **C:** Stage III: AGs involve the anterior cingulate gyrus, septum, accumbens, gyri recti, insular cortex, and hypothalamus in addition to the medial temporal lobe.

and the gyri recti beyond the boundary of the temporal lobe. Pretangles in the fascia dentata increase in number. Tau-immunoreactive ballooned neurons are scattered in the affected area, including the anterior cingulate gyrus and the entorhinal area. Bush-like astrocytes are frequent in the amygdala, the gyrus rectus, and the nucleus accumbens and are scattered in the other affected areas. Superficial spongy degeneration associated with grains is present most prominently in the ambient gyrus, followed by the cortical subnucleus of amygdala, the posterior entorhinal area, and the medial temporal pole. In the terminal stage, marked atrophy of the junction between the amygdala and the anterior temporal lobe is a characteristic feature (2). The size of AGs apparently increases with advanced staging. However, in the terminal stage, the number of grains appears to be decreased in the areas where severe neuronal loss is present (1).

Clinical Correlation with the Staging of AGs

Cases from Group A were categorized as follows: stage 0, 792 cases (63.8%); stage I, 234 cases (18.9%); stage II, 118 cases (9.5%); and stage III, 97 cases (7.8%).

CDR was available in 1,105 out of 1,241 cases in Group A as follows: CDR0, 436 cases; CDR0.5, 190 cases; CDR1, 193 cases; CDR2, 124 cases; and CDR3, 162 cases. Further analysis was done for these CDR cases.

Among the 479 cases of dementia (CDR 1, 2, and 3), 50 cases presented with AGs as the only morphological substrate that might explain the cognitive decline. Forty-seven of these 50 cases were classified as argyrophilic grain stage III and the remaining 3 cases as stage II. The 3 stage II cases presented with sparse NFTs (Braak stage I) and SPs (Braak stage A) without Lewy bodies (stage 0) or any vascular lesions possibly contributing to cognitive decline (17, 18).

Among the 66 stage III cases whose CDR was available and who did not have any other degenerative or vascular dementing lesions, 47 cases had a clinical description of dementia as stated above, 17 cases were classified as CDR0.5, and 2 cases as CDR0. The rate of dementia (CDR> = 1) among argyrophilic grain stage III cases was 71.2%, and the percentage of cases with cognitive impairment (CDR> = 0.5) reached 97%. One of the 2 CDR0 cases had a history of suicide attempt and the degeneration of the ambient gyrus was milder, and the remaining case showed marked right-sided predominance of grains with right-handedness. The difference between the CDR0.5 and CDR3 cases was macroscopically distinct atrophy of the ambient gyrus in the latter.

ApoE Genotyping and AGs

There was no correlation between the staging of AGs and apoE genotyping or apoE allelic frequency (Table 1). However, comparing the average argyrophilic grain stage of each allele, the heterozygotes for the ε2 allele (0.64) tended to have higher stage than the combinations of the other alleles (ε3: 0.54 and ε4: 0.44, Mann-Whitney *U*-test, p = 0.094) and homo- or heterozygotes for ε3 tended to have higher stage than homozygotes for ε4 (p = 0.056), although no statistical significance of these differences was found.

TABLE 1
Apolipoprotein E Status and Staging of Argyrophilic Grains (AGs)

	AG Stage			
Genotype	0	I	II	III
23	4.8	1.7	0.8	0.6
33	48	14.5	7	5.6
34	10.7	3.1	1.3	0.4
44	1.2	0.1	0	0.2
Allelic frequency				
2	2.4	0.9	0.4	0.3
3	55.8	17	8	6.1
4	6.5	1.6	0.6	0.4

The percentage of each apoE genotype and allelic frequency with argyrophilic grain (AG) stage. The total number was 1,114 cases. There was no significant difference among the AG stages.

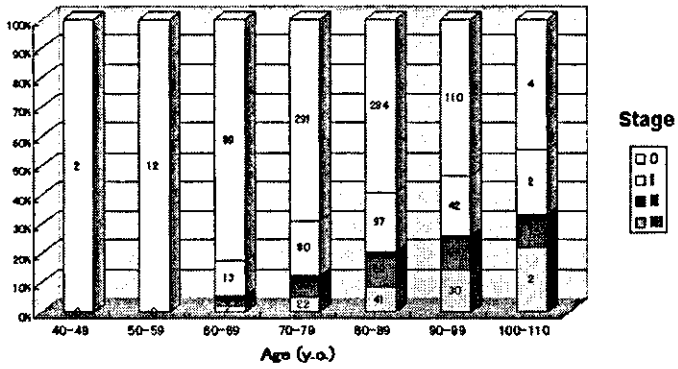

Fig. 2. The correlation between age and the argyrophilic grain stage. The argyrophilic grain stage significantly increased with age (Spearman correlation coefficient by rank, p < 0.0001).

The Influence of Age, Gender, and Brain Atrophy on AGs

The percentage of cases carrying AGs and the average staging both increased with age (Spearman rank correlation coefficient, p < 0.0001, Fig. 2). As for gender difference, the stage was significantly higher (Mann-Whitney *U*-test, p = 0.003) in females (average = 0.69) than in males (average = 0.54) and the frequency of grains was also significantly higher (χ^2 test, p = 0.0049) in females (40%) than in males (32%). However, no gender difference was detected in stage III cases (χ^2 test, p = 0.053) (Fig. 3). The average brain weight from each grain staging was as follows: stage 0, 1,227 ± 139 g; stage I,

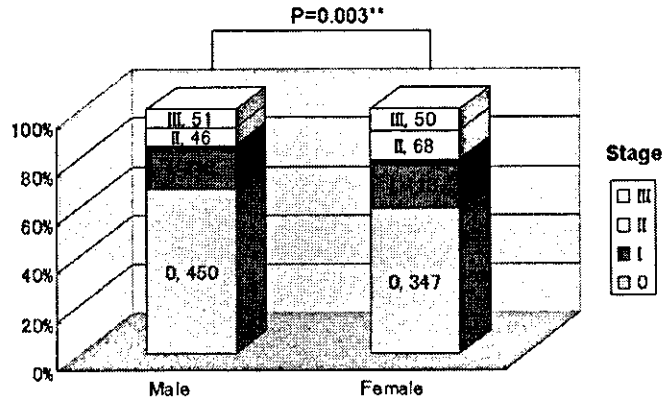

Fig. 3. The correlation between each gender and the stage of argyrophilic grains (AGs). Both average AG stage (Mann-Whitney *U*-test, p = 0.003) and frequency (χ^2 test, p = 0.0041) were higher in females than males. No statistically significant gender difference was found among stage III cases (χ^2 test, p = 0.53).

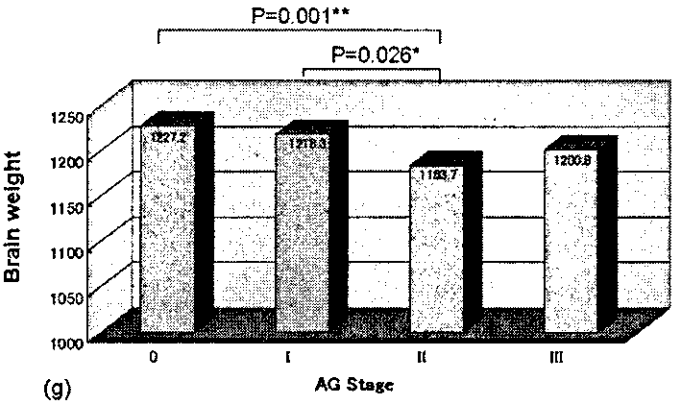

Fig. 4. The stage of argyrophilic grains and the brain weight. The average brain weight was significantly lower in argyrophilic grain stage II than stage 0 (Student *t*-test, p = 0.001) and I (p = 0.026). However, there were no significant differences between stage 0 and I (p = 0.39), stage 0 and III (p = 0.65), stages I and III (p = 0.25), or stages II and III (p = 0.31).

1,218 ± 134 g; stage II, 1,183 ± 136 g; and stage III, 1,201 ± 109 g. The average brain weight in stage II was significantly lower than in stage 0 (Student *t*-test, p = 0.001) and I (p = 0.026) (Fig. 4).

The average NFT stage (Braak) was significantly higher in argyrophilic grain-positive cases than in negative ones (Fig. 5A), but NFT stage was not correlated with argyrophilic grain staging. There was no relationship between the staging of AGs and Braak staging of SPs or our staging of Lewy bodies (Fig. 5B, C).

AGs in Other Neurodegenerative Diseases

The average staging of AGs in several neurodegenerative diseases is shown in Table 2. Both the frequency (Fisher exact probability test) and stage (Mann-Whitney *U*-test) were significantly higher in progressive supranuclear palsy (p < 0.0001 and p < 0.0001, respectively), neurofibrillary tangle-predominant form of dementia (p = 0.0048 and p = 0.0017), and DLB (p = 0.001 and p = 0.0017) compared with those of the background (Table 2).

DISCUSSION

This is the first report proposing a system for the staging of AGs and demonstrating the usefulness of this staging system for examining the contribution of AGs to cognitive decline.

In this report, we confirmed our previous finding (1) that dementia with grains is associated with grain-associated spongy degeneration of the ambient gyrus, spreading to the more anterior medial temporal lobe and to the more posterior parahippocampal gyrus. In this stage, AGs are observed beyond the boundary of the medial temporal lobe to the anterior cingulate gyrus, the gyri recti, the septal nuclei, the nucleus accumbens, the hypothalamus, and the insular cortex. In turn, when the cognitive state of the cases with AGs showing such widespread distribution was examined, 97% of the cases presented with cognitive impairment. Therefore, we categorized this phase as advanced stage (stage III). In more than 50 percent of cases, AGs were found only in the ambient gyrus and its vicinity, confirming the ambient gyrus as an initial site of involvement in argyrophilic grain-related senile change. Thus, we categorized these cases as the early stage (stage I). The relatively uniform progression of AGs may result in progression to the intermediate stage II. This staging method is quite convenient and only requires a section of the ambient gyrus as the minimal requirement, in addition to the sections recommended for use in CERAD and Braak methods.

The age-dependent increase in the incidence and severity of AGs that we observed here is in accordance with a previous report (21), although some exceptional cases of dementia with grains with either younger onset or with neocortical involvement have been reported (2, 22, 23).

Our statistical analysis showed that AGs were independent of SPs or Lewy bodies. NFT stage was significantly higher in argyrophilic grain-positive cases than negative ones, suggesting a mutual interaction in the deposition of tau. However, since NFT stage was not related to argyrophilic grain stage, the interaction may not be strong. The preponderance of AGs in females but a lack of gender difference in dementia with grains was first noted in this study and will require further confirmation in other groups.

The genetic effect of ApoE genotype on AGs is controversial (1, 24–26). In dementia with grains, a higher frequency of apoE ε2 and a lower frequency of apoE ε4

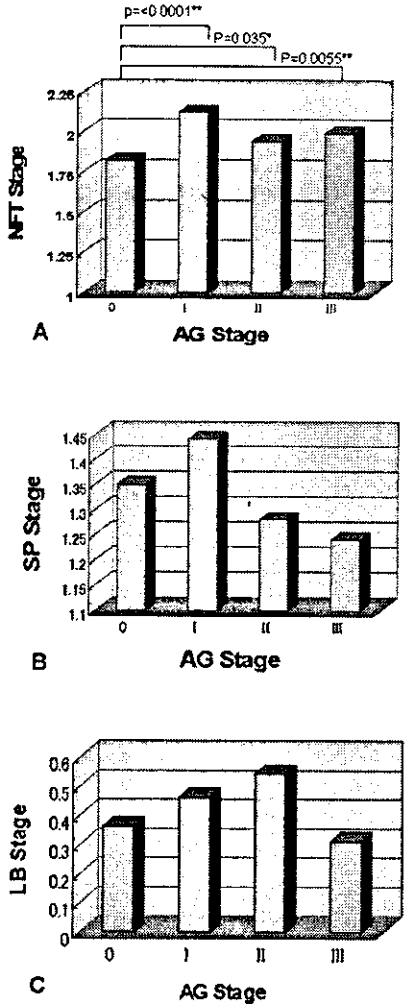

Fig. 5. The correlation between the stage of argyrophilic grains (AGs), and each stage of neurofibrillary tangles (NFTs), senile plaques (SPs), or Lewy bodies (LBs). **A:** The average NFT stage (Braak) was significantly higher in AG-positive cases than in negative cases (Mann-Whitney *U*-test, stage I: $p < 0.0001$, stage II: $p = 0.035$, stage III: $p = 0.0055$), but NFT stage was not related to AG staging. **B:** Average SP stage (Braak) was not different in the different AG stages. **C:** Average LB stage showed no difference among AG stages.

were reported (1, 24–26). We could not find any significant correlation between the staging of AGs and apoE genotyping in this study. Previously, we reported that dementia with grains with minimal senile changes was more common in subjects with apoE ε2 (1). The exclusion of stages B and C of SPs (Braak), which was done to highlight pure cases of dementia with grains in that study, might have contributed to the increase of the ε2 allele rather than dementia with grains itself. In this study, we strictly excluded cases with any vascular lesions possibly contributing to dementia (17, 18) in order to highlight the independent contribution of argyrophilic grain stages to cognitive decline. This difference in the selection of the cases of dementia with grains may also have influenced the difference in the correlation with apoE genotype.

The distinction between argyrophilic grain stages II and III is the involvement of the frontal lobe beyond the boundaries of the medial temporal lobe, as well as the presence of grain-associated spongy degeneration involving the ambient gyrus. For strictly accurate evaluation of this advanced stage, sections of the insular cortex, the anterior cingulate gyrus, the septal nuclei with the nucleus accumbens, and the gyri recti should be investigated with the Gallyas-Braak method for the presence of grains. It is still controversial whether AGs really contribute to cognitive decline. Our study showed that it is highly probable that the pathology in argyrophilic grain stage III contributed to cognitive decline. It is worth noting that approximately one fourth of stage III cases were categorized into CDR0.5 or the mild cognitive impairment level. The macroscopically distinct atrophy of the ambient gyrus separated more advanced dementia with grain cases from these CDR0.5 cases, confirming the importance of the ambient gyrus in cognitive decline associated with grains. However, the distinction between CDR0.5 and CDR1 cases was often very difficult and may indicate the limitation of this type of retrospective study. Prospective studies are now ongoing in our institute. Dementia with grains was the second most common form of degenerative dementia in our series as well as in studies by Braak and Tolnay (personal communication with Drs. Braak and Tolnay). Cases in these series represent data from the general geriatric population and have been analyzed by morphological examination able to detect AGs. Since many of dementia with grains cases present clinically with a milder form of dementia or mild cognitive impairment, prospective studies with special attention to clinical cognitive decline as well as morphological appearance of AGs may confirm the biological significance of AGs in human aging.

Argyrophilic grains have frequently been reported to be associated with other neurodegenerative diseases (27). In this large series, cases of progressive supranuclear palsy, neurofibrillary tangle-predominant form of dementia, and DLB clearly had a higher incidence as well as more advanced staging of grains compared with the background.

In conclusion, our staging method may contribute to better understanding of the role of AGs in the age-associated cognitive decline involving the human central nervous system.

ACKNOWLEDGMENTS

We thank Dr. Yasuo Ihara (Department of Neuropathology, University of Tokyo), Dr. Peter Davies (Department of Pathology, Albert Einstein College of Medicine, Bronx, NY), Dr. Hiroshi Mori (De-

TABLE 2
Argyrophilic Grains in Neurodegenerative Disorders

	Stage 0	Stage I	Stage II	Stage III	Frequency		Average stage
PSP	0	4	4	2	10/10	(100%)**	1.8++
NFTD	5	5	3	4	12/17	(71%)**	1.35++
DLB	12	12	7	3	22/34	(65%)**	1.029+
MSA	1	1	1	0	2/3	(67%)	1
AD	75	20	7	3	30/105	(29%)	0.4
ALS	7	1	1	0	2/9	(22%)	0.33
PD	8	1	1	0	2/10	(20%)	0.3
CBD	3	1	0	0	1/4	(25%)	0.25
Group A	792	234	118	97	449/1,241	(36%)	0.53

Stage: argyrophilic grain stage, PSP: progressive supranuclear palsy, NFTD: neurofibrillary tangle-predominant form of dementia, DLB: dementia with Lewy bodies, MSA: multiple system atrophy, AD: Alzheimer disease, ALS: amyotrophic lateral sclerosis, PD: Parkinson disease and CBD: corticobasal degeneration; Group A: total cases in Group A.

**: Significantly high incidence compared with background, Fisher exact probability test, $p < 0.01$. +: Mann-Whitney *U* test, $p < 0.05$, ++: Mann-Whitney *U* test, $p < 0.01$.

The average AG stage of 1,241 cases was 0.53. The incidence of AG in the background was 36%. Both the frequency and stage of argyrophilic grains were higher in PSP, NFTD, and DLB.

partment of Neuroscience, Osaka City University Medical School, Osaka, Japan), and Dr. Takeshi Iwatsubo (Department of Neuropathology and Neuroscience, Graduate School of Pharmaceutical Science, University of Tokyo), for donation of the antibody; Mr. Naoo Aikyo, Ms. Mieko Harada, and Ms. Nobuko Naoi for the preparation of sections; Ms. Hiroko Matsuoka for molecular techniques and Dr. Kinuko Suzuki (Department of Pathology and Laboratory Medicine, the University of North Carolina at Chapel Hill) for helpful discussions and comments. Part of this study was presented at the 77th Annual Meeting of the American Association of Neuropathologists held in Denver, Colorado, June 2002.

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Received February 13, 2004
Revision received April 27, 2004
Accepted May 7, 2004

特集 神経病理：最前線

軽度認知機能障害の神経病理*

村山繁雄** 齊藤祐子** 笠畑尚喜**

軽度認知機能障害とは，正常と痴呆の中間に位置し，適切な介入により，痴呆への進展を防ぐことが重要とされる群と定義されるが，生物学的背景に関する研究はほとんどない。筆者らは，東京都高齢者ブレインバンク内1,120名の連続剖検例より後方視的に，軽度認知障害疑い例を抽出，神経病理学的背景を検討した。170例が軽度認知機能障害疑い例と抽出された。男女比1.44，平均年齢82.0歳，平均脳重1,260gで，いずれも正常と痴呆の中間に属し，変性型では，アルツハイマー病変化，高齢者タウオパチー変化，レヴィー小体型痴呆様変化，さらに血管障害型においては多発性脳梗塞，Binswanger型白質脳症型変化とも，痴呆例と正常例との中間の病理を示すことが明らかとなった。方法論的限界はあるが，この結果は，軽度認知機能障害の概念および，その群への早期介入への妥当性を支持する所見と考えられる。

キーワード：神経原線維変化，老人斑，レヴィー小体，嗜銀顆粒

I．軽度認知機能障害とは

軽度認知機能障害(mild cognitive impairment：MCI)とは，正常群と痴呆群の間に属する集団とする概念として，最初提出された[1]。Petersenらはこの概念を発展させ，記憶障害の訴えがあり，それが同伴者ないしは介護者により確認され，確立したメモリースケールで1.5 SD以下の低下を示し，他の知的機能のドメインは1 SD以下の低下にとどまり，日常生活に障害がなく，痴呆はなく，簡易認知機能評価指標であるclinical dementia rating (CDR)[2]では0.5である群と定義した。MCIは高齢者の5%に存在し，年率10～15%でアルツハイマー病に移行するため，痴呆予防のために介入する上で，極めて重要な対象であるというのが彼の主張である[3]。なおDSM IV-Rによる痴呆の定義は，記憶障害を必須とし，失語・失行・失認・実行機能の障害の1つ以上の領域で低下を認め，日常生活が障害されている状態である。Petersenらにより，MCIが普遍的に抽出できる操作的定義にのっとった概念となり，中枢性抗アセチルコリンエステラーゼ剤の出現により，アルツハイマー病の記憶障害への早期介入の重要性が強調された結果，MCIという名称が特に米国と日本で重用視される結果をもたらした[4]。

しかし，MCIという概念は，Petersenらが基盤としたもの忘れ外来においては有用であるが，一般のコホートを対象とした場合，頻度が極めて低くなり，国際老年精神医学会で定められた，本人または同伴・介護者からの認知障害の訴え，記憶・言語・注意・視空間機能，論理(推論)の5つのカテゴリーのいずれかで1 SD以下の低下，半年以上にわたる緩徐進行性の経過，痴呆および認知障害を来す精神・神経疾患および薬物の副作用の除外を定義とする，年齢相応知的機能

2004年3月30日受稿

* Neuropathology of mild cognitive impairment.

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0001-8724/04/¥500/論文/JCLS

低下(ageing associated cognitive decline：AACD)[5]の概念のほうが，痴呆への進展予防の点では有用であるという意見が欧州では根強い[6]。AACDの場合問題となるのは，最後の認知機能障害を来す精神・神経疾患，ないしは薬物副作用の除外の項目である。もの忘れ外来を神経内科ベースで行っていると，MCIの範疇に入る疾患の中に，慢性呼吸不全，慢性心不全，糖尿病，高血圧，骨系統疾患に伴う運動機能障害，無症候性脳梗塞および症候性脳梗塞後遺症，心臓手術をはじめとする全身麻酔手術の既往[7]等があり，適切な治療により認知機能障害が改善することが明らかとなってきている。これらを二次性MCIという概念でくくり，見逃がさないことのほうが重要である。血管障害性MCIの概念は，降圧剤の大規模スタディからの知見，無症候性脳梗塞の数の増加が認知障害の増悪と関係するとするデータ[8]等より提出された概念であり，脳血管障害の危険因子をコントロールすることで，痴呆への進展を予防しうる。一方パーキンソン病のように，運動機能障害を主訴とするが，特徴的認知機能障害を呈することが知られている疾患で，機械的にPetersenらの定義をあてはめると，高齢発症の場合はほとんどがMCIの範疇に入る。MCIという概念を，正常と痴呆の中間に属し，痴呆への進展予防対象とするという当初の定義に基づけば，内科・神経内科的疾患に附随し出現するMCIを二次性MCIと分類し，認知機能障害に配慮しながら治療を行うことが必要であろう。

一方，Clinical Dementia Rating (CDR)は痴呆を評価する観察法として提出されたが，記憶，見当識，判断力，問題解決，社会適応，家庭状況および趣味・関心，介護状況の6項目を基にする。本人の診察以外に，介護者のインタビューでもある程度評価可能であること，日常生活に即した複数のパラメーターを基にしているので，医師以外でも評価可能であることより，有用性は高い。CDR0.5に記憶検査で1.5 SD以下という指標を加えても，介入する対象を制限するだけで実際的ではないことから，この指標単独を重視すべきであるという意見も，特にフィールドワークで痴呆予防を試みているグループの中では強い。

MCIの概念の混乱の原因として，高齢で標準化された記憶テストがほとんどなく，1.5 SDの低下という基準自体が実際的でないことがあげられる。痴呆研究において，発症前の知的機能レベルが大きく影響することが明らかになってきたことより，ある年齢での平均値の意味が，本来本人の生理学的なゆるやかな低下とどのくらい偏位しているかが最も重要とされ，この指標は介護者の判断にゆだねられることになる。また，正常コントロールとする高齢者の数が少なく，かつその記憶テストの結果が著しくばらつくため，通常の知能検査の平均化の考え方が適用しにくいことも背景にある。

最近のコホート研究によっては，高齢者の知的機能には，明らかな地域差があることも明らかになってきている。Petersen自身，自らが提唱した記憶障害を主体とするMCIを，amnestic MCI (記憶障害を主体とする軽度認知障害)という名で呼ぶことで，MCIの概念を拡大している。そして，amnestic MCIは依然として，アルツハイマー病前駆状態を多く含むという考え方を継承している。

以上の点をふまえ，本稿では，軽度認知機能障害を，正常と痴呆の中間に属し，介入により痴呆への進展を予防することが最も有効な群とする，本来に立ち返った定義で用いることとする。

II．軽度認知機能障害の神経病理学的研究：前方視的研究の文献的考察

MCIの病理を得るためには，ある集団を前方視的に追跡し，その認知機能の経過を追い，できるかぎり剖検をとる努力をする以外ない。

Morrisらは，ワシントン大学の正常と痴呆の前方視的研究より，4例の知的機能正常者，4例のCDR 0.5症例，6例のアルツハイマー病の剖検所見を呈示し，Consortium to Establish Registry for Alzheimer Disease (CERAD)の診断基準をあてはめた時には，CDR0.5例はアルツハイマー病の診断基準を満たし，知的に正常な例はアルツハイマー変化が極めて軽度であり，CDR0.5の病理の中核は，アルツハイマー病であるという発表をし[9]，さらにこれを支持する発表を最近行っている。しかし，筆者らの高齢者連続剖検例からの検討では，認知機能正常例でもアルツハイマー変化を認めることが一般的である。また，後述するように，後方視的に抽出したCDR0.5症例には，アルツハイマー病以外に多数の疾患が含まれることより，前方視的研究にリクルートする段階でのバイアスが想定される。

前方視的研究としては，Nun Studyが最も有名であるが，MCIの概念を超高齢者にあてはめることが難しいせいか，MCIを直接のテーマとした研究は見当たらない。MCIを理解する上で重要と思われるのが，若い時の言語機能が高いほどアルツハイマー病変化が抑制されること[10]，栄養状態がよいこと[11]や血管障害を伴わないこと[12]が，同等のアルツハイマー病変を有していても，知的機能障害の程度を減じることなどの報告

である。

Mayo Clinicにおける前方視的研究から得られた知見として，MCIはアルツハイマー病以外に，タウオパチーを主体とする症例を含むことが報告されている[13]。また知的に正常とされた症例群の病理が同じグループからなされ，後述する神経原線維変化のBraak stageはⅢ以下であること，CERADの基準でいうとneuritic plaqueが100倍視野で6個以下であることが報告されている[14]。

また，the Religious Orders Studyにおいては，嗅内野の神経原線維変化とneuropil threadsに代表されるタウの病理が，認知機能正常者においては低く，MCIないしはアルツハイマー病において強いこと，またその程度はepisodic memoryの障害と相関するとする報告がなされている[15]。

これら前方視的検討に基づく症例の蓄積は，極めて重要な知見を与えるが，症例数が絶対的に少ないこと，最後の知的機能の検索と死亡までの間には必ず間隔が存在すること，死亡直前の入院時の知的機能評価が信頼性がないことより，結果の信頼性にやはりある程度制限が加わる。

Ⅲ．軽度認知機能障害の神経病理：後方視的研究

1．背景

筆者らの施設は，一般在宅医療対象高齢者の背景神経病理を明らかにする目的で，神経疾患の有無にかかわらず，剖検を得る努力を継続的に行っている。また，医師・看護サイドともに，認知機能が臨床経過に大きく影響を与えるという認識のもとに，MMSE，Basic ADL，Instrumental ADL（IADL）を評価していくことを継続しており，またカルテや画像が全て保存されている。

神経病理学的には，代表的検索部位を網羅するかたちで症例が蓄積されており，検索を追加することで評価レベルを一定に保つことが可能である。以上より，以下の方法で，軽度認知機能障害相当例の検討を行った。

2．方法

1995年よりの当施設1,120名の連続剖検例を対象とした。

病歴の検索は，神経内科専門医が2名独立して検索した。両者が一致しない場合は，討論し，必要に応じ，主治医，場合によっては介護者に主治医を通じて問い合わせ，確認するかたちをとった。他院での既往病歴が参考になる可能性がある場合は，遺族の文書同意を得た上で参照した。CDR0.5で，かつ年齢相応の物忘れ，あるいはぼけの記載があり，かつそれに基づく病気療養上の問題を起こしているが，痴呆の診断を受けていないか，あるいは家族・医師・看護師間で，診断に相違がある症例を，MCI相当者として抽出した。この場合，せん妄の疑われる症例，うつ病等精神疾患が疑われる症例は厳密に除外した。また，最終入院時の死に連続する身体状況での認知機能障害は評価しなかった。

表1　脳血管障害の評価

臨床情報データベース	
・脳卒中発作の回数	0〜3
・放射線画像の有無と回数	CT，MRI，SPECT，PET
神経病理データベース	
・塞栓（embolism）：	E，e
・血栓（thrombosis）：	T，t
・ラクナ梗塞（lacuna）：	L，l
・脳内出血（hemorrhage）	H，h
・クモ膜下出血	SAH

心臓弁膜症にならい，臨床症状に寄与していれば大文字，死亡直前におきたと考えられるものは（　）内に入れた。血管障害は，いかなるものも何らかの機能障害をもたらし，繰り返すとさらに障害をもたらすという観点で整理した。塞栓性梗塞か血栓性梗塞かについては，臨床的判断を重視した。

これら全例にApoE4遺伝子多型の検索を行った。遺伝子研究の同意は解剖承諾書より得，また東京都老人総合研究所，東京都老人医療センター双方の倫理委員会の承認のもとに行った。

神経病理学的検索には，ブレインカッティングカンファランスで臨床・画像と肉眼病理所見を正確に対応させることで，血管障害性病変の確認，脳萎縮等を慎重に評価した。組織学的には，前頭・側頭・頭頂・後頭葉，扁桃核・海馬，基底核，乳頭体，視床，中脳，橋，延髄，小脳，脊髄等代表的部位を，通常標本部位とし，局所性病変を伴う場合には，適宜追加した。Hematoxylin & Eosin染色とKlüver-Barrela染色に加え，神経原線維変化の検出にGallyas-Braak染色，老人斑の検出にmodified methenamine銀染色，アミロイドアンギオパチーの検出にコンゴ赤染色，血管の評価にElastica-Masson染色を行った。免疫組織化学的には，抗リン酸化タウ（AT8），Aβ（11-28），ubiquitin，α-synucleinに抗体を採用し，Ventana NX20自動免疫染色装置を用いた。適宜，電子顕微鏡による検索を追加した。

血管障害の評価には，臨床的な脳卒中発作の回数に加え，臨床症状と二次変性を伴う場合は大文字にし，

表2 東京都高齢者ブレインバンク臨床神経病理データベース

A/G	CDR	PMI	NFT	SP	Grain	AA	Lewy	τ-astro	ubq	apoE	NPD
92F	3	15：35	6	3	0	3	2L	0	1	44	AD/AA

A/G	年齢，性	49-108/M：男性，F：女性
CDR	clinical dementia rating	0-3
PMI	死後時間	時間：分
NFT	神経原線維変化，Braak ステージ[16]	0-6
SP	老人斑，Braak ステージ[16]	0-3
Grain	嗜銀顆粒[19]，筆者ら（東京都高齢者ブレインバンク）のステージ	0-3
AA	アミロイドアンギオパチー，筆者らのステージ	0-3
Lewy	レヴィー小体病，筆者らのステージ[17]	0-5
τ-astro	タウ免疫染色陽性アストログリア，筆者らのステージ	0-3
ubq	抗ユビキチン抗体陽性顆粒，筆者らのステージ	0-3
apoE	apoE 遺伝子多型	ε2，3，4/3，4
NPD：	神経病理学的所見	略号表記

嗜銀顆粒のステージ（0：なし，0.5：免疫染色のみで検出，1：迂回回およびその近傍に限局し出現，2：側頭葉内側面に出現，3：前帯状回，島回，中隔野，側坐核に進展）。アミロイドアンギオパチーのステージ（0.5：免疫染色のみで検出，1：小血管壁に沈着，2：血管平滑筋に沈着し，変性を伴う，3：アミロイドアンギオパチーによる出血・梗塞を伴う）。レヴィー小体病のステージ（0：なし，0.5：免疫染色のみで検出，1：偶発的レヴィー小体，2：レヴィー小体に基づく変性を認めるが，それに伴う臨床記載がない，3．痴呆を伴わないパーキンソン病，4．レヴィー小体型痴呆と痴呆を伴うパーキンソン病で，レヴィー小体スコアから移行型に分類されるもの，5．レヴィー小体型痴呆と痴呆を伴うパーキンソン病で，レヴィー小体スコアから新皮質型に分類されるもの）。タウ免疫染色陽性アストログリアのステージ（0：なし，1：thorn shaped astrocytes が出現，2：bush-like astrocytes が出現，3：tuft-shaped astrocytes ないし astrocytic plaque が出現）。抗ユビキチン抗体免疫染色陽性顆粒のステージ（0：なし，1：嗅内野に少数出現，2：嗅内野に多数出現，3：CA1 の錐体細胞周囲にも出現）。

かつ血栓か塞栓かラクナ梗塞かを明記することで，全てを記載する方針とした（表1）。

一方，変性型病変については，神経原線維変化，老人斑については，Braak らの分類[16]を，レヴィー小体[17]と嗜銀顆粒性痴呆[18]に関しては，東京都高齢者ブレインバンクの分類を用いた（表2）。

3．結果

170例がMCI相当症例として抽出された。内訳として，男女比 100：70＝1.44，平均年齢 82.0 歳，平均脳重 1,220 g で，知的正常例 395 例の男女比 241：154＝1.56 との間には有意差はないが（図1），平均年齢 77.2 歳（$p<0.0001$）（図2），脳重 1,260 g（$p=0.001$）（図3）で有意差を認めた。一方，痴呆例 430 例とは，男女比 197：233＝0.84（$p=0.00031$）（図1），平均年齢 83.8 歳（$p=0.0174$）（図2），平均脳重 1,170 g（$p=0.0004$）（図3）のいずれにおいても有意差を認めた。結果として，MCI相当症例は，いずれの項目でも正常と痴呆の中間に入った。

ApoE 多型は，正常 2/3：38例，3/3：307例，3/4：50例，4/4：0例；MCI，2/3：18例，3/3：121例，3/4：29例，4/4：2例；痴呆 2/3：0例，3/3：346例，3/4：70例，4/4：14例で，多型遺伝子頻度は，ε2が，9.6%，10.5%，0%，ε4が12.6%，19.3%，22.1%であり，ε2に関しては痴呆と明らかな頻度差あり，ε4に関しては正常との有意差（$p=0.0031$）がある結果であった（図4）。

病理診断として，変性疾患およびその初期と考えられる例は 62 例（36.8%）であった（図5）。AD 関連としては，CERAD definite で，Braak tangle ステージVの症例1例，IVの症例2例，IIIの症例14例，IIの症例1例，CERAD probable で Braak ステージIIIの症例6例の計24例であった（表3）。一方，嗜銀顆粒関連では，痴呆（DG）のレベルの病理をとるもの9例，嗜銀顆粒を多数認めるが変性が明らかでないもの2例の計11例であった。神経原線維変化優位型痴呆（NFTD）関連では，NFTD の病理を示すもの4例，程度は軽い

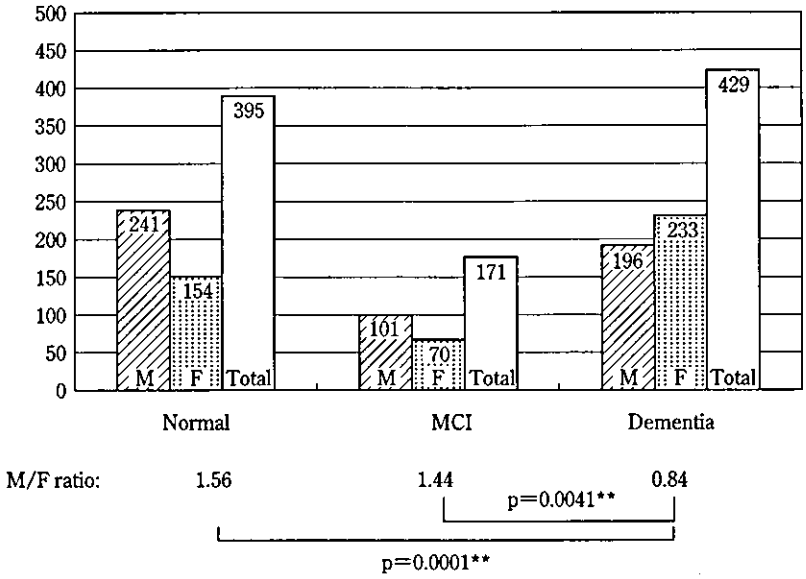

図 1　軽度認知機能障害の男女比

全体が男性優位であるが，痴呆は女性が多く，正常者は男性が多く，軽度認知機能障害はその中間に入る．認知機能正常者と痴呆，軽度認知機能障害と痴呆の間で有意差を認める（M：男性，F：女性，MCI：軽度認知障害）．

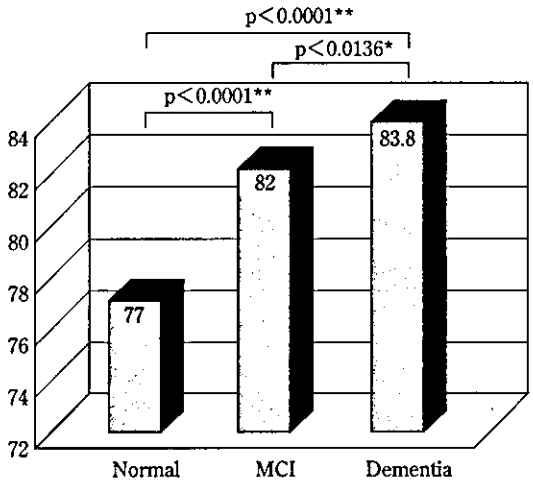

図 2　軽度認知機能障害の平均年齢

軽度認知機能障害（MCI）の平均年齢は，正常認知機能障害（Normal）と痴呆（Dementia）の中間に入り，全ての群間に有意差を認める．

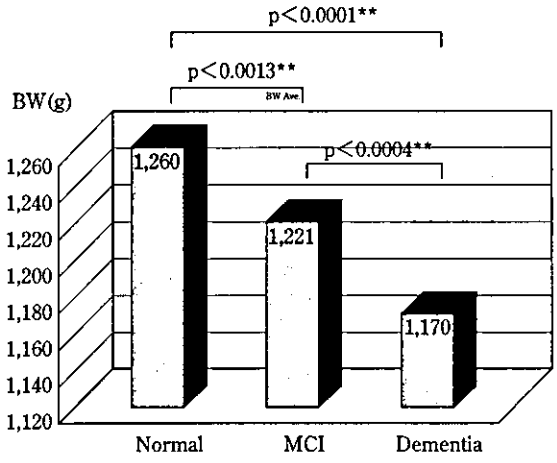

図 3　軽度認知機能障害の平均脳重

軽度認知障害（MCI）患者の平均脳重は，認知機能正常者と痴呆の中間に入り，全ての群間で有意差を認める．

が神経原線維変化優位の老年性変化を示すもの（NFTC）7例の，計11例が該当した。またDG+NFTCの病理を示すものが1例あった（表4）。レヴィー小体病関連では，パーキンソン病（PD）1例，PDの病理を示すがパーキンソン症状の記載がないもの5例，レヴィー小体型痴呆（DLB）移行型の病理を示すもの3例，新皮質型の病理を示すもの2例の計11例であった（表5）。その他，筋萎縮性側索硬化症2例，SCA6 1例，黒質変性を示すがレヴィー小体を欠くもの1例であった。

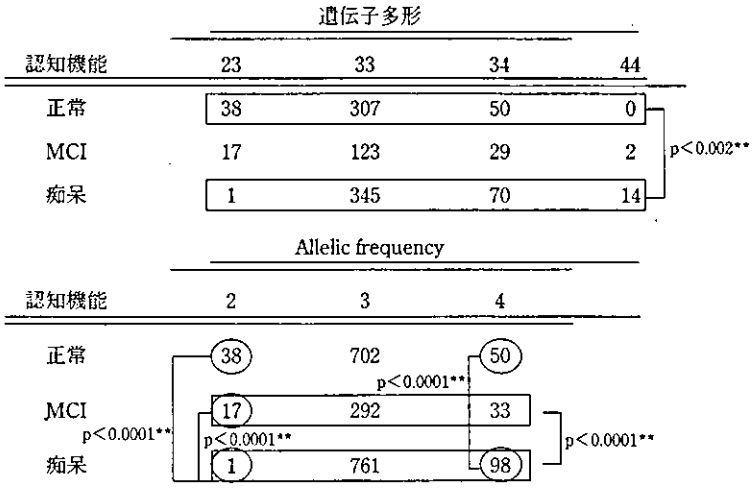

図 4　ApoE 遺伝子多型と認知機能との関係

軽度認知機能障害（MCI）患者は正常と痴呆の中間に位置する結果であった．遺伝子多型では，正常と痴呆の間に有意差を認めた．Allelic frequency では，MCI と痴呆との間に群間有意差を認めた．個別には，apoE e2 の頻度について，正常と痴呆，MCI と痴呆との間に有意差を認めた．apoE e4 については，正常と痴呆，群の間に有意差を認めた．

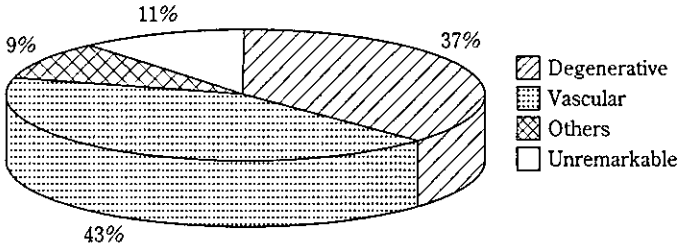

図 5　軽度認知機能障害の病理診断

Degenerative：変性型，Vascular：血管障害型，Others：その他．血管障害が一見多くみえるが，無症候性脳梗塞を半数近く含んでおり，その意義付けが問題．また，責任病巣が認められない症例が11%に及んでおり，脳の基質性疾患に起因できないか，これまでの病理学的検索法では検出できない病変による可能性の両方が考えられる．

表 3　軽度認知機能障害相当例におけるアルツハイマー病変

(老人斑 Braak ステージ C)	
神経原線維変化 Braak　ステージV	2 例
Ⅳ	2 例
Ⅲ	14 例
Ⅱ	1 例
(老人斑 Braak ステージ B)	
神経原線維変化 Braak　ステージⅢ	6 例
計	25 例

他の随伴病変の程度が，ここに記載したアルツハイマー病変の程度より軽い症例を抽出した。Braak ステージで，老人斑 C，神経原線維変化Ⅱの症例は，これ以外に神経病理学的異常所見を伴わない症例であること，CERAD の基準では definite Alzheimer disease の基準を満たすため含めた。

表 4　軽度認知機能障害とタウオパチー

(嗜銀顆粒性疾患)	
ステージ 3	9 例
ステージ 2	2 例
計	11 例
(神経原線維変化優位型疾患)	
高度	4 例
中等度	7 例
計	11 例
(両者の合併)	1 例
計	23 例

嗜銀顆粒性痴呆については筆者らのステージ3，神経原線維変化優位型痴呆については，Braak の神経原線維変化ステージ 3-4 で老人斑のステージが A 以下，さらに他の随伴病変が軽いものを抽出した。

一方，脳血管障害の変化を主体とするものは 71 例，41.5%であった。臨床症状を伴う脳梗塞 26 例，うち Binswanger 型白質脳症様病変を示すもの 2 例，無症候性脳梗塞 35 例，脳出血 7 例，クモ膜下出血 1 例で，41.5%であった。無症候性脳梗塞の部位は多岐にわたっており，左視床，左乳頭体視床路，左海馬傍回等，いわゆる戦略拠点破壊梗塞（strategic infarct）は少数で，大部分が，皮質・白質・基底核にランダムに存在しており，実際原因となっているかどうかの確認は困難であった（表 6）。

表5 軽度認知機能障害におけるレヴィー小体病変

レヴィー小体型軽度認知障害	
(パーキンソン症状の記載なし)	
脳幹型	5例
移行型(辺縁型)	3例
新皮質型	2例
(パーキンソン症状あり)	
パーキンソン病	3例
計	13例

パーキンソン症状の記載のない例については，レヴィー小体が出現し，変性を認める症例(筆者らのステージII)の中で，軽度認知機能障害を呈し，他の随伴病変の程度が相対的に軽いものを選んだ。パーキンソン病については，後方視的にもMCI該当例が存在することが確認された。

IV. 軽度認知機能障害の神経病理：前方視的研究からの推察

特定の高齢者のコホートを前方視的に追求することは，東京都老人医療センターと老人総合研究所が共同して，5年前より長期プロジェクト，「老年性痴呆の克服」において，痴呆症例の前方視的追求を行っている。さらに2年前より，厚生労働省長寿科学総合研究事業の研究費援助を受けて，MCIレベルの症例の前方視的追求を開始した。実質の研究期間がまだ1年間であるため，前方視的に追求したMCI例の剖検はまだ得られていない。しかし，筆者らは，volumetric MRI, positoron emission tomography (PET)，髄液バイオマーカー(HVA, 5HIAA, タウ，リン酸化タウ，amyloid beta)による検索を行っているため，かなり確実にアルツハイマー病かそれ以外であるかの鑑別が可能である。

この過程で明らかになってきたのは，全体的知能を評価する指標としてMini Mental State Examination (MMSE)を採用し，記憶障害の指標として高齢者標準値をもつRivermead Behavioral Memory Test (RBMT)以下を採用し，MMSE 24以上で，RBMT 1.5 SD以下を軽度認知機能障害として機械的に抽出した場合，基礎疾患を問わないのであれば，最も頻度が高いのはパーキンソン病，ついで脳血管障害である。一方，もの忘れ外来受診者について，軽度認知機能障害レベルの症例を検索した場合，アルツハイマー病と考えられる症例の他に，Lewy小体型痴呆初期と思われる群，嗜銀顆粒性痴呆，あるいは神経原線維変化優位型痴呆と思われる例がやはり存在する。

表6 軽度認知機能障害と血管病変

脳卒中発作を伴う脳梗塞	27例
(うちBinswanger様病変)	2例
無症候性脳梗塞	35例
脳出血	7例
クモ膜下出血	1例
計	72例

変性型変化が軽い症例を抽出した。無症候性脳梗塞については，知的に正常の症例にも認めるため，この病変が軽度認知障害に関与しているかどうかについては，前方視的検討による確認が必要と考えられる。

V. 考察

筆者らのMCIの前方視的・後方視的研究から明らかなように，MCIはアルツハイマー病を中核とするが，それ以外に多くの疾患を含む。血管障害性の頻度が高いのは，再発しなければ進行はしないため，軽度にとどまる症例が多くなることは十分理解できる。中にBinswanger病様変化を示すものを含んでいる点は，血管障害性軽度認知障害の概念の妥当性を支持する。また左内頸動脈閉塞に伴い，血栓内膜除去術で回復可能な症例の存在については，今後軽度認知機能障害の臨床上注意すべき事項と考えられる。無症候性脳梗塞が多い点に関しては，脳病変を認めないのにMCI類似群に分類された例が11%存在することを考慮すると，解釈に慎重である必要がある。Lewy小体関連病態については，頻度からいって，MCIの範疇に一定数入ってくると考えられる。問題は高齢者タウオパチーと呼ばれる，嗜銀顆粒性痴呆と神経原線維変化優位型痴呆群であり，両者を併せるとADに匹敵する。これらは，筆者らの観察では，痴呆を呈しても程度が軽く，進行が遅い印象があり，MCIとして一断面で切ると，頻度的に多くなると考えられる。これらの非AD群をいかにADと分離し，介入・治療を考えていくかが今後の重要な課題である。

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Abstract

Neuropathology of mild cognitive impairment

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Mild cognitive impairment (MCI) is defined as the intermediate state between normal cognition and dementia. MCI has been highlighted as the main target for preventive scheme against cognitive decline in the elderly. However, there are very few studies about neuropathological background of this state. Moreover, many of these studies only reported MCI as substrate of early Alzheimer disease (AD).

We screened clinical records of 1,120 serial autopsy cases from Tokyo Metropolitan Brain Bank for Aging Research in these five years. These cases represent serial autopsy cases from a general geriatric hospital for community care and reasonably represent a cohort of the elderly in Tokyo metropolitan urban district. The cases equivalent to MCI were retrospectively selected by two professional neurologists, on the following criteria : 1) the description of memory impairment incurring problems for medical care, 2) no definite description of dementia, and

Shinkei Kenkyu no Shinpo (Advances in Neurological Sciences), Vol. 48, No. 3, pp441-449, 2004.
IGAKU-SHOIN Ltd., Tokyo, Japan.

3) CDR 0.5. One hundred and seventy cases were pulled out. A male to female ratio was 1.44, the average age, 82.0 years and the average brain weight, 1,260 g respectively. These values were just between those of the cases with normal cognition and with dementia. The frequency of apoE ε4 allele is also between cognitively normal cases and cases with dementia.

The background pathology of these MCI cases were evaluated on our standard pathology protocol. The areas examined followed the recommendations of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and the consensus guidelines for the diagnosis of dementia with Lewy bodies. Six-μm-thick sections were routinely stained with hematoxylin and eosin (H & E) and the Klüver-Barrera (KB) method. Selected sections were stained with the modified methenamine silver and Gallyas-Braak silver method for senile changes, with Congo red for amyloid deposition, and with elastica Masson trichrome for vascular changes. Immunohistochemically employed were the antibodies raised against amyloid β, phosphorylated tau (AT8), phosphorylated α-synuclein, ubiquitin, phosphorylated neurofilament, glial fibrillary acidic protein and HLA-DR. The description of the location, size and nature (thrombotic, embolic or lacunar) of all vascular lesions and the semi-quantitative assessment of each abnormally accumulated proteins (tau, amyloid beta protein, alpha-synuclein and ubiquitin) were applied to all the cases examined.

Among the 170 cases, 25 cases mainly presented with Alzheimer-type pathology including neurofibrillary tangles and senile plaques. 10 cases presented with Lewy-related neuronal degeneration without parkinsonism. 11 cases each show dementia with grain-or neuforibrillary tangle predominant form of dementia-type changes and one case showed the combination of these two changes. All of these changes were generally milder than the cases with dementia.

In addition to cases with mainly degenerative changes, 72 cases presented with mainly vascular lesions. Among them, 27 cases experienced stroke events, among whom 2 cases exhibited with Binswanger-type white matter changes. These vascular changes were generally milder than those of cases with vascular dementia. Moreover, 35 cases carried asymptomtic cerebral infarcts as only morphological substrates of cognitive decline.

Our data indicate that MCI comprised heterogeneous background pathology of probable pre-symptomatic phase. Similar data have been now accumulating in the literature. Thus, the prospective studies of MCI is quite important to develop the tools for the prevention of the progression to dementia from MCI stage.

(*Received : March 30, 2004*)

Journal of Neuropathology and Experimental Neurology

Vol. 62, No. 6
June, 2003
pp. 644–654

Accumulation of Phosphorylated α-Synuclein in Aging Human Brain

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Abstract. α-Synuclein in Lewy bodies (LBs) is phosphorylated at Ser129. We raised monoclonal and polyclonal antibodies to this phosphorylation site (psyn) and examined 157 serial autopsy brains from a geriatric hospital. Anti-psyn immunoreactivity was observed in 40 of these cases (25.5%). Immunohistochemistry revealed 4 novel types of pathology: diffuse neuronal cytoplasmic staining (pre-LB); neuropil thread-like structures (Lewy threads); dot-like structures similar to argyrophilic grains (Lewy dots); and axons in the white matter (Lewy axons). This novel pathology was abundantly present around LBs and also involved the limbic subcortical white matter, the cerebral cortical molecular layer, and the spongiform changes of the medial temporal lobe associated with cases of dementia with LBs (DLB). The phosphorylated α-synuclein was limited to the temporal lobe in cases of Parkinson disease, spread from the temporal lobe to the frontal lobe in cases of DLB transitional form and further spread to the parietal and occipital lobes in DLB neocortical form. Our findings suggest that LB-related pathology initially involves the neuronal perikarya, dendrites, and axons, causes impairment of axonal transport and synaptic transmission, and later leads to the formation of LBs, a hallmark of functional disturbance long before neuronal cell death.

Key Words: α-Synucleinopathy; Axon; Dementia with Lewy bodies; Immunohistochemistry; Parkinson disease; Synapse.

INTRODUCTION

Lewy body (LB)-related pathology was originally recognized in the brains of patients with Parkinson disease. Since it was discovered that ubiquitin (1) and α-synuclein (2, 3) are components of LBs, the locations of LB-related pathology and the corresponding specific neurological abnormalities have received considerable attention. Involved sites include the peripheral autonomic nervous system in cases with autonomic failure (4), the dorsal motor nucleus of the vagus nerve in cases with dysphagia (5), and the limbic system and neocortex in cases with cognitive decline (i.e. dementia with Lewy bodies [DLB]) (6). The severity of clinical abnormalities in these cases parallels the number of LBs (7) rather than neuronal cell loss (8), especially in cases with DLB neocortical form.

Recent immunohistochemical studies with anti-α-synuclein antibodies suggest that the dorsal motor nucleus of the vagus is the initial site of involvement in Parkinson disease (9). In contrast, α-synuclein-positive structures preferentially localize in the amygdala in familial (10) and sporadic (11) Alzheimer disease (AD). Further clarification of the relationship between these 2 reported types of α-synucleinopathy has been difficult, because α-synuclein is a normal constituent of presynaptic structures (12), and interpretation of abnormal accumulations based on staining intensity may be influenced by the conditions of staining or fixation.

We recently reported that the α-synuclein accumulated in LBs (13) is phosphorylated at Ser129, and that a polyclonal anti-phosphorylated α-synuclein antibody (anti-PSer129) produced strong staining of LBs and Lewy neurites (13). We have now raised a monoclonal antibody (psyn#64) that specifically recognizes this phosphorylation site. Immunohistochemistry with this highly specific monoclonal antibody produces intense staining of LBs and Lewy neurites without staining normal presynaptic structures.

In the current study, we examined serial autopsy brains from an aging population to assess the progression of the 2 reported types of LB-related α-synucleinopathy. We also correlated the morphological changes with the severe functional impairment that occurs prior to cell loss in LB-related cognitive decline.

MATERIALS AND METHODS

Tissue Source

One hundred and fifty-seven serial autopsy brains from Tokyo Metropolitan Geriatric Hospital (TMGH) were studied in the present work. The patients' ages ranged from 48 to 100 years. The mean age was 81.1 ± 8.6 years and the male to female ratio was 89:68.

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Sources of support: Foundation of Tokyo Metropolitan Institute of Gerontology.

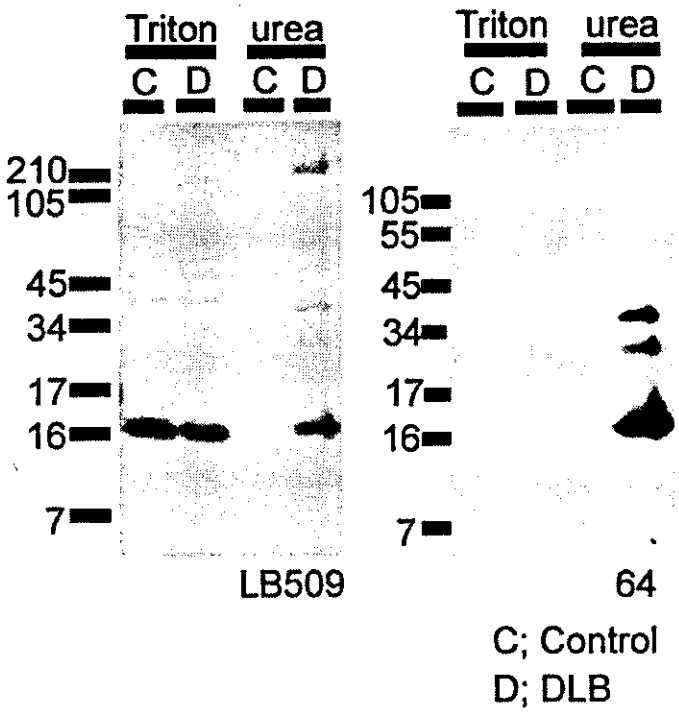

Fig. 1. Western blot analysis of α-synuclein differentially extracted with Triton X-100 (Triton), Sarkosyl, or urea from cerebral cortices of a patient with DLB, neocortical form (Case 4) and a normal control individual probed with LB509 (left panel) or psyn#64 (right panel). Sarkosyl soluble fractions that did not contain detectable amounts of α-synuclein are not shown. Molecular weight markers are shown in kilodaltons at the left side of the panels. A ~15 kDa polypeptide, labeled by LB509 (3), was detected in TX-soluble fractions of DLB and normal control brains and represents normal α-synuclein, as previously reported (13). A major ~15 kDa polypeptide and additional minor higher molecular weight polypeptides were specifically detected by LB509 in Sarkosyl-insoluble, urea-soluble fractions from DLB cortices. Monoclonal antibody psyn#64 did not label TX-soluble α-synuclein, but strongly reacted with the urea-soluble α-synuclein in DLB brains.

Neuropathology

Sections of the right substantia nigra, amygdala, anterior hippocampus and frontal, temporal, parietal, and occipital lobes were fixed in 4% paraformaldehyde for 48 hours and embedded in paraffin. The left half of the brain was fixed in 20% neural buffered formalin (Wako, Osaka, Japan) for 7 to 13 days and representative areas were embedded in paraffin.

Six-μm-thick sections were stained with hematoxylin and eosin (H&E) and by the Klüver-Barrera method. Selected sections were examined with modified methenamine and Gallyas-Braak silver staining for senile changes, with Congo red staining for amyloid deposition, and with elastica Masson staining for vascular changes.

Preparation and Characterization of Anti-Phosphorylated α-Synucliein (psyn) Monoclonal Antibody (psyn#64)

Anti-psyn monoclonal antiobody (psyn#64) was raised against a synthetic peptide corresponding to residues 124–134 of human α-synuclein containing phosphoserine at position 129 and screened by ELISA. For the characterization of the antibody, neocortex from the medial temporal lobe of brains with DLB neocortical form and of normal control brains from the present autopsy series were differentially extracted as previously described (13), with some modifications. Briefly, the neocortices were directly homogenized in 1% Triton X-100 (TX) containing protease inhibitors (instead of Tris saline) and then extracted with Sarkosyl and urea. TX- or urea-soluble fractions, in which normal and deposited α-synuclein is extracted, respectively, were separated by SDS-PAGE and analyzed by immunoblotting with LB509 (3) or psyn#64 as primary antibodies. In addition, the immunoreactivity was confirmed by immunoblot with nonphosphorylated recombinant human α-synuclein and α-synuclein phosphorylated in vitro by casein kinase 2, which specifically phosphorylates Ser129 (3) (A.K., H.F., and T.I., unpublished observation).

Immunohistochemistry

Six-μm-thick serial sections were obtained from paraffin blocks and immunohistochemically stained using a Ventana 20NX autostainer (Ventana, Tucson, AZ) for single or double immunolabeling as previously described (14). Two antibodies specific for phosphorylated α-synuclein (mouse monoclonal antibody psyn#64 and polyconal antibody anti-Pser129 [13]) were used. In addition, other antibodies against α-synuclein (LB509, monoclonal [3] & and S1, recognizing the C terminus [a kind gift from Dr. Y. Ihara]); phosphorylated tau (AT8, monoclonal, Innogenetics, Temse, Belgium); Aβ11-28 (12B2, monoclonal, IBL, Maebashi, Japan); ubiquitin (polyclonal, Sigma-Aldrich, St. Louis, MO); glial fibrillary acidic protein (GFAP, polyclonal, DAKO, Carpinteria, CA); and HLA-DR (CD68, monoclonal, DAKO) were also used. The sections were pretreated with 99% formic acid for 5 min for psyn#64 and anti-Aβ, or heated in a microwave oven (Nisshin EM, Tokyo, Japan) for 10 min in citrate buffer before incubation with LB509, CD68, or anti-ubiquitin antibody. Areas selected for staining with psyn#64 included all of the paraformaldehyde-fixed tissues, as well as areas from the formalin-fixed tissues recommended by the consensus guidelines for dementia with Lewy bodies (DLB) (6) (lumbar, thoracic, and cervical spinal cord, medulla oblongata at the level of the dorsal motor nucleus of the vagus nerve, upper pons at the level of the locus ceruleus, midbrain, and basal ganglia, anterior cingulate and entorhinal cortex, amygdala, and second frontal, temporal and supramarginal gyri).

Phosphorylated α-synuclein was detected in dendrites or axons with confocal double immunofluorescence using anti-Pser129 combined with anti-MAP2 (HM2, monoclonal, Sigma, St. Louis, MO) or anti-phosphorylated neurofilament (SMI 31, monoclonal, Sternberger Immunochemicals, Bethesda, MA) antibody. Primary antibodies were visualized with anti-rabbit Alexa 568 Fluor™ and anti-mouse IgG Alexa 488™ (Molecular Probes, Eugene, OR) using a confocal laser microscope (BioRad, Hercules, CA). SMI 31 was diluted up to 1:10,000 in order to avoid possible cross-reaction with phosphorylated tau protein.

Evaluation of Lewy Body-Related Neuropathology

The brains were initially evaluated and their LB scores calculated from sections stained with H&E and anti-ubiquitin immunohistochemistry, as recommended by the consensus guidelines for DLB (6). The presence of LB-related pathology was

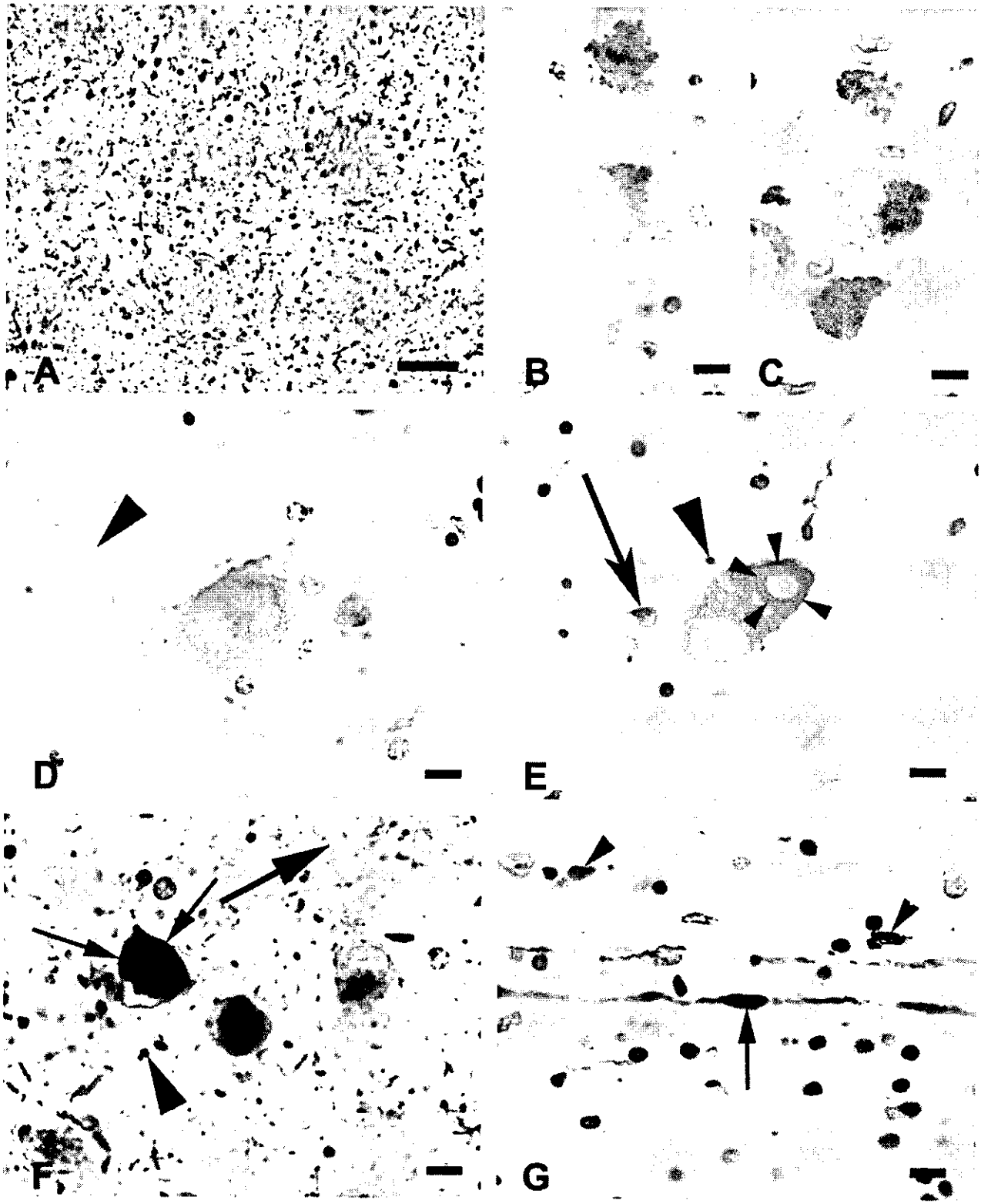

Fig. 2. Novel morphological alterations visualized by immunohistochemistry with anti-phosphorylated α-synuclein (psyn) antibodies. **A:** Numerous dots and threads within the spongiosis in the entorhinal cortex (case 1, bar = 50 μm). **B–E:** Possible morphologic progression in the formation of LBs in melanin-containing neurons of the substantia nigra (Ventana red staining for alkaline phosphatase, bars = 10 μm). **B:** Weak cytoplasmic staining (case 13). **C:** Diffuse cytoplasmic staining (case 14). **D:** Focal cytoplasmic aggregate and positive axon (arrowhead) (case 13). **E:** Typical lamellar staining (small arrowheads), consistent with LBs, associated with neuropil dots (large arrowhead) and a glial inclusion (arrow) (case 6). **F:** Anti-psyn-immunoreactive threads (thick arrow) and dots (arrowhead) with cortical LBs (thin arrows) in the entorhinal cortex (stained with diaminobenzidine) (case 2). **G:** Axons in the white matter of the amygdala (fibrae amygdalofugal) with focal swellings (arrow), positive for anti-psyn antibodies. Anti-psyn-immunoreactive glial inclusions are also visible (arrowheads, case 2).

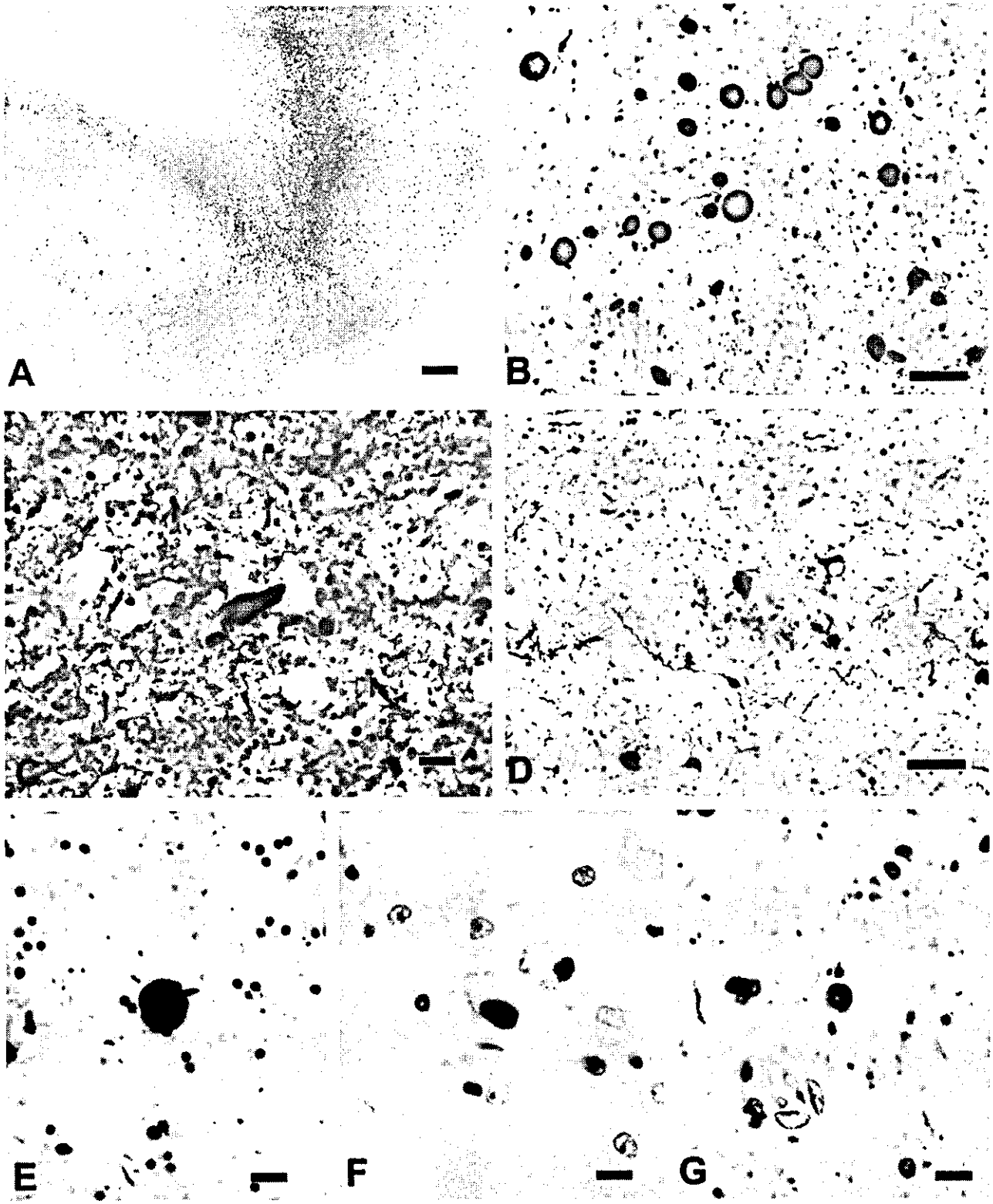

Fig. 3. Morphological alterations visualized by immunohistochemistry with anti-phosphorylated α-synuclein (psyn) antibodies. **A:** Prominent positive staining in the anterior alveus (bar = 0.25 mm, case 2). **B:** Clustered thick neurites in the molecular layer of the anterior subiculum (bar = 50 μm, case 2). **C:** Higher magnification of the alveus shown in (A), showing numerous axons with focal thickening (bar = 10 μm, case 2). **D:** Intracytoplasmic neuronal inclusions with neuritic threads and dots in CA2 and CA3 (bar = 50 μm, case 2). **E:** A large immunoreactive spheroid in the dorsomedial putamen (bar = 10 μm, case1). **F:** Neuronal intracytoplasmic inclusions in the inferior olivary nucleus (bar = 10 μm, case from stage 1B). **G:** Lewy dots, threads, and globules in the molecular layer of the striated cortex (bar = 15 μm, case 1).