Staging of Argyrophilic Grains: An Age-Associated Tauopathy

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Abstract. We have reported that the ambient gyrus is the site with the greatest accumulation of argyrophilic grains (AGs) and that the degeneration of the ambient gyrus is responsible for dementia with grains. Here we analyzed 1,405 serial autopsy cases from 2 hospitals and detected AGs only in cases older than 56 years of age. The distribution of AGs followed a stereotopic regional pattern. Thus, we propose the following staging paradigm: stage I: AGs restricted to the ambient gyrus and its vicinity; stage II: AGs more apparent in the anterior and posterior medial temporal lobe, including the temporal pole, as well as the subiculum and entorhinal cortex; and stage III: abundant AGs in the septum, insular cortex, and anterior cingulate gyrus, accompanying spongy degeneration of the ambient gyrus. Sixty-three of 65 (96.9%) argyrophilic grain stage III cases without other dementing pathology were classified as 0.5 or higher in the clinical dementia rating. Forty-seven of 50 dementia with grains cases (94%) were stage III and 3 were stage II. No association with apoE genotyping was detected. Our study further confirms that dementia with grains is an age-associated tauopathy with relatively uniform distribution and may independently contribute to cognitive decline in the elderly.

Key Words: Alzheimer disease; Clinical dementia rating; Dementia with Lewy bodies; Medial temporal lobe; Neurofibrillary tangle-predominant form of dementia; Progressive supranuclear palsy.

INTRODUCTION

We have reported that the ambient gyrus, which is situated between the amygdala and the anterior medial temporal lobe, is the site with the greatest propensity to accumulate argyrophilic grains (AGs), and that the degeneration of the ambient gyrus is responsible for dementia with grains (1, 2). There is only 1 report discussing the correlation between the grade of cognitive decline and the distribution and amount of grains (3). Also, as far as we know there has been no report of attempts to stage the grains.

By screening serial autopsy cases from Tokyo Metropolitan Geriatric Hospital and Yokohama Rosai Hospital, we observed a relatively uniform distribution of AGs in a given brain region as follows: the localized presence of grains in the ambient gyrus in earlier stages; the more apparent existence of grains in the anterior and posterior medial temporal lobe in intermediate stage; and the involvement of the septal area, the anterior cingulate gyrus, and the insular cortex beyond the boundaries of the temporal lobe in the later stage. There was a strong correlation between the distribution of AGs and the grade of cognitive impairment. Argyrophilic grains were never observed in subjects younger than the mid-fifties. Further analysis using this staging method provided additional new information about the significance of AGs in the human aging process.

MATERIALS AND METHODS

Tissue Source

In the present work, 1,241 serial autopsy brains from Tokyo Metropolitan Geriatric Hospital (TMGH) (Group A) and 164 serial autopsy cases younger than 65 years of age from Yokohama Rosai Hospital (YRH) (Group B) were studied. YRH is a community center general hospital and neuropathological diagnosis was carried out by two of the authors (YS and SM). The patients’ ages ranged from 48 to 104 years in Group A and 0 to 64 years in Group B. The mean age and the male to female ratio were 80.6 ± 8.9 and 663:578 for Group A and 45.0 ± 20.2 years and 101:63 for Group B.

Cognitive State

Clinical information was retrospectively obtained from the medical charts as well as interviews with the patients’ attending physicians and caregivers. The Mini-Mental State Examination (MMSE) (4) or Hasegawa dementia scale (5, 6) was used for evaluation of cognitive function, and the clinical dementia rating scale (CDR) (7) was employed for the grading of cognitive decline as previously reported (1).

Neuropathology

Representative areas in the central nervous system were examined as previously reported (1). Briefly, the areas recommended by CERAD (8) and Braak (9) were stained by the Gallyas-Braak modified methenamine silver (10) and Bielschowsky

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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-a-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 stage equal to or above IV combined with plaque stage C, diagnoses of neurofibrillary tangle-predominant form of 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 non-parametric 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. Oligodendrogial 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,
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: CDR 0, 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 e2 allele (0.64) tended to have higher stage than the combinations of the other alleles (e3: 0.54 and e4: 0.44, Mann-Whitney U-test, p = 0.094) and homo- or heterozygotes for e3 tended to have higher stage than homozygotes for e4 (p = 0.056), although no statistical significance of these differences was found.

**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 (χ² test, p = 0.0049) in females (40%) than in males (32%). However, no gender difference was detected in stage III cases (χ² 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 (\( \chi^2 \) test, \( p = 0.0041 \)) were higher in females than males. No statistically significant gender difference was found among stage III cases (\( \chi^2 \) 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 e2 and a lower frequency of apoE e4
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.

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Argyrophilic Grains in Neurodegenerative Disorders

**TABLE 2**

Argyrophilic Grains in Neurodegenerative Disorders

<table>
<thead>
<tr>
<th>Stage</th>
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<th>NFTD</th>
<th>DLB</th>
<th>MSA</th>
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<th>ALS</th>
<th>PD</th>
<th>CBD</th>
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<td>8</td>
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**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**.

**REFERENCES**


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