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（障害者対策総合研究開発事業（精神障害分野））

双極性障害の神経病理学に基づく診断法の開発

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研究要旨：

福島県立医科大学では、ブレインバンク事業において、双極性障害患者の生前登録を進め、2015年3月までに、35名の双極性障害患者が登録された。登録者の分布は、北海道から九州まで全国に及んでいた。一方、実際の死後脳の集積に関しては、2015年3月現在で、6名の双極性障害患者の脳が集積された。

国立精神・神経医療研究センターでは、リサーチリソースネットワークおよび高齢者ブレインバンクの双極性感情障害例の過去の13症例を見直し、タウオパチーをはじめとする神経変性疾患が一部にあることを報告した。また、生前同意登録システムに基づくブレインバンクに精神疾患を加えるべく倫理申請を行った。

高齢者ブレインバンクでは、これまで行ってきた気分障害の病歴に関する後方視的な病歴確認に加え、本年度から、認知症スクリーニングにGeriatric Depression Scaleを加えることにより、前方視的検討も開始した。後方視的に大うつ病の病歴を確認した6例について検討したところ、タウオパチー2例（嗜銀顆粒性疾患、神経原線維変化優位型老年性変化、1例ずつ）、脳血管障害2例、神経病理学的有意所見なし2例であった。双極性障害、うつ病で神経病理学的異常のない各二例をさらに検討したが、高感度免疫組織化学的検討において、対象タンパクを拡げても、異常所見は得られなかったことから、これらの神経病理学的異常を認めない症例の脳リソースは、気分障害の生物学的研究に貢献すると期待される。

理化学研究所脳科学総合研究センターでは、モデルマウスを用いて開発した、ミトコンドリアDNA(mtDNA)欠失に伴うシトクロムc酸化酵素（Cox）蛋白サブユニット減少を反映するCox陰性細胞を検出する方法をヒト死後脳に応用し、ヒト死後脳でCox陰性細胞を免疫組織化学的に検出する方法を確立した。マウスでCox陰性細胞が見られた候補部位に相同なヒト脳の部位を明らかにするため、抗カルレチニン抗体および抗アセチルコリンエステラーゼ抗体を用いた免疫染色により検討を行い、その解剖学的な特徴づけを進めた。双極性障害患者死後脳における予備的な検討を進め、双極性障害患者においても、Cox陰性細胞が存在する可能性が示唆された。

研究分担者

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A 研究目的

双極性障害は、躁状態、うつ状態を繰り返し、社会生活の障害を引き起こす重大な精神疾患である。他の精神疾患に比べて、比較的均一な一群であると考えられたことから、ゲノム研究、脳画像研究が進められてきたが、未だ確実な所見は乏しく、その原因については不明な点が多い。そのため、確実な生物学的診断法がなく、診断は面接によって行われるため、初発のうつ状態は大うつ病と診断せざるを得ない。そのため、潜在的な双極性障害患者において、抗うつ薬治療によって躁転・急速交代化などの問題が引き起こされる可能性があり、これが経過の悪化につながっている。初発時に双極性障害と診断できるような診断法の開発が急務である。

我々は、双極性障害患者における磁気共鳴スペクトロスコピー研究、死後脳研究、遺伝学研究、及び動物モデルの研究などから、双極性障害にミトコンドリア機能障害が関与することを示した。

さらに、平成21～23年度の厚生労働科学研究費（気分障害の神経病理学に基づく分類を目指した脳病態の解明）により、脳内の情動関連部位へのミトコンドリア機能障害細胞の蓄積が、気分障害の病態に関与する可能性を示した。一方、双極性障害患者死後脳と気分障害モデル動物の遺伝子発現解析により共通点を探索した結果、Sfpq/PSFが共通に変化していることを突き止めた(Kubota et al, 2010)。最近、SFPQがタウ遺伝子のスプライシングに関わることが見いだされ(Ray et al, J Mol Neurosci　2011)。リチウムがタウリン酸化酵素であるGSK-3βの阻害作用を持つことと併せ、タウと双極性障害の関連が注目された。

村山らは、同研究費により気分障害患者の脳を蓄積し、最近、齊藤らと共に、タウ蓄積による嗜銀顆粒(Saito et al, JNEN 2004)が双極性障害患者死後脳で脳幹部などに多い可能性を示した。

死後脳において、特定の脳部位のこれらの変化が双極性障害に特異的な変化であることが確認されれば、双極性障害の脳画像診断が可能になると期待される。

本研究の目的は、双極性障害における局在性ミトコンドリア機能障害およびタウ蓄積の意義について、患者死後脳を用い、動物モデルと比較しながら明らかにすることである。

B 研究目的

本研究では、病態としては、ミトコンドリア機能障害とタウを中心として解析し、研究の手法としては、死後脳の収集と、その免疫組織化学的な解析に焦点を絞る。

加藤は、死後脳および動物モデルにおける定量的かつハイスループットな免疫組織化学的解析法を開発すると共に、モデルマウスを用いて、病変候補脳部位を同定する。一方、國井と共に、双極性障害患者の献脳登録の推進に向けて、啓発活動を行う。啓発活動は、これまでに進めてきた福島精神疾患ブレインバンクを基盤として、生物学的精神医学会のブレインバンク委員会と連携して進める。

村山、齊藤、國井は、各々が関わるブレインバンク（高齢者ブレインバンク、リサーチリソースネットワーク、福島精神疾患ブレインバンク）において、生前登録した患者の剖検を推進し、双極性障害患者の死後脳の蓄積を進めると共に、既に得られた試料を用いた神経病理学的な解析を行う。

（倫理面への配慮）

本研究については、参加施設の倫理委員会の承認を受けている。これらの研究においては、ヒトゲノム指針、死体解剖保存法など、関連の法規および指針を遵守して行う。

動物実験においては、施設内の動物実験委員会の承認を得て研究を進めると共に、3Rの原則に基づき、使用する動物を最小にすること、可能な限り代替法を利用すること、苦痛を軽減することに努める。

福島精神疾患ブレインバンクの生前登録者には、献脳について説明の上、書面にてインフォームドコンセントを得ると共に、家族の同意を得る。死亡後は家族のインフォームドコンセントに基づいて剖検を行う。剖検は、基本的に、東京都健康長寿医療センター、国立精神神経医療研究センター、福島県立医科大学の3カ所で行う。

国立精神神経医療研究センターにおける精神疾患患者の生前登録については、倫理委員会に申請中である。

C.研究結果

福島県立医科大学では、ブレインバンク事業において、双極性障害患者の生前登録を進め、平成27年3月までに、福島県内からの7名に加え、福島県外からも28名の登録を受け、合計35名の双極性障害患者が登録された。これは、生前登録者168名の20.8%を占めており、特に平成26年度に新規に生前登録された24名のうち双極性障害患者は13名（54%）にも上り、本研究の取り組みにより双極性障害患者の登録は大きく増加している。一方、実際の死後脳の集積に関しては、2015年3月現在で、6名の双極性障害患者の脳が集積された。これは、同ブレインバンクに脳が集積された50例の12%を占めている。6例のうち1名は悪性腫瘍に罹患したことを機に生前登録し、剖検に至ったケースであった。これらの症例の脳については、今後、神経病理学的な検討を進め、診断確定を行う予定である。

国立精神・神経医療研究センターでは、双極性障害では嗜銀顆粒のステージが高く、対照群に比して、若年より、高いステージの嗜銀顆粒を示すことが確認され、若年の双極性障害患者群においては嗜銀顆粒が扁桃体、縫線核、青斑核等に多いなどの特徴から、タウオパチーが一部の症例に関係していることがわかった。この所見を受け、国立精神・神経医療研究センターのバイオバンクと共同で、双極性障害を含めた精神疾患の症例における髄液中の総タウ、リン酸化タウ測定に関する共同研究の準備を開始した。一方、生前登録システムに基づくブレインバンクは現在神経疾患を対象としているが、実際は、精神疾患の方からの登録希望者が多いことから、まず国立精神・神経医療研究センター病院外来通院中の方を対象として、倫理委員会申請を行っている。準備段階の時点で、既に、生前登録を希望している者は、精神疾患で13名あり、そのうち双極性障害の方は6名である。

高齢者ブレインバンクでは、在宅高齢者支援救急総合病院の特質を生かし、高齢者コホート全体の死後脳リソースを構築している。リソースの中に気分障害も一定の頻度で存在することから、剖検例において、精神科専門医による病歴の後方視的な確認により、臨床診断を確認し、気分障害例の半脳凍結リソースの構築を行っている。今年度は、病歴確認により気分障害と診断された症例のうち、神経病理学的に有意の病変を見いだし得ない症例の抽出を試みた。高齢者ブレインバンク連続登録例中、気分障害の既往を持つ例の病歴を、ご遺族の同意を元に後方視的に収集し、うつ病、双極性障害と推定できる症例を抽出した。登録例は全例、高齢者ブレインバンクプロトコールに従い老化性蛋白の網羅的検討を行っている。それらの検討から、有意病変の得られない群を抽出した。それらの例にさらに抗p62、FUS抗体等通常のスクリーニングには含めていない抗体を用いた免疫染色を行うことで、何らかの差異が認められないかを検討した。

これまでに、双極性障害半脳凍結例4例（表1）、うつ病例6例が抽出された（表2）。

表1. 双極性障害

	歳・性	脳重（g）	神経病理診断
1.	90・男	1,160	嗜銀顆粒性認知症
2.	67・男	1,325	著変なし
3.	79・男	1,266	皮質基底核変性症
4.	71・男	1,360	著変なし

表2. うつ病

	歳・性	脳重（g）	神経病理診断
1.	77・男	1,240	脳梗塞
2.	78・男	1,170	微小血管障害変化
3.	90・男	1,186	嗜銀顆粒性認知症
4.	81・男	1,216	著変なし
5.	85・女	925	神経原線維変化優位型変化
6.	86・女	1,192	脳出血・梗塞

双極性障害は、タウオパチー2例、著変なし2例であった。またうつ病は、タウオパチー2例、脳血管障害2例、神経病理学的有意所見なし2例（微小血管障害例を含む）であった。有意な変性型老化性病理を欠く、双極性障害2例（症例2, 4）、うつ病2例（症例2, 4）に関し、p62、FUSによる免疫染色を行ったが、通常の陽性所見以外の異常な蓄積は認められなかった。

理化学研究所脳科学総合研究センターでは、気分障害モデルマウスを用いて開発した、ミトコンドリアDNA(mtDNA)欠失蓄積に伴い、mtDNAにコードされたシトクロムc酸化酵素（COX）蛋白サブユニットが減少している細胞（COX陰性細胞）を免疫組織化学的に検出する方法を用いて、視床室傍核においてmtDNA欠失が特に多く蓄積していることを見いだしたが、この所見を元に、COX陰性細胞をヒト死後脳で検出する方法の確立を進めた。

同じ所見が患者死後脳でも見られるかどうかを確認するには、マウスの視床室傍核と相同な部位がヒト脳でどこにあたるのかを明らかにする必要がある。そこで、ヒト視床室傍核の解剖学的特徴を明らかにするため、マウスでこの部位の同定に用いられている、抗カルレチニン抗体および抗アセチルコリンエステラーゼ抗体を用いた免疫染色を行って、その解剖学的な特徴付けを進めた結果、ヒト視床室傍核は、マウスと異なった形状を持つ可能性が示唆された。

D.考察

啓発活動により、双極性障害患者の生前登録は確実に増加しており、実際に生前登録を経て剖検に至ったケースもあった。今後は、国立精神・神経医療研究センター病院でも生前同意システムに基づくブレインバンクを推進すべく、次年度中の倫理委員会承認を目指すと共に、啓発活動を行って行く予定である。

髄液中のタウについては、被検者が若いこともあり、上昇症例はわずかである可能性が高いが、今後、共同研究を行う予定である。

これまでの研究で、気分障害患者死後脳において老化性変性蛋白蓄積が見られることが明らかとなってきたが、これらの蓄積が原因なのか結果なのかは判断困難である。これらの変化を欠く症例については、気分障害の最上流の変化を示している可能性がある。今回神経変性疾患に伴う異常蛋白蓄積の検出に汎用されるようになったp62と、新たな異常蛋白であるFUSを用いて検出を試みたが、異常構造は見られなかった。これらの症例は、原因解明に貢献しうる可能性を持つ、貴重なリソースであると考えられ、今後多くの研究者に提供することにより、研究が進展すると期待される。

ヒト視床室傍部の解剖学的な検証により、その形態が明らかになりつつあり、今後、視床室傍部についての神経病理学的な検討が可能になると期待される。今後、集積した双極性障害患者死後脳を用いて、候補部位である視床室傍核のミトコンドリア機能障害に関する神経病理学的な検討を進めていく予定である。

E.結論

双極性障害患者死後脳の蓄積は困難な課題ではあるが、生前登録活動を通して啓発を進め、脳の集積も進みつつある。

最終年度には、双極性障害における視床室傍核のミトコンドリア機能障害についての検討をさらに進め、結論を得たい。

F.健康危険情報

特記事項なし。

G.研究発表

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H.知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

研究成果の刊行一覧表（平成 26 年度）

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資　　料

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Original Article

Neurodegenerative changes in patients with clinical history of bipolar disorders

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Neurodegeneration in bipolar disorder (BPD) is poorly understood. Therefore, the current study was designed to assess the immunohistochemical changes in neurodegenerative markers in patients with BPD. Eleven consecutive autopsy cases diagnosed with BPD were analyzed. Sections were obtained from archival paraffin blocks of representative areas and stained using conventional methods, as well as immunostained with several antibodies to screen for neurodegenerative diseases. Age- and non-argyrophilic grains (AGs) degeneration matched controls were selected for each case. Clinical information was retrospectively collected from medical charts. All patients were men, and the average age of death was 70 years. Neuropathological diagnoses included dementia with grains (2), argyrophilic grain disease (2), corticobasal degeneration (CBD, 1), Lewy body disease (1), hypoxic encephalopathy (1) and cerebral infarction (1). All cases showed AGs to various degrees. Three patients died in their 50s; one demonstrated dementia with Lewy bodies, while the other two showed abundant AGs in the thalamus and amygdala. Of the three patients who died in their 60s, one showed AGs preferentially in the thalamus and amygdala, while the others demonstrated limbic predominance. The patients who died in/after their 70s demonstrated AGs similar to controls, except for the patient with CBD. Our data provides potentiality that neurodegenerative diseases may be an underlying pathology in certain cases of BPD.

Key words: argyrophilic grain, chronic traumatic encephalopathy, lithium carbonate, mood disorder, tau.

INTRODUCTION

Bipolar disorder (BPD) is an endogenous psychiatric disease that involves the repetition of manic and depressive states,[1] and has a lifetime prevalence of 0.8–2.6%.[2] BPD is treated with various medications, including lithium carbonate, which has been shown to be an effective mood stabilizer. Over the past half century, lithium carbonate has been used as a prophylactic treatment with both antimanic and antidepressant effects,[3] most likely due to its multiple biochemical and biological effects. Several mechanisms of action have been proposed for lithium carbonate, including inhibition of glycogen synthase kinase-3 beta (GSK-3β),[4] 5-HT_{1B} serotonin receptor binding,[5] modulation of glutamate uptake and release,[6] and induction of Bcl-2, a neuroprotective protein.[7] Among these, GSK-3β is a tau protein kinase.[8] Several lines of evidence indicate that BPD could be a circadian rhythm disorder mediated by a GSK-3β abnormality. For example, transgenic mice overexpressing GSK-3β exhibit hyperactivity.[9] In addition, *Drosophila* overexpressing GSK-3β demonstrate a shortened circadian cycle that can be extended by lithium.[10] Lastly, the symptoms of BPD are thought to be associated with circadian rhythm disruptions. Cell culture studies have also shown that the action of GSK-3β is suppressed by lithium. Lithium inhibits the phosphorylation of tau, promotes the binding of tau with microtubules, and eventually promotes microtubule aggregation.[11] This would suggest

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that pathological tau metabolism may have a role in the etiology of BPD.

Diseases with neuropsychiatric symptoms caused by the accumulation of abnormally phosphorylated tau are generally called tauopathies. One of these, dementia with grains (DG), was recognized as an independent disease relatively recently, thanks to the development of methods such as Gallyas-Braak staining. DG was first reported by Braak *et al.* in 1987.[12] It is characterized by the appearance of argyrophilic grains (AGs) in the neuropil of the limbic system gray matter and in oligodendroglia-derived coiled bodies in the white matter. The pathological significance of these structures has since been elucidated by the discovery that they are composed of phosphorylated tau[13] and deposits of 4-repeat tau, which can bind four microtubules.[14] At present, cases tend to be labeled as DG when dementia is caused solely by AGs, while cases are labeled argyrophilic grain disease (AGD) if pathological AGs are observed. However, with the latter, no clear connection to dementia has been established. The clinical symptoms of AGD are characterized by comparatively well preserved cognitive functions, but prominent psychiatric symptoms.[15] In addition, another tauopathy, chronic traumatic encephalopathy (CTE), a disease that is caused by repeated brain trauma and has received attention in recent years, shows psychiatric symptoms such as irritability, impulsiveness, depressive states and memory disorders, which are thought to appear 8–10 years after the actual trauma.[16] Neuropathological findings of CTE include atrophy of the cerebral cortex, medial temporal lobe, diencephalon, and mammillary body, as well as deposits of phosphorylated tau and phosphorylated transactive response DNA binding protein 43 kDa (TDP-43) over a wide area. In particular, phosphorylated tau is found throughout the frontotemporal lobe, limbic system and brainstem. These findings suggest a connection between diseases with prominent psychiatric symptoms and pathological tau metabolism.

In the current study, an immunohistological examination was performed on archival tissue specimens of patients who had previously been clinically diagnosed with BPD in order to clarify the relationship between BPD and neurodegenerative diseases, particularly AGD.

MATERIALS AND METHODS

Tissue source

From the cases registered at our brain bank networks (the Brain Bank for Aging Research (BBAR) and Research Resource Network (RRN)), we selected 11 consecutive autopsy cases that had been diagnosed with BPD. The controls included 1240 consecutive cases (ages 48–104 (mean ± SD, 80.6 ± 8.9) years; men : women, 662:578) registered to the BBAR and 164 consecutive autopsy cases under the age of 65 (ages 0–64 (45.0 ± 20.2 years); men : women, 101:63) from the Yokohama Rosai Hospital, a community-based hospital in the BBAR network.[17] The study was approved by the Institutional Review Board of the National Center of Neurology and Psychiatry (NCNP).

Clinical information

Clinical diagnosis of BPD followed the "Diagnostic and Statistical Manual, 4th Edition-Text Revision" (DSM-IV-TR) with retrospective analysis of each medical chart. Eight cases were classified to have had Bipolar I Disorder (cases 1, 2, 3, 4, 5, 6, 8 and 9) and the other three case were unclassifiable (cases 7, 10 and 11). The history of medication and cognitive function was also studied, and the Mini-Mental State Examination (MMSE)[18] was used as an index of cognitive function.

Neuropathology

The brains of cases 4, 5, 7, 9, 10 and 11 and all of the control cases from the BBAR were prepared with the BBAR protocol as previously reported.[19] Briefly, one side of each brain was frozen for biochemical and molecular biological studies, while the other side was used for pathological studies. At the time of autopsy, 7-mm coronal sections of one side of the cerebrum were obtained for freezing. In addition, 5-mm axial slices were obtained from the brainstem and 5-mm sagittal slices were obtained from the cerebellum. Representative areas from the frozen side, including the amygdala, hippocampus, midbrain, motor cortex, frontal cortex, temporal pole, parietal lobe and occipital pole, were then fixed for 48 h in 4% paraformaldehyde and subsequently embedded in paraffin. The other side of the brain was fixed in 20% neutral formalin for 7–13 days and sliced in the same manner as the opposite side. The representative areas were then embedded in paraffin. For Case 1, parts of one side of the brain (frontal, temporal and occipital lobes) were freeze-fixed, and the remaining tissue was post-fixed for approximately 1 year in acidic formalin. For cases 2, 3 and 6, the entire brain was fixed in acidic formalin for approximately 4 months, 20 days and 50 days, respectively. For case 8, the entire brain was fixed for approximately 11 days in neutral formalin. After fixation, the brains were sliced as described above and the representative areas embedded in paraffin. The control brains from the community-based hospital followed the BBAR protocol, skipping the recovery of frozen tissue. For this study, the following regions were intensively analyzed: the medulla oblongata, pons, midbrain, cerebellum, amygdala, hippocampus, thalamus, basal nucleus of Meynert and cerebral neocortex (frontal, temporal, parietal and occipital

lobes). Slices were cut at a thickness of 6 μm and stained with HE or KB stain, or were silver-impregnated using the Gallyas-Braak method on all of the sections.

Immunohistochemistry

Immunostaining was performed with an automated immunostainer (Ventana XT DISCOVERY; Ventana, Tucson, AZ, USA). As described in detail previously,[20] the antibodies used included anti-phosphorylated tau (ptau; AT8, monoclonal; Innogenetics, Temse, Belgium), 3-repeat/4-repeat tau-specific (RD3/RD4, Upstate, Lake Placid, NY, USA), anti-phosphorylated α-synuclein (psyn; monoclonal; Psyn#64, WAKO, Osaka, Japan), anti-amyloid-β (Aβ) (12B2, monoclonal; IBL, Maebashi, Japan), and anti-phosphorylated TDP-43 antibodies (pS409/410-2, polyclonal; COSMO BIO CO., LTD, Tokyo, Japan). Anti-phosphorylated tau immunostaining was performed on all areas. For the other forms of immunostaining, the BBAR protocols[17] were followed.

Neuropathological diagnosis

AGs were classified into four stages based on Saito's staging.[17] Neurofibrillary tangles (NFTs) were classified into seven stages (0–6), and senile plaques into four stages (0 and A–C), based on Braak's staging.[21] Lewy body pathology was diagnosed based on the dementia with Lewy bodies (DLB) consensus guideline – revised (DLBCG-R).[22] Amyloid angiopathy was diagnosed based on BBAR (http://www.mci.gr.jp/BrainBank/index.cgi) staging.[23] The original stages (none, A, B and C) were converted to 0, 1, 2 and 3 for quantitative analysis. Diagnosis of corticobasal degeneration was based on National Institutes of Health criteria.[24]

Argyrophilic grain examination

The AG density for all BPD cases was semi-quantified. Specifically, the representative areas were immunostained with anti-phosphorylated tau and the field of view (FOV) with the greatest number of AGs at 200× magnification was semi-quantified (score 0 = no AGs; 1 = 1–30 AGs/200× FOV; 2 = 31–60 AGs/200× FOV; 3 ≥ 61 AGs/200× FOV) (Fig. 1). Cases were extracted from the control group that largely matched these cases in age and non-AG senile degeneration (NFTs, senile plaques, Lewy body pathology and amyloid angiopathy). The AG density of these cases was also semi-quantified as described above.

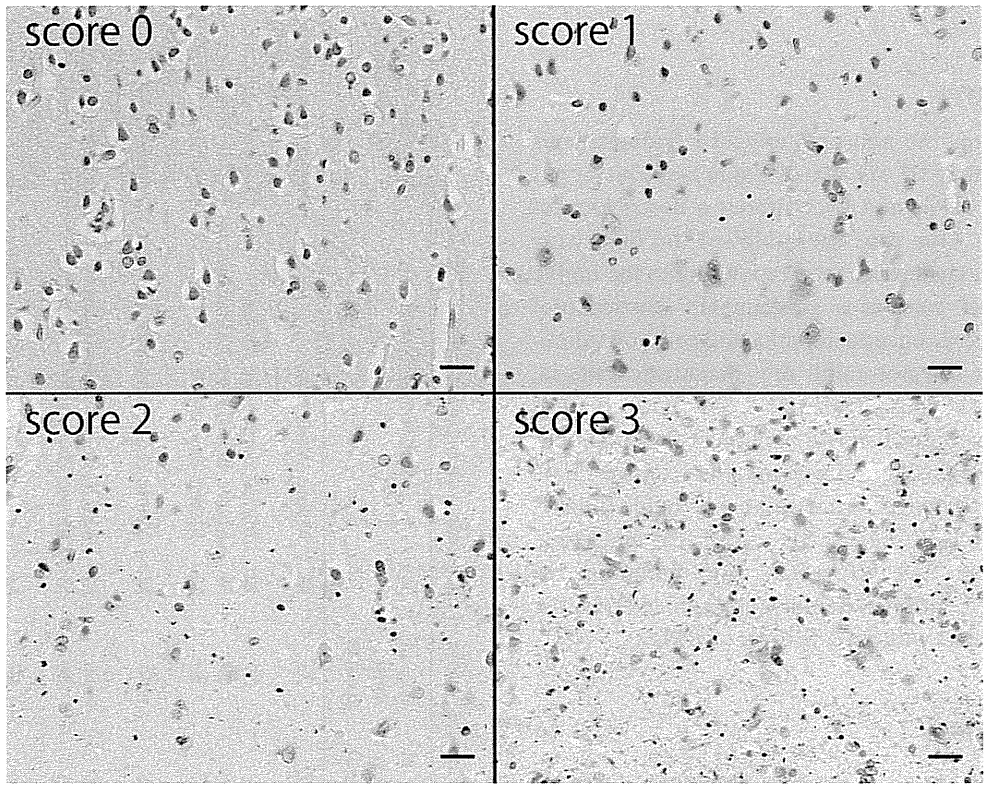

Fig. 1 Semi-quantification of argyrophilic grains (AGs). AT8 staining and high-power fields (HPF; generally 200×) were used to measure AG density. Score 0 is the absence of AGs. Score 1 is 1–30 AGs/HPF, score 2 is 31–60 AGs/HPF, and score 3 is ≥ 61 AGs/HPF. Bar = 20 μm.

RESULTS

Clinical information

Table 1 provides the detailed clinical information of the 11 BPD patients. The age of onset of BPD ranged from 22–56 years (mean ± SD = 41.8 ± 12.6 years), age at death from 52–90 years (70.3 ± 12.9 years) and disease duration from 13–34 years (27.2 ± 7.1 years). All of the patients were male. Three cases (cases 2, 5, 6) had violent behavior episodes in their manic phase, and three cases (cases 3, 8, 10) had hallucinations and delusional episodes. Regarding other diseases, case 2 was diagnosed with diabetes mellitus, case 3 with and convulsive seizures, cases 4 and 9 with malignant lymphoma, case 5 with alcoholic liver cirrhosis and alcohol dependency, case 6 with bronchial ectasia, case 8 with corticobasal syndrome, and case 11 with atrioventricular blockage. Cognitive impairment, including mild forms, was observed in four cases (cases 1, 5, 8 and 11) (36.4%). Five cases (cases 2, 4, 5, 7 and 8) had taken lithium carbonate for BPD, five had taken antipsychotic drugs (cases 2, 3, 4, 5 and 8), and five had taken antidepressant drugs (cases 2, 3, 4, 5 and 9). Three of the cases which had parkinsonism (cases 3, 7 and 10) and one case, which received diagnosis of corticobasal syndrome (case 8), had taken levodopa and other antiparkinsonian drugs.

Neuropathology

Table 2 summarizes the neuropathological findings. Four cases presented Stage III AG, two of whom had a history of cognitive decline (DG) and two without a description of dementia (AGD). One case each fulfilled the diagnostic criteria of Lewy body disease (corresponding to the limbic form in the DLB consensus guidelines),[22] corticobasal degeneration, hypoxic encephalopathy and cerebral infarction. Three cases did not fulfill any diagnostic criteria, including one case with incidental Lewy body disease (Figs 2 and 3). Except for the case of hypoxic encephalopathy (652 g brain weight), brain weights ranged from 1160–1470 g. Alzheimer changes were relatively mild: Braak's NFT[21] Stage being I–II (mean ± SD = 1.5 ± 0.5) and senile plaque (SP) stage 0–A (0.5 ± 0.5). The pathology of corticobasal degeneration was indistinguishable from the pathology of patients without a history of BPD.

Argyrophilic grains

AGs were observed in all 11 cases, with a mean stage[17] of 2.1 (Stage 0.5: two cases (18.2%); Stage I: one case (9.1%); Stage II: three cases (27.3%); and Stage III: five cases (45.5%)). In the control group, 68.2% (958 individuals)

Table 1 Clinical profile of each case with bipolar disorder (BPD)

Case	Age at death (years)	Age at onset (years)	Sex	Mental manifestation	Comorbidity other than bipolar disorder	Cognitive impairment	MMSE	Lithium carbonate treatment duration	Antipsychotic or antidepressant treatment	
									Antipsychotic	Antidepressant
1	52	22	M	Manic, depressive	Hypoxic encephalopathy, after suffocation	Yes	ND	ND	ND	ND
2	52	34	M	Manic, depressive, violent	Diabetes mellitus	ND	ND	Over 7 years	(+)	(+)
3	58	28	M	Manic, depressive, hallucination	Parkinsonism, seizure	ND	ND	ND	(+)	(+)
4	67	33	M	Manic, depressive	Malignant lymphoma, diabetes mellitus	ND	ND	Over 1 year	(+)	(+)
5	68	45	M	Manic, depressive, violent	Alcohol abuse, alcoholic liver cirrhosis	Yes	22 points (63 years)	5 years	(+)	(+)
6	69	56	M	Manic, depressive, violent	Bronchial ectasia	ND	ND	ND	ND	ND
7	71	38	M	Manic, depressive	Heavy use of alcohol, parkinsonism	ND	ND	Over 1 year	ND	ND
8	79	50	M	Manic, depressive, hallucination	Corticobasal syndrome	Yes	24 points (76 years)	2 years	(+)	ND
9	83	Young age	M	Depressive main	Malignant lymphoma	ND	ND	ND	ND	(+)
10	84	56	M	Manic, depressive, delusion	Parkinsonism	ND	ND	ND	ND	ND
11	90	56	M	Depressive main	AV block	Yes	12 points (87 years)	ND	ND	ND

AV, atrioventricular; M, male; ND, not described; MMSE, Mini-Mental State Examination.

Table 2 Summary of neuropathological findings

Case	Age at death (left)/duration of disease (right) (years)	Neuropathologic diagnosis	Brain weight (g)	Argyrophilic grain stage[17]	NFT stage[21]	Lewy body stage[22]	Senile plaque stage[21]	Amyloid angiopathy stage[23]
1	52 / 30	Hypoxia	652	II	I	None	None	None
2	52 / 18	Argyrophilic grain disease	1,430	III	I	None	A	None
3	58 / 30	Lewy body disease	1,470	0.5	I	Limbic	None	1A
4	67 / 34	Unremarkable	1,325	II	II	None	None	None
5	68 / 23	Dementia with grains	1,116	III	I	None	A	None
6	69 / 13	Unremarkable	ND	II	II	None	None	None
7	71 / 33	Unremarkable	1,360	0.5	I	Incidental	A	1C
8	79 / 29	Corticobasal degeneration, argyrophilic grain disease	1,266	III	II	None	None	None
9	83 / –	Acute cerebral infarction	1,458	I	I	None	None	1A
10	84 / 28	Argyrophilic grain disease	1,300	III	II	None	A	1A
11	90 / 34	Dementia with grains	1,160	III	II	None	A	None

ND, not described.

belonged to Stage 0, 0.1% (two individuals) to Stage 0.5, 16.4% (230 individuals) to Stage I, 8.1% (114 individuals) to Stage II and 7.1% (100 individuals) to Stage III in both genders. Among the male gender, 72.1% (550 individuals) belonged to Stage 0, 15.3% (117 individuals) to Stage I, 6.0% (46 individuals) to Stage II, and 6.6% (50 individuals) to stage III. The incidence and extent of AG appeared to be more severe in the BPD group than in the control group, although the number of cases in the BPD was too small for statistical analysis. The youngest case in the BPD group was 52 years old (at the time of death) with an AG of Stage III, which was definitely more severe than the Stage I of the youngest brain with AGs at 56 years of age in the control group. We selected 4–7 cases of each decade with similar NFT and SP stage and semi-quantified for AGs using the method described in the "Materials and Methods" section (Fig. 1). The detail of the control cases were as follows: five cases for 50 control cases (death age 56.0 ± 1.9 years old), seven cases (death age 66.7 ± 2.9 years old) for 60 control cases, and four case (death age 84.3 ± 1.0 years old) for more than 70 control cases. The patients who died in their 50s (cases 1 and 2) and had BPD onset in their 20s and 30s, respectively, tended to have higher AG density than the control group. Case 2, in particular, showed a prominent presence of AGs in the limbic system, including the hippocampus proper, subiculum, amygdala and anterior thalamic nucleus, as well as the locus coeruleus. Among the patients who died in their 60s (cases 4, 5 and 6), case 5 presented the similar unique distribution of AGs, while cases 4 and 6 followed the usual distribution, but had more dense AG deposition compared with the control group. The patients who died above the age of 70 also presented the latter pattern. There was no apparent difference between cases that were administered lithium and those that were not (Fig. 4).

Corticobasal degeneration (CBD)

Neuropathological diagnosis of CBD was reached in a manner blind to this research project. Distribution of tauopathy is typical for CBD and could not point out any unique features.

Lewy body-related α-synucleinopathy

Case 3 exhibited limbic-type Lewy body pathology according to Dementia with Lewy Bodies Consensus Guidelines – Revised (DLBCG-R).[22] Lewy bodies and Lewy neurites were scattered in the brainstem, including the dorsal motor nucleus of the vagus nerve, locus coeruleus and substantia nigra. There was a heavy burden of phosphorylated α-synuclein-immunoreactive structures in the amygdala, corpus striatum and hypothalamus. In the neocortex and hippocampus, Lewy body pathology was minimal.

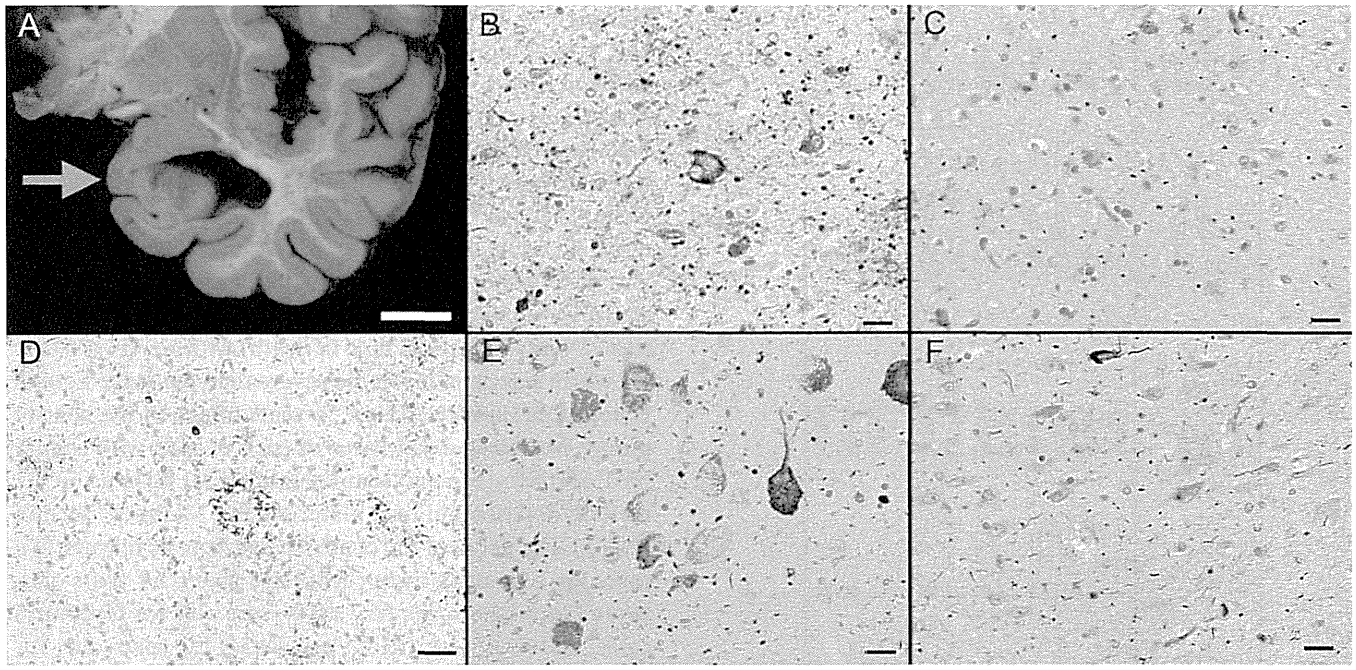

Fig. 2 Case 11 (argyrophilic grain disease: AGD) and case 8 (corticobasal degeneration and AGD). A, B, C. Case 11 (AGD). A. Macroscopic observation confirmed prominent atrophy of the ambient gyrus and amygdala (arrow) (bar = 10 mm). B. Histopathology of the amygdala showing many AGs, accompanied by ballooned neurons and pre-tangles (AT8 immunostaining, bar = 20 μm). C. The medial temporal lobe contained many AGs (Gallyas-Braak (GB) staining, bar = 20 μm). D, E, F. Case 8 (corticobasal degeneration and DG). D. The precentral gyrus had an astrocytic plaque (AT8 immunostaining, bar = 50 μm). E. The locus coeruleus had abundant AGs with pre-tangles and neurofibrillary tangles (NFTs) (AT8 immunostaining, bar = 20 μm). F. Amygdala had many AGs (GB staining, bar = 20 μm).

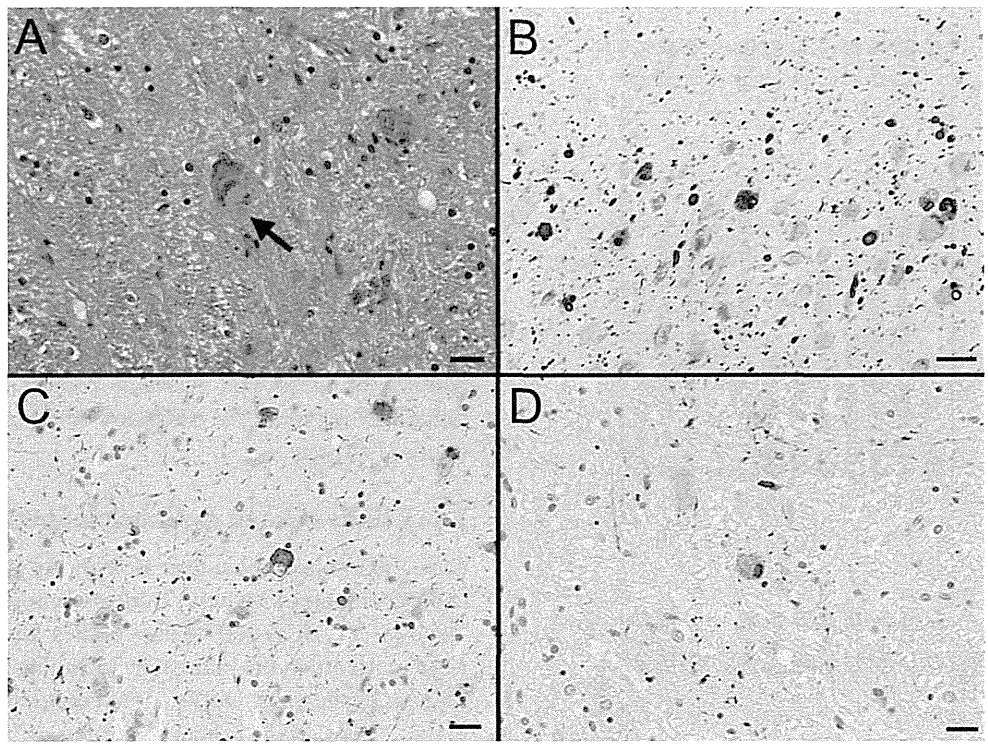

Fig. 3 Case 3 (Lewy body disease). A. Substantia nigra: a brainstem-type Lewy body (arrow) accompanied by melanophages and free melanin (HE staining, bar = 20 μm). B. Locus coeruleus. C. Amygdala. D. Nucleus raphe obscurus: Lewy bodies and dots were present (pSyn#64 immunostaining, Bar = 50 μm (B), bar = 20 μm (C, D)).

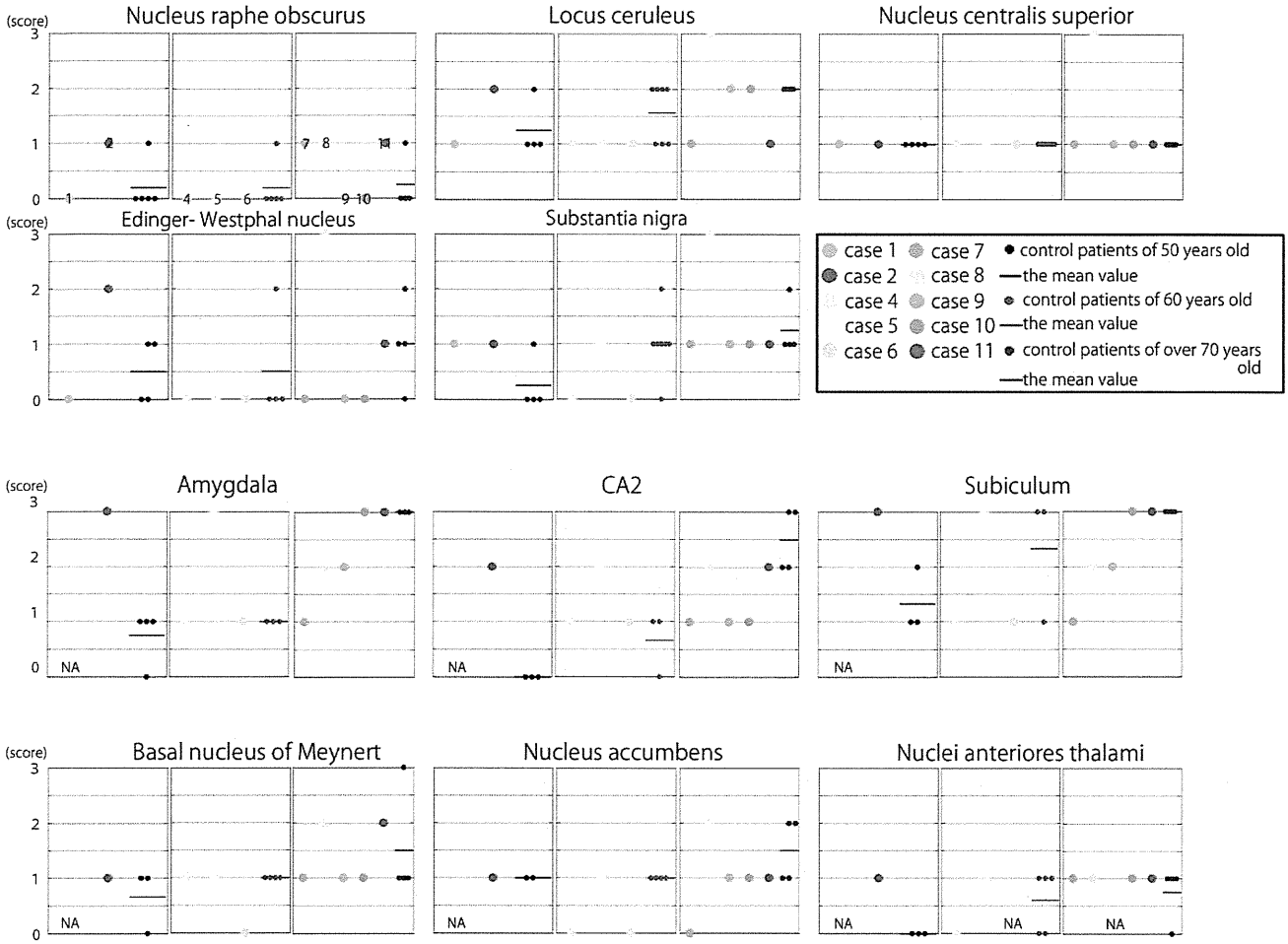

Fig. 4 A comparison of the semi-quantified density of argyrophilic grains (AG) in cases of bipolar disorder (BPD) and age-matched controls with similar Alzheimer pathology. Case 1 had superimposed hypoxic encephalopathy and only the brainstem was evaluated. Compared to the controls, patients who died in their 50s exhibited higher AG density in the amygdala, hippocampus proper, subiculum, Edinger-Westphal nucleus, locus ceruleus and anterior thalamic nucleus. Of the cases with death in their 60s, case 5 exhibited AG density and distribution similar to that of patients who died in 50s, while cases 4 and 6 showed distribution patterns similar to that of age-matched controls, as did the cases with death in their 70s. NA: not available.

DISCUSSION

This study is the first to demonstrate the neurodegenerative outcome of some cases in bipolar disorder, including argyrophilic grain-type tauopathy and Lewy body-related alpha-synucleinopathy.

AG was present in all our cases. The distribution of AG in the younger group was unique. In the older group except case 8 with a diagnosis of CBD and AGD, AG distribution was similar to that observed in the control group, but the staging was higher. Our observation may be consistent with those of Nagao *et al.*[25] who reported an association of AG with late-onset schizophrenia and delusional disorders (LOSD).

A group of patients with BPD who died in their 50s and 60s exhibited a heavy burden of AGs in the amygdala, thalamus and locus coeruleus, which did not follow Saito's extention paradigm.[17] In this group, we could not find patterns of chronic traumatic encephalopathy (CTE) associated with neocortical NFTs, which does not follow Braak's extention paradigm. Instead, our observation of a unique distribution of AG-type tauopathy (as observed in patients with BPD who died at a younger age) could suggest that these anatomical sites are involved in AG formation during aging. Alternatively, the mere presence of AGs may contribute to the psychiatric symptoms.

It is also worthy to comment that one case who received diagnosis of BPD later developed into CBD. It is difficult to deny the possibility of coincidence, but further accumulation of cases may be indicative.

In the current study, Lewy body pathology was observed in two cases in the BPD group, with one case corresponding to the DLBCG-R[22] limbic stage. Although depression is regarded as one of the prodromal symptoms of Lewy body