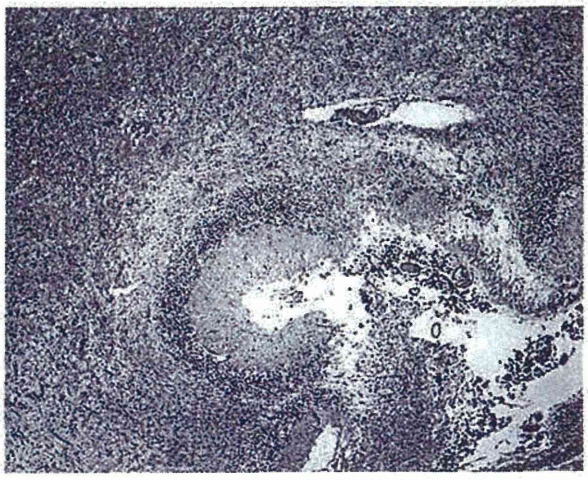

Fig. 1　Cerebellar cortex of superficial hemosiderosis. A photomicrograph shows severe loss of the cerebellar cortex and numerous hemosiderin deposits. H&E stain.

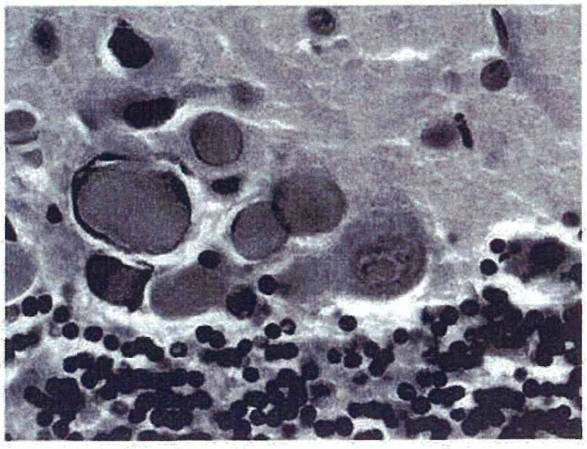

Fig. 2　Cerebellar cortex of neuroferritinopathy. A photomicrograph shows round bodies in the nucleus of glial and Purkinje cells. H&E stain.

チン軽鎖，変異型フェリチン軽鎖，およびフェリチン重鎖に対する抗体による免疫染色を施行したところ，すべての抗体で封入体が陽性反応を示し，生化学的解析ともあわせ，封入体が上記3種類のフェリチンをふくむことが明らかにされている[6]．

中枢神経系以外では，皮膚乳頭層の線維芽細胞，尿細管上皮の核内，横紋筋内血管の内皮細胞にも，フェリチン免疫染色陽性の構造物が報告されることから，本症を全身性疾患と捉え，神経フェリチン症ではなくフェリチン症と呼ぶことが望ましいかもしれない．

神経フェリチン症が神経細胞死をきたす原因として，変異フェリチンによる gain of toxic function と loss of toxic function が想定されている[10]．ここで紹介した両者の神経病理所見は，大きくことなるものの，鉄と神経変性との関連を解決する糸口になりうる疾患であると考えられ，今後の展開が期待される．

本研究の一部は厚生労働省難治性疾患克服研究事業（H23-難治（難）一般-062，H24-難治等（難）一般-063）によった．

※本論文に関連し，開示すべき COI 状態にある企業，組織，団体はいずれも有りません．

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Abstract

Neuropathology of superficial hemosiderosis and neuroferritinopathy

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Neuropathology of superficial hemosiderosis. Gross finding shows diffuse brownish discoloration at the surface of the cerebrum, cerebellum, brainstem and spinal cord. Severe atrophy and necrosis is present in the cerebellum. Extensive deposits of hemosiderin that are well recognized with Berlin blue and ferritin immunohistochemistry are present at the surface and in the superficial parenchyma of the cerebrum, brainstem, cerebellum and spinal cord. Although the pathomechanism of this disease remains unresolved, continuous or recurrent subarachnoid hemorrhage may be important for developing diffuse hemosiderin deposition. Neuroferritinopathy. Intranuclear and intracytoplasmic bodies are seen in glia and subsets of neurons in the central nervous system as well as in extraneural tissue. They are stained by Perls' method for ferric iron. The bodies are immunopositive against antibodies raised against mutated and wild type of ferritin light polypeptide as well as ferritin heavy polypeptide. It is suggested that loss of normal function and gain of toxic function may be crucial for neurodegeneration of neuroferritinopathy. Both diseases could be helpful to understand and clarify the pathomechanism of neurodegeneration associated with iron metabolism.

(Clin Neurol 2012;52:959-961)

Key words: Superficial hemosiderosis, neuroferritinopathy, iron, hemsosiderin, ferritin

＜シンポジウム (1)—5—4＞鉄と神経疾患

鉄沈着をきたす疾患の画像診断

百島　祐貴

(臨床神経 2012;52:955-958)
Key words：MRI，鉄沈着，T_2*強調画像

1. 鉄沈着を診断するためのMRI撮像法

脳内に沈着する非ヘム鉄の多くは，フェリチン，ヘモシデリンの形で存在し，MRIはこれらの物質の常磁性によるT_2短縮を利用して鉄沈着を診断する[1]．

a. GRE法 vs. SE法

一般に，鉄沈着の診断には勾配磁場法（Gradient Echo, GRE）法によるT_2*強調画像が有用である．現在，T_2強調画像の多くは高速スピンエコー（Fast Spin Echo, FSE）法で撮影されるが，この原型である（古典的な）スピンエコー（Conventional Spin Echo, CSE）法は鉄沈着の検出能が高く，大脳基底核，中脳などの鉄沈着はGRE法よりCSE法の方が明瞭にみとめられるばあいがある（Fig. 1）．一方，陳旧性血腫などによる鉄沈着は，GRE法がすぐれている．これは，大脳基底核に沈着するフェリチンは，粒子が小さく，均等に分布するために磁化率効果が相対的に少なく，一方ヘモシデリン粒子は大きいためにT_2*緩和効果が明瞭に現われるためと考えられる[2]．

b. GRE法 vs. SWI法

最近，多くの装置で利用可能となっている磁化率強調画像法（Susceptibility-weighted Imaging）は，位相画像に後処理を加えることにより，磁化率のことなる部位をより明瞭に描出する方法で，脳内鉄沈着については通常のT_2*強調画像よりも感度が高い事が知られており，積極的に利用する価値がある（Fig. 2）[3]．

c. 磁場強度の影響

T_2（T_2*）緩和効果は，撮影装置の静磁場強度に依存して大きくなる．現在のところ1.5T装置がもっとも多く，3T装置もかなり普及している．一般に前述のような適切な撮影法を選択するかぎり，1.5T以上の装置では診断上の問題はない．しかし，0.2～1.0Tの低磁場装置のばあいは，検出率が低下するので注意が必要である[4]．

2. 陳旧性微小出血巣の診断

諸疾患における鉄沈着の画像診断については他稿に譲り，本稿では陳旧性微小出血巣（microbleeds）のMRI診断について述べる．

a. 陳旧性微小出血巣の画像所見

陳旧性微小出血巣は，様々な病態でみとめられるが，無症候性出血巣としてみとめられるばあいが多く，長期にわたる高

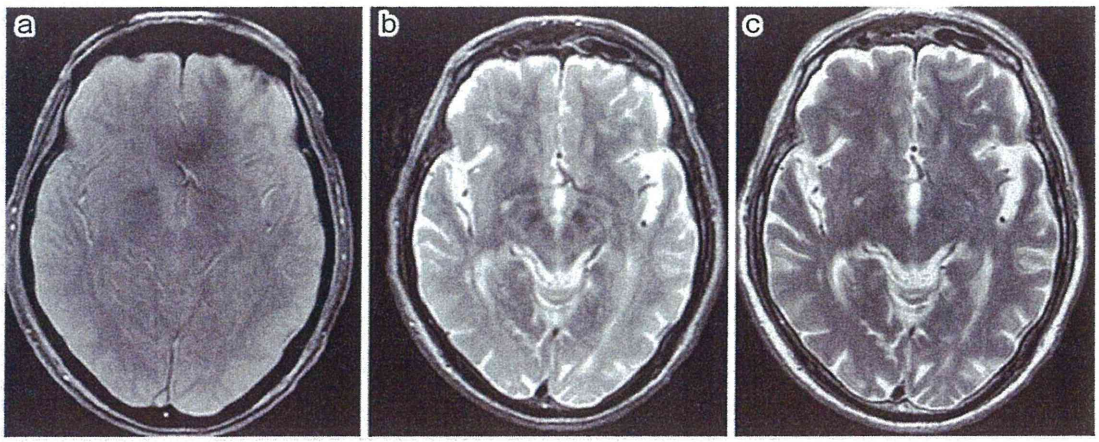

Fig. 1　撮影法による違い—GRE法 vs. SE法．
a. GRE法（T_2*強調画像），b. CSE法（T_2強調画像），c. FSE法（T_2強調画像）．赤核，黒質の鉄沈着は，GRE法よりCSE法の方が明瞭にみえる．

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（受付日：2012年5月23日）

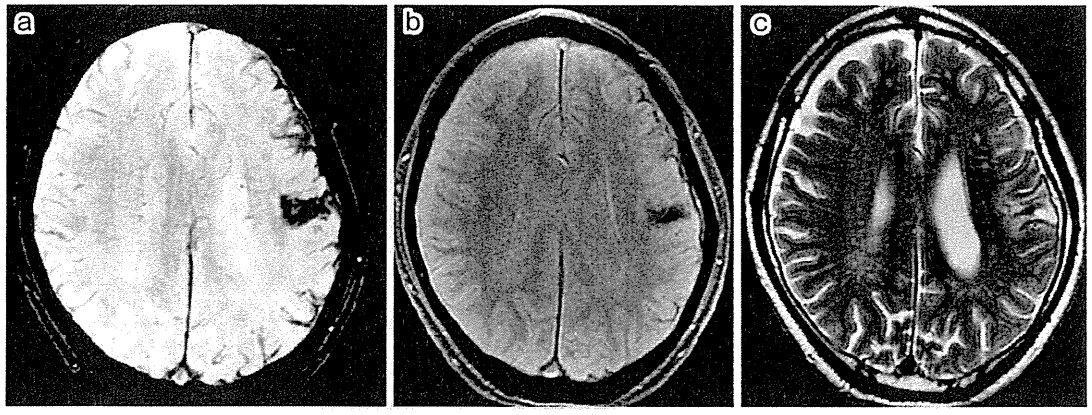

Fig. 2　撮影法による違い—GRE 法 vs. SWI 法.
a. SWI 法（磁化率強調画像），b. GRE 法（T_2*強調画像），c. FSE 法（T_2 強調画像）
外傷性クモ膜下出血後の限局性脳表ヘモシデローシス．脳表の低信号が，SWI 法では GRE 法より明瞭である．

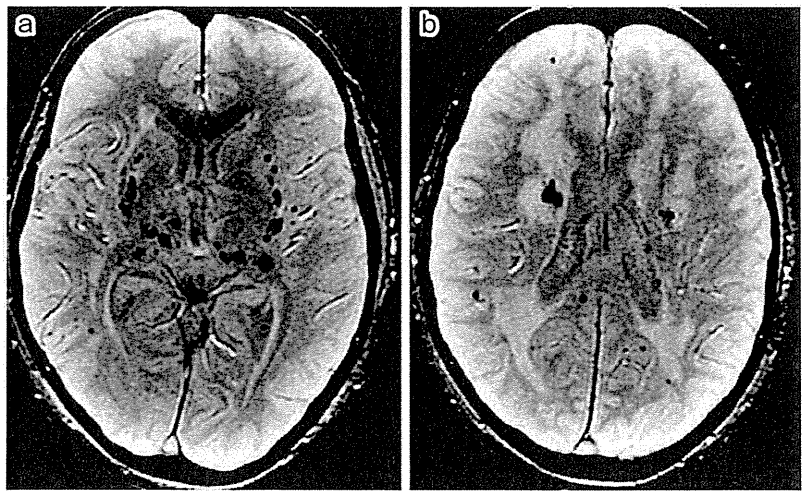

Fig. 3　陳旧性微小出血巣—慢性高血圧.
a, b. T_2*強調画像．70 歳男性．無症状．20 年前より高血圧，服薬無し．T_2*強調画像でテント上下に小さな低信号が多発している．

Table 1　陳旧性微小出血巣をきたす疾患.

Hypertensive angiopathy
Amyloid angiopathy
Hemorrhagic thromboemboli
Diffuse axonal injury
Cavernous malformation
Post-radiation teleangiectasia
CADASIL
Parry-Romberg syndrome

血圧の症例に多いことが知られている．T_2*強調画像，磁化率強調画像では，数 mm 大の多発低信号としてみとめられるが，その一部が T_2 強調画像でもみえることがある（Fig. 3）．

b. 鑑別診断

この所見がみとめられる病態としては，Table 1 のようなものが挙げられる[4]．多くのばあい，病歴，臨床所見から鑑別が可能であるが，無症候性に偶然発見される例では，慢性高血圧に続発するものと，多発海綿状血管奇形（血管腫）との鑑別が問題となることがある．このようなばあい，海綿状血管奇形では，T_2*強調画像，T_2 強調画像でしばしば低信号に加えて高信号の混在による不均一な内部構造がみとめられること，T_2 強調画像で高信号をともなうことが多いことが鑑別の参考になる（Fig. 4）[4][5]．

びまん性軸索損傷でも慢性期に多発低信号がみとめられ，外傷との関連が問題となることがあるが，びまん性軸索損傷のばあいは，大脳半球の皮髄境界，脳幹，視床など深部構造，硬膜近傍など，外傷による物理的ストレスがかかりやすい部

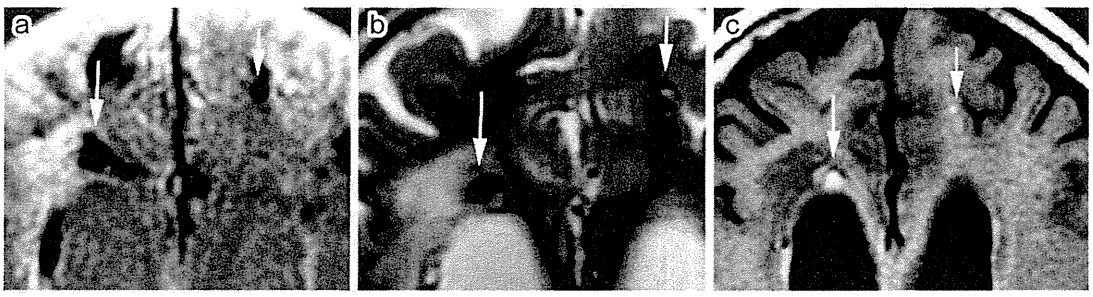

Fig. 4　陳旧性微小出血巣—海綿状血管奇形.
a. T_2*強調画像，b. T_2強調画像，c. T_1強調画像．75歳男性．無症状．30年前に後頭葉の脳出血切除術，海綿状血管奇形と診断されている．大脳半球に多発する微小出血巣があるが，T_2強調画像で高信号をともなう内部構造があり，T_1強調画像では高信号がみとめられる（矢印）.

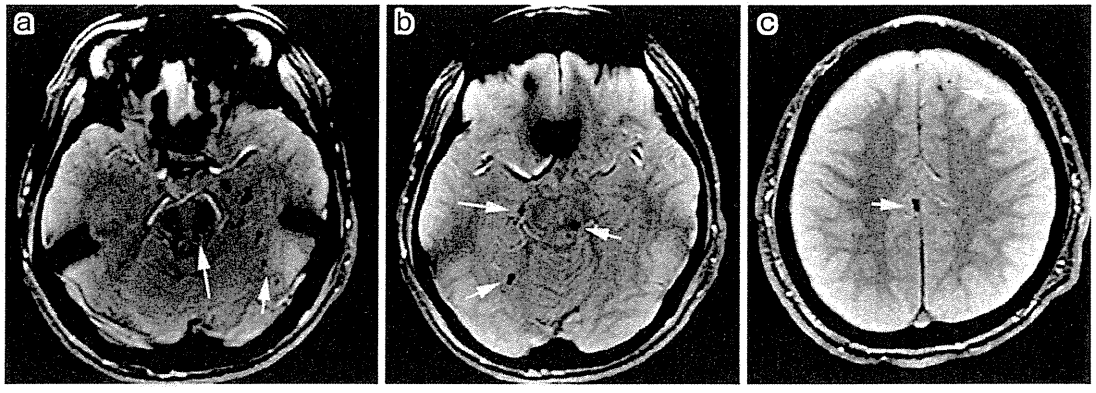

Fig. 5　陳旧性微小出血巣—びまん性軸索損傷.
a, b, c.　T_2*強調画像．32歳男性．交通事故後，高次脳機能障害．微小低信号が多発しているが，皮髄境界，脳幹，硬膜（大脳鎌，小脳テント）近傍に限局しており，外傷との関連が示唆される（矢印）.

位に限局することが参考となる（Fig. 5）[6].

※本論文に関連し，開示すべきCOI状態にある企業，組織，団体はいずれも有りません.

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Abstract

Imaging of diseases with iron deposition

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Some fundamental technical aspects of magnetic resonance imaging (MRI) in evaluation of iron deposition were discussed. MRI is an imaging modality sensitive to iron deposition of the brain tissue. T_2 weighted imaging (T_2WI), T_2^* weighted imaging (T_2^*WI), and susceptibility-weighted imaging (SWI) are particularly available for this purpose. They are different in sensitivity and availability, and should be used in the right places respectively. Susceptibility to iron deposition is also dependent on the strength of static magnetic field, which should be taken into account in the interpretation of the images.

(Clin Neurol 2012;52:955-958)

Key words: magnetic resonance imaging, iron deposition, T_2^* weighted image

＜シンポジウム（1)—5—2＞鉄と神経疾患

脳表ヘモジデリン沈着症の臨床

山脇　健盛

(臨床神経 2012;52:947-950)
Key words：脳表ヘモジデリン沈着症，MRI，T_2*強調画像，感音性難聴，小脳失調

はじめに

脳表ヘモジデリン沈着症（以下SS）は，小脳失調，感音性難聴，ミエロパチー，などを主徴とし，小脳，脳幹部などの軟膜下にヘモジデリンが沈着する疾患である．発症後は進行性の経過をとり，小脳失調と難聴のためにADLはいちじるしく障害される．剖検例も少なく診断基準もないことから，臨床医の理解は低く，他疾患と誤診され加療される症例も少なくない．

病態と症候

SSは1908年にHamillによりはじめて報告[1]され，本邦では1965年小田らにより最初に報告され[2]，1967年湯浅により脳表ヘモシデリン沈着症と命名された[3]．原因としては，クモ膜下腔へ持続的にあるいは反復して出血することにより，赤血球中の鉄がヘモジデリンとして小脳，脳幹，大脳，脊髄の軟膜下や上衣下に沈着することによる[4]．

症候は，感音性難聴，小脳失調，錐体路障害が多く，その他，嗅覚低下，視覚障害，認知機能障害など多彩である[5][6]．Levyらの270例の文献報告[6]の統計では，難聴と小脳失調がもっとも多く81%，ミエロパチー（主に錐体路障害）が53%，排尿障害，頭痛，嗅覚障害がそれぞれ14%であった．

またSSは，何らかの基礎疾患を有することが多い．出血原因としては，髄膜癌や偽髄膜癌をふくめた脳脊髄液貯留が多い[7]とされ，その他脳血管疾患，腫瘍，外傷，アミロイドアンギオパチー（CAA）などがある．前述の270例の統計[6]では，原因不明の特発性が35%，原因が明らかなものでは，腫瘍が21%，頭部/背部の外傷が13%，動静脈奇形が9%，腫瘍以外の術後が7%，腕神経叢/神経根の外傷が6%であった．一方，Linnらの報告[8]では，CAAでは38例中23例（61%）でSSをみとめたが，非CAA脳内出血22例では1例もみとめずSSはCAAの特徴のひとつともされている．なかには，明らかな基礎疾患なく偶然に発見される例も増えている[9]．ただ，これらCAA例や偶然発見例の多くは小脳や脳幹でなく，大脳皮質のみにSSをみとめており病態がことなるものと考えられる．

画像診断

以前は病理解剖や生検，脳外科手術時などに発見されてきたが，MRIの登場により非侵襲的に生前診断が可能となった．反対に，剖検や生検・手術がなければ，MRIなくしては診断は不可能であり，本疾患の診断にはMRI所見はきわめて重要な位置を占める．

ヘモジデリン沈着はT_2強調画像（T_2WI）で脳表に沿って縁取るように低信号として描出されるが，磁化率変化の影響を受けやすいT_2*強調画像（T_2*WI）ではより明瞭となる．Susceptibility weighted image（SWI）ではさらに明瞭となるが，磁化率変化に鋭敏になると副鼻腔，乳突蜂巣などの空気によるartifactの影響を受けやすくなる（Fig. 1）．

治療と予後

SSは一般に進行性の経過をとる．SSの治療は，まず出血源を検索し，それに対する治療をおこない進行を阻止することである．腫瘍や血管奇形があれば手術摘出を，脳脊髄液貯留や硬膜欠損も可能なかぎり手術治療を考慮する．出血源が明らかでないばあいは，止血剤やキレート剤を投与することにより，進行が停止したり，改善したとする報告もある．なかでも血液脳関門を通過しうる鉄キレート剤であるdeferiproneにより，著明に画像および臨床症候が改善した例が報告され，臨床試験もおこなわれており期待される．

SSの予後は，一般に生命予後は比較的良好であるが，感音性難聴は進行することが多く，完全聾に陥ることが多くQOLはいちじるしく障害される．難聴に対しては，人工内耳埋め込み術がおこなわれることがあるが，その効果は一定していない．

診断基準の作成

平成23年度難治性疾患克服研究事業「脳表ヘモシデリン沈着症の診断基準の構築と調査に関する研究班」（主任研究者：高尾昌樹）では，診断基準の作成を試みた．

文献的検討から，これまでSSとして報告されてきた例の

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（受付日：2012年5月23日）

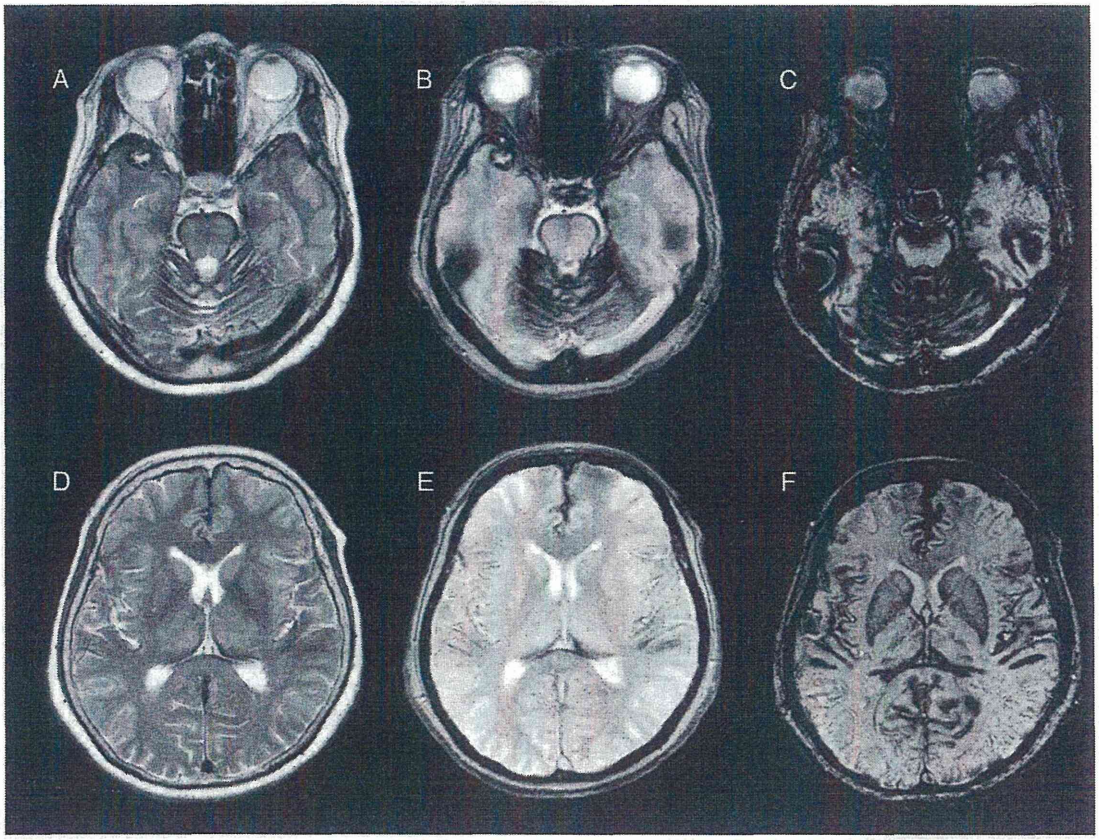

Fig. 1　MRI of a 66 year-old woman with hearing loss and cerebrellar ataxia for 12 years. T_2 weighted image (A, D), T_2* weighted image (B, E) and susceptibility weighted image (C, F).

ほとんどは，その特徴として，感音性難聴または小脳失調を主徴として，MRIにて小脳，脳幹部を中心にびまん性，対称性にヘモジデリン沈着がみられるが，一方でこれらの症候をきたさずMRIにて皮質などのごく一部にヘモジデリン沈着がみられる例も少なからず存在することが明らかとなった．今回これらは分けて検討することとし，前者を古典型，後者を限局型とした．Table 1に診断基準を示す．

全国調査

本診断基準をもとに，わが国における病像把握のため，上記研究班において，神経内科（761施設）教育施設，脳神経外科（387施設）教育施設に調査票を送付し，アンケート調査を実施した．回収率は38.4%で，神経内科施設40.3%，脳神経外科施設33.3%であった．

集積された脳表ヘモジデリン沈着症例は，66例で，内訳は古典型53例，限局型7例，非典型例4例，不明2例であった．古典型は，男性32例，女性20例，不明1例で，発症年齢63.3±10.4（28～81）歳，罹病期間は6.8±6.9年であった．古典型の臨床症候は，小脳失調が44例（83%），感音性難聴が39例（74%），ミエロパチーが24例（53%），認知症が12例（23%），その他，嗅覚障害，てんかん，頭痛などがみられた．初発症状としては，小脳失調または感音性難聴で約90%を占めていた．原因疾患は，脊椎・脊髄疾患13例（25%），脳血管疾患7例（14%），脳腫瘍5例（10%），外傷3例（6%）で，原因不明のものも25例（45%）と半数近くを占めていた．治療については，1/3の例ではとくに治療はなく，止血剤，キレート剤などが使用されていた．

考　察

今回の全国調査では，全体の回収率は38.4%，とくに神経内科施設では40.3%と比較的高い回収率であった．しかし，回答のあったSSの症例は，全体で66例，古典型は53例と思ったより少なかった．この数値から予測すると，わが国におけるSS患者（古典型）は，100～200人と考えられる．難聴のみを呈する例もあり，今後調査をおこなうばあいは，耳鼻咽喉科施設における症例も検討する必要があろう．一方，限局型については，偶然みつかることが多く，放射線科施設における検討も必要と考えられる．

古典型の発症年齢は，63.3±10.4歳，男女比は約3：2で，これまでの欧米での報告では，40歳代～50歳代，男女比は2～3：1であり[5][6]，それよりもやや年齢が高く，やや男性が少ない結果であった．今回の診断基準を適用したばあい，66

Table 1　Criteria of superficial siderosis.

概念

脳表ヘモジデリン沈着症は，鉄（ヘモジデリン）が脳表，脳実質に沈着することにより，神経障害をきたす疾患で，小脳，脳幹など後頭蓋窩を中心に中枢神経系にびまん性・対称性に病変が生じるタイプ（古典型）と，限局性（例：一側の前頭葉など）に生じるタイプ（限局型）の2種類があり，主に前者が検討されることが多い．

脳表ヘモジデリン沈着症診断基準（試案）

古典型の診断には臨床症候の1から4のいずれかをみとめ，画像診断の1を満たすことが必須条件である．

臨床症候（古典型）

1. 感音性難聴
2. 小脳失調
3. 脊髄症（錐体路障害，排尿障害，など）
4. 認知機能障害

注：1か2が初発症状（あるいは1か2で気付かれる）であることが圧倒的に多い．画像診断の1を満たすが神経症候がないばあいあるいは1から4以外の症候のみのばあいは非典型例として別に記載する．限局型に特徴的な症候は明らかでない．

画像診断

現在臨床的に診断をおこなうためには，MRIによる診断に依拠する以外なくMRIで発見されることも多い．

1. MRIのT_2強調画像，T_2*強調画像において，脳，脊髄の表面を縁取る明瞭な低信号をびまん性・対称性にみとめ，とくに小脳，脳幹など後頭蓋窩に優位に分布する．脳神経，脊髄にもみとめられ，病変部には萎縮をともなう．
2. 原因疾患として脳動脈瘤，脳動静脈奇形，アミロイド血管症，脳および脊髄腫瘍，外傷，脳脊髄液減少症，脊柱管内の囊胞性疾患・硬膜異常症などの合併が報告されていることから，それらを検索する撮像方法を適宜考慮する．

ただし，2にあげた疾患を原因とする限局性のヘモジデリン沈着症（たとえば一側前頭葉のみなど）がみられること（限局型）があるが，1でいうところの，対称性・びまん性（古典型）のヘモジデリン沈着症とは区別する．

例中4例（6%）で非典型例が存在した．これらについては詳細な検討が必要であり，今後診断基準の見直しも必要かもしれない．

今回のアンケートでは，初発症状は難聴and/or小脳失調が約90%を占めた．これまでの報告では，初発症状に関するデータはないが，臨床症状としては，この両者が8〜9割にみられ，今回のアンケートでも概ね同程度であった．嗅覚低下はこれまでの報告では10数%であるが，今回のアンケートでは6%であった．嗅覚については，詳しく検査されることが少なく，また難聴のために検査も難しいことも多く，過小評価されている可能性がある．認知機能低下は，これまでの報告同様20数%であり，重要な症候のひとつと考えられる．

おわりに

SSは，MRIの普及とともに生前診断例が飛躍的に増加している．さらに古典的な三徴（難聴，小脳失調，錐体路障害）を呈する例以外に無症候例の報告も増えているとはいえ，比較的まれな疾患である．QOLをいちじるしく阻害する難聴の進行を阻止するために，本疾患の病態解明と，さらなる早期発見と治療法の確立が望まれる．

本研究は平成23年度厚生労働科学研究　難治性疾患克服研究事業「脳表ヘモシデリン沈着症の診断基準の構築と調査に関する研究」（主任研究者：高尾昌樹）の助成を受けておこなった．

注：「脳表ヘモジデリン沈着症」の名称について

もともと，湯浅が脳表ヘモシデリン沈着症（以下「シ」）と命名したが，その後多くの論文では，脳表ヘモジデリン沈着症（以下「ジ」）が使われ，日本神経学会用語集でも「ジ」が明記されている．今回の研究班およびシンポジウムでは，「シ」とされたが，本稿では原則として「ジ」を使用した．

※本論文に関連し，開示すべきCOI状態にある企業，組織，団体はいずれも有りません．

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Abstract

Clinical features of superficial siderosis

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Superficial siderosis (SS) of the central nervous system is a rare condition in which hemosiderin is deposited in the subpial layer of the brain and/or spinal cord. It is supposed that hemosiderin deposition is a result of recurrent or persistent hemorrhage in the subarachnoid space. The causes of hemorrhage are tumor, vascular abnormality, injury, dural defect, and others. The source of hemorrhage is not apparent despite of extensive examinations in about a half of the patients with SS. Patients with SS usually reveal slowly progressive and irreversible cerebellar ataxia and/or sensorineural hearing loss. MRI of T_2WI or T_2*WI demonstrates characteristic linear low intensity along surface of the brain and the spinal cord. There are two types of SS. One is a classical type, in which low intensity of MRI is diffuse and symmetrical. The other is a localized type. We attempted to make a clinical criteria of SS according to the world literature. Then, the criteria was applied to cases (53 cases of classical type and 7 cases of localized type) which are collected from Japanese nationwide questionnaires. The causes and symptoms of Japanese SS are similar with those of Western countries.

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Key words: Superficial siderosis, MRI, T_2* weighted image, sensorineural deafness, cerebellar atxia

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Original Article

Neuropathologic analysis of Lewy-related α-synucleinopathy in olfactory mucosa

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We analyzed the incidence and extent of Lewy-related α-synucleinopathy (LBAS) in the olfactory mucosa, as well as the central and peripheral nervous systems of consecutive autopsy cases from a general geriatric hospital. The brain and olfactory mucosa were immunohistochemically examined using antibodies raised against phosphorylated α-synuclein. Thirty-nine out of 105 patients (37.1%) showed LBAS in the central or peripheral nervous systems. Seven patients presented LBAS (Lewy neurites) in the olfactory lamina propria mucosa. One out of the seven cases also showed a Lewy neurite in a bundle of axons in the cribriform plate, but α-synuclein deposits were not detected in the olfactory receptor neurons. In particular, high incidence of α-synuclein immunopositive LBAS in the olfactory mucosa was present in the individuals with clinically as well as neuropathologically confirmed Parkinson's disease and dementia with Lewy bodies (6/8 cases, 75%). However, this pathologic alteration was rare in the cases with incidental or subclinical Lewy body diseases (LBD) (one out of 31 cases, 3.2%). In the olfactory bulb, the LBAS was usually present in the glomeruli and granular cells of most symptomatic and asymptomatic cases with LBD. Our studies further confirmed importance of the olfactory entry zone in propagation of LBAS in the human aging nervous system.

Key words: α-synuclein, Lewy body, neuropathology, olfactory mucosa, Parkinson's disease.

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INTRODUCTION

Sporadic Parkinson's disease is a neurodegenerative disorder characterized clinically by resting tremor, rigidity, bradykinesia and gait disturbance, as well as neuropathologically by the loss of neurons in several brainstem nuclei and the presence of Lewy bodies formed by abnormal accumulation of α-synuclein.[1–5] Of the many types of neurons in the central and peripheral nervous systems, a specific subset of neurons is vulnerable to accumulation of α-synuclein, which takes the form of aggregates such as Lewy bodies and Lewy neurites (LBs/LNs).[6–8]

Based on studies of a large number of autopsy cases, the initial sites involved in Lewy-related pathology are reported to be the dorsal motor nucleus of the vagus, the intermediate reticular zone in the lower brainstem and olfactory bulb.[9,10] We previously reported that in the earliest stage of Lewy-related α-synucleinopathy (LBAS), abnormal α-synuclein accumulation extends from the peripheral part of the olfactory bulb to the anterior olfactory nucleus as well as the amygdala.[11] From a clinical standpoint, impaired olfactory function constitutes one of the earliest symptoms of sporadic Parkinson's disease.[12,13] Therefore, the olfactory system may be one of the vital regions in the development of Lewy body disease (LBD).

In the olfactory bulb, α-synuclein accumulation is observed in the anterior olfactory nucleus as well as the mitral, tufted, and granular cells of individuals with clinical Parkinson's disease or dementia with Lewy bodies (DLB). Even in the early stages of these diseases, LNs, LBs or both, can be seen in the olfactory bulbs.[11,14,15] Based on the results of a neuropathologic study, Beach *et al.* suggested that the olfactory bulb may be a candidate region of biopsy study to

confirm the diagnosis of LBD.[16] However, the biopsy of olfactory bulb is too invasive and difficult to carry out for patients without risk.[17,18]

The olfactory epithelium is composed of paraneurons and neurites from which the glomeruli of the olfactory bulb originate. However, a neuropathologic analysis of LBAS has not been carried out adequately for LBD. Duda *et al.* reported that normal α-synuclein is expressed in the basal cells, olfactory receptor neurons, supporting cells, and Bowman's glands of the olfactory epithelium in normal controls, as well as patients with Parkinson's disease, Alzheimer disease and multiple system atrophy.[19] However, pathologic α-synuclein accumulation is rare (3.7%) among both normal controls and individuals affected by DLB, Alzheimer disease or Parkinson's disease.[20] According to a biopsy study of the olfactory epithelium in individuals with Parkinson's disease and younger hyposmic controls, no specific pathologic alteration was found.[21]

Therefore, it is still controversial whether abnormal α-synuclein accumulation in the olfactory epithelium precedes the formation of LBs/LNs in the olfactory bulb and contributes to olfactory dysfunction in sporadic Parkinson's disease. The aim of this study was to clarify the neuropathologic alterations of the olfactory mucosa in LBD by immunohistochemical analysis of a series of autopsied individuals.

MATERIALS AND METHODS

Tissue source

Tissue samples were obtained from autopsy materials that were collected at the Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology between October 2008 and August 2010. This hospital is located at the center of Tokyo city and is a geriatric general emergency hospital with 579 beds. This hospital provides community-based medical service to the aged population 24 h/day in cooperation with local general practitioners. The number of autopsy cases was 162 in the above duration. In addition to the general organs, we could obtain the brains and spinal cords from 105 cases in that period, that were registered to the Brain Bank for Aging Research (BBAR) with the deceased's relatives' informed consent. The BBAR is approved by the ethics committee of the Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology to carry out comprehensive research.

Clinical information

All clinical information, including the presence or absence of Parkinsonism as well as dementia, was retrospectively obtained from medical charts and reviewed by two board-certified neurologists.[11,22–26] First, we evaluated Parkinsonism such as bradykinesia, resting tremor, rigidity and postural instability. In this study, when individuals had two or more of these four clinical symptoms, we defined them as having Parkinson's disease-related symptoms.[27] Second, we analyzed scores for the Mini-Mental State Examination[28] or the Hasegawa Dementia Scale (or its revised version),[29,30] the Instrumental Activities of Daily Living,[31] and the Clinical Dementia Rating (CDR).[32] When individuals were not assigned to a category of CDR, we retrospectively determined CDR using medical records, including the battery of cognitive tests above, as well as interviews with attending physicians and caregivers when necessary. Based on these results, we assigned a clinical diagnosis to each patient. The clinical diagnosis of Alzheimer disease was carried out based on the criteria of the National Institute of Neurological and Communication Disorders and Stroke-Alzheimer Disease and Related Disorders Association.[33] The diagnosis of DLB and Parkinson's disease with dementia conformed to the third report of the DLB consortium.[34]

Histology

We examined the brain and olfactory epithelium, olfactory bulb, esophagogastric mucosal junction, sympathetic ganglia, thoracic spinal cord, adrenal glands, anterior wall of the left ventricle of the heart, and abdominal skin.[22,26] The brains and spinal cords were examined as previously reported.[22,24,25] Briefly, the cerebral and cerebellar hemispheres as well as brainstem were dissected in the sagittal plane at the time of autopsy. In each case, half of the brain was preserved at −80°C for further biochemical and molecular analyses. The other half of the brain and abdominal skin were fixed in 20% buffered formalin (WAKO, Osaka, Japan) for 7–13 days and sliced in the same manner as the contralateral hemisphere. The adrenal gland and anterior wall of the left ventricle of the heart were fixed in 20% formalin. The representative areas were embedded in paraffin. Six-micrometer-thick serial sections were cut and stained with HE and KB. Sections of the amygdala, hippocampus, parahippocampal gyrus and temporal cortex were stained with the modified Gallyas-Braak method for senile plaques, NFTs and argyrophilic grains.[35]

Immunohistochemistry

Sections were immunostained using the following antibodies raised against phosphorylated tau protein (p-tau) (AT8, monoclonal; Innogenetics, Temse, Belgium): synthetic peptide corresponding to amino acids 11–28 of amyloid-beta protein (12B2, monoclonal; IBL, Maebashi, Japan); phosphorylated α-synuclein (pSyn#64, monoclonal[25] and

Table 1 Antibodies used for immunohistochemistry

Antibody	Epitope	Source	Clone	Dilution ratio	Antigen method	Retrieval (min)
pSyn#64	α-synuclein phosphorylated ser 129	T. Iwatsubo	Monoclonal	1:20000	99% formic acid	5
PSer129	α-synuclein phosphorylated ser 129	T. Iwatsubo	Polyclonal	1:100	None	
PGP9.5	PGP9.5	Biomol	Polyclonal	1:5000	microwave	30
SMI31	phosphorylated neurofilament	Sternberger	Monoclonal	1:20000	None	
Tyrosine hydroxylase	Anti-tyrosine hydroxylase, rat	CALBIOCHEM	Monoclonal	1:10	microwave	30
AT8	Phosphorylated tau protein	Innogenetics	Monoclonal	1:1000	None	
12B2	Aβ11–28	IBL	Monoclonal	1:50	99% formic acid	5

PSer129 polyclonal[36]), ubiquitin (polyclonal, Sigma-Aldrich, St. Louis, MO), Protein Gene Product 9.5 (PGP9.5, polyclonal; ENZO Life Sciences International, Farmingdale, NY USA); phosphorylated neurofilament (SMI31, monoclonal; Sternberger Immunochemicals, Bethesda, MA, USA); and tyrosine hydroxylase (Anti-Tyrosine Hydroxylase, Rat, monoclonal; Calbiochem-Novabiochem Corporation, Darmstadt, Germany) (Table 1). The signals from monoclonal and polyclonal antibodies were detected by using the automatic system on a VENTANA NX20 with the I-View DAB Universal Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Sections were counter-stained with hematoxylin.

LBAS

CNS

In order to analyze LBAS,[22] we carried out immunohistochemical analysis with phosphorylated α-synuclein antibodies for the following sections: the medulla oblongata at the level of the dorsal motor nucleus of the vagus, the upper pons at the level of the locus coeruleus, and the midbrain including the substantia nigra, amygdala, anterior hippocampus and the peripheral nervous system from all cases (described in the next section). When immunopositive deposits were observed in these anatomic regions, we carried out additional immunohistochemical analysis for sections of the basal nucleus of Meynert, anterior cingulate gyrus, entorhinal cortex, the second frontal and temporal gyri and the supramarginal gyrus, using antibodies raised against phosphorylated α-synuclein.

Peripheral nervous system

To analyze LBAS of the peripheral nerve, tissue sections from epicardium and epicardial fat of the left ventricle of the heart, sympathetic ganglia, esophagogastric mucosal junction, adrenal gland[22] and abdominal skin[26] were examined by using antibodies raised against phosphorylated α-synuclein.

Olfactory mucosa

At the time of autopsy, the olfactory mucosa, bony septae and contiguous cribriform plate were removed en bloc (Fig. 1). The cribriform plate was dissected in the sagittal plane of the midline by using an electric jigsaw. The left side was fixed for 24 h in 4% paraformaldehyde. After fixation, the olfactory mucosa was removed, dehydrated in a graded alcohol series, cleared in xylene and embedded in paraffin. The right side was fixed for 24 h in 4% paraformaldehyde, decalcified with EDTA for 2 weeks, and dehydrated and embedded in paraffin. Serial 6-μm-thick sections were stained with HE and immunolabeled with antibodies against phosphorylated α-synuclein, PGP9.5, phosphorylated neurofilament, tyrosine hydroxylase, phosphorylated tau and amyloid β (Table 1). In particular, the olfactory receptor neurons of the olfactory epithelium were identified by using PGP9.5 immunohistochemistry.[19] The normal anatomical appearance of the olfactory system is shown in Figure 2.

Olfactory bulb

The olfactory bulbs were prepared for histologic sections to analyze the presence of LBAS. By using HE stain and α-synuclein antibodies, LBAS were identified in the glomeruli, mitral cells, tufted cells and granular cells as previously reported.[11] Mitral and tufted cells were distinguished by their specific shapes. Each neuron was identified when it had an apparent nucleus containing a prominent nucleolus and Nissl substance.

Semiquantitative scoring system of Lewy-related pathology

For each section, we semi-quantitatively graded the immunohistochemical staining with antibody raised against phosphorylated α-synuclein. Our grading system was modified based on the scoring system of the third report of the DLB consortium[34] because we used both the HE stain and immunohistochemistry using monoclonal antibody for phosphorylated α-synuclein to identify LBAS.

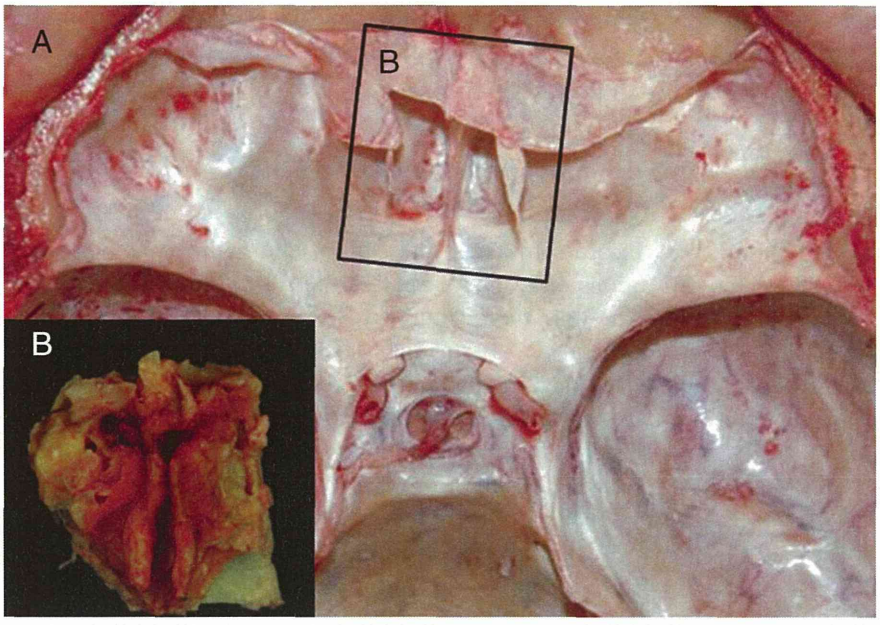

Fig. 1 (a) The anterior cranial fossa after removal of the brain. In order to obtain the olfactory mucosa, the bony septae and contiguous cribriform plate (the rectangular area) were dissected using an electric jigsaw. (b) An inset shows the olfactory mucosa and cribriform plate from the opposite side of the rectangular area.

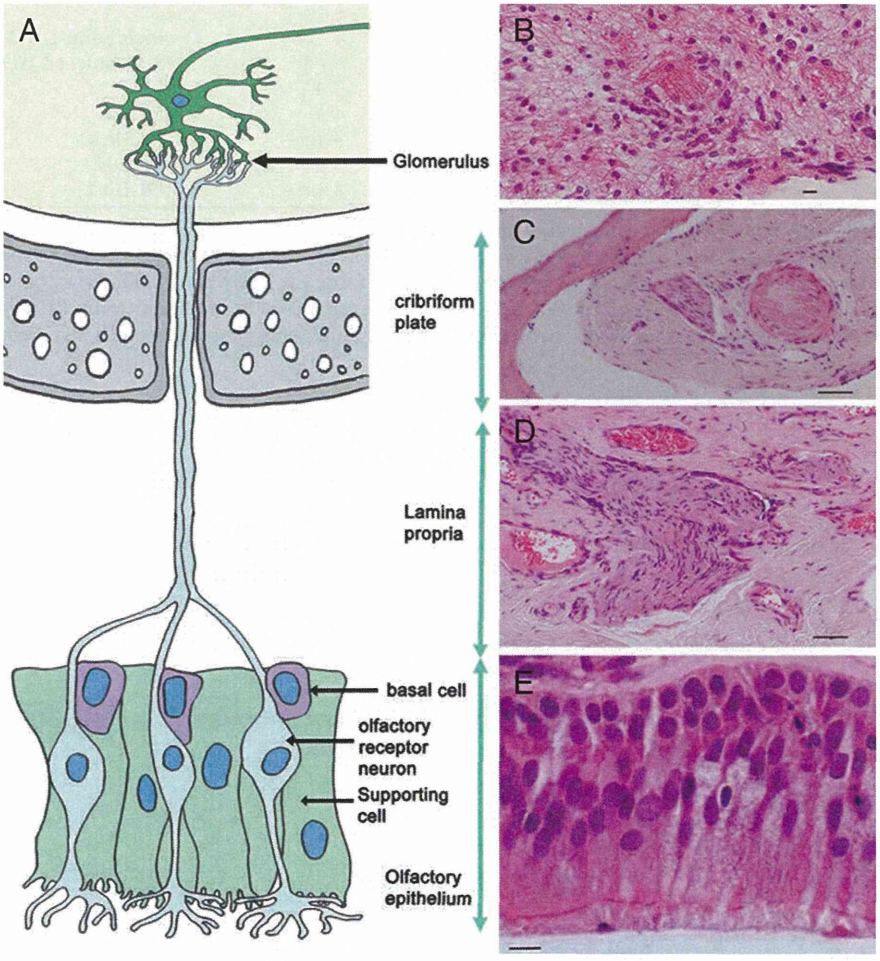

Fig. 2 Scheme of the normal olfactory pathway (a) and photomicrographs of representative histologies of each region (b–e). The olfactory epithelium is composed of three cell types: the basal cells, olfactory receptor neurons and supporting cells (a). The basal cells are the progenitor of the olfactory receptor neurons (a, e). In general, the turnover rate of the olfactory receptor neurons is approximately 30–90 days. Nerve fibers are present in the lamina propria and cribriform plate (c and d, respectively). They consist of either the axons of the olfactory receptor neurons or postganglionic sympathetic nerve fibers. There are glomeruli in the olfactory bulb (b). Glomeruli are the synaptically connected structures of the axons of the olfactory receptor neurons and mitral/tufted cells in the olfactory bulb. (b, e), scale bar = 10 μm; (c), scale bar = 50 μm; (d), scale bar = 100 μm.

For example, 'Stage 1' of the original scoring system was defined as 'sparse Lewy bodies or neurites.' On the other hand, 'Grade 1'of our methodology is defined as 'sparse Lewy neurites without Lewy bodies.'

Grade 0 = neither LNs nor LBs detected using anti-phosphorylated α-synuclein antibody.

Grade 1 = sparse phosphorylated α-synuclein immunopositive dots or neurites, or diffuse granular cytoplasmic stain in the neuron, neither LBs nor phosphorylated α-synuclein-immunopositive neuronal intracytoplasmic dense aggregations.

Grade 2 = 1–3 LBs or phosphorylated α-synuclein-immunopositive intracytoplasmic dense aggregations and scattered LNs in a low-power field (×10).

Grade 3 = more than four LBs and scattered LNs in a low-power field (×10).

Grade 4 = numerous LBs and neurites with severe immunoreactivity for phosphorylated α-synuclein in the neuropil or background.

LB staging system of our BBAR (BBAR LB stage)

In order to assess the clinical and neuropathologic alterations of LBD, we applied the following rating system to our BBAR for all autopsy cases (Table 2, Fig. 3). The original BBAR LB staging system was developed in order to track the individual data of our brain bank.[24,25] This rating system requires clinical symptoms, gross and microscopic neuropathologic alterations, and LB scores used in the consensus guidelines for the clinical and pathologic diagnosis of DLB.[27] In this staging system, Parkinson's disease with

Table 2 Lewey body stage of Brain Bank for Aging Research

Stage	Psyn-IR	LB	SN: loss of pigmentation	LB score	Dementia	Parkinsonism	Diagnosis
0	–	–	–				
0.5	+	–	–				
1	+	+	–				Incidental LBD
2	+	+	+	0–10	–†	–†	Subclinical LBD
3	+	+	+	0–10	–	+	PD
4	+	+	+	3–6	+	+	PDDL
	+	+	+	3–6	+	+ or –	DLBL‡
5	+	+	+	7–10	+	+	PDDN
	+	+	+	7–10	+	+ or –	DLBN‡

†Neither dementia nor Parkinsonism associated with Lewy body-related α-synucleinopathy. ‡Differential diagnosis of PDD and DLB was based on the '1-year rule' according to the consensus guidelines (34). DLBL, dementia with Lewy bodies and a Lewy body score corresponding to the limbic form; DLBN, dementia with Lewy bodies and a Lewy body score corresponding to the neocortical form; LB, Lewy body; LBD, Lewy body disease; PD, Parkinson's disease; PDDL, Parkinson's disease with dementia and a Lewy body score corresponding to the limbic form; PDDN, Parkinson's disease with dementia and a Lewy body score corresponding to the neocortical form; Psyn-IR, phosphorylated alpha-synuclein immunoreactivity; SN, substantia nigra.

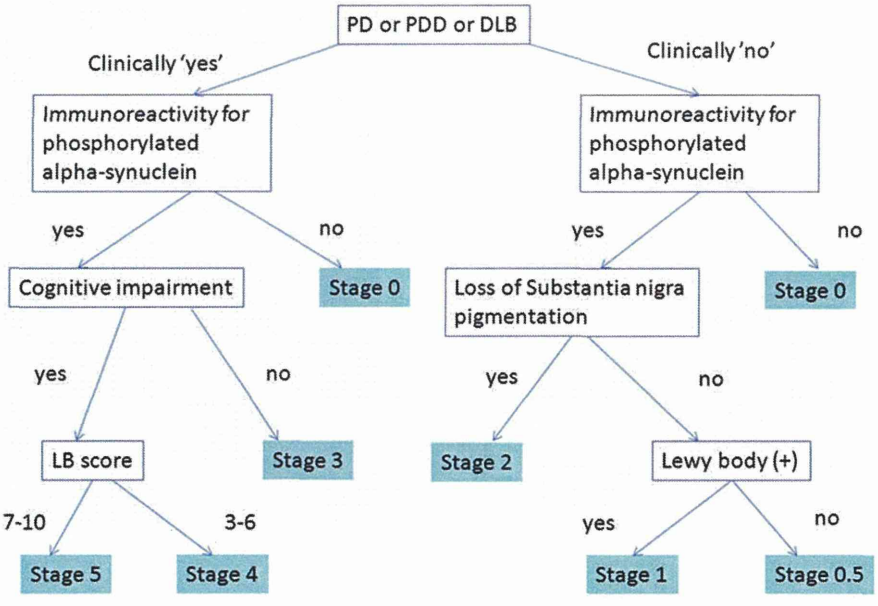

Fig. 3 Flow-chart of the Lewy body staging system of the Brain Bank for Aging Research (BBAR). PD, Parkinson's disease; PDD, Parkinson's disease with dementia; DLB, dementia with Lewy bodies; SN, substantia nigra; LB score, Lewy body score. See Table 2 for detailed description of each stage.

dementia was differentiated from DLB by applying the '12-month (1-year)' rule noted in the Consensus Guidelines (i.e., 'dementia appears more than one year after the onset of Parkinsonism').[27]

Evaluation of senile changes and neuropathologic diagnosis

NFTs were classified according to Braak and Braak's staging system using modified Gallyas-Braak staining[37] and AT8 immunohistochemistry.[38] The staging system for senile plaques (SPs) comprises four stages (0–C). Argyrophilic grains were classified into our four stages (0–III), as reported previously.[23] The neuropathologic diagnosis of Alzheimer disease was based on our previous definition,[39] which proposed a modification of the National Institute on Aging and Reagan Institute criteria.[40,41] The diagnoses of dementia with grains and NFT-predominant forms of dementia were based on the previously described definitions.[42,43]

Statistical analysis

Fisher's exact test was carried out to compare the number of cases having LBAS pathology in the olfactory mucosa.

RESULTS

Clinical information

Of the 105 consecutive autopsy patients, 58 were men and 47 were women. The patient ages at death ranged from 65 to 104 years (82 ± 37, mean ± SD). Twelve patients showed Parkinson's disease-related symptoms according to the clinical criteria in this study. Six out of 105 patients were clinically diagnosed as LBD including Parkinson's disease, Parkinson's disease with dementia and DLB.

Neuropathologic diagnosis

The neuropathologic diagnoses consisted of Alzheimer disease ($n = 15$), dementia with grains ($n = 11$), NFT-predominant form of dementia ($n = 8$), Parkinson's disease ($n = 2$), Parkinson's disease with dementia ($n = 2$), and DLB ($n = 1$), as well as one case each of dentatorubral-pallidoluysian atrophy, neuronal hyaline inclusion body disease, frontotemporal lobar degeneration with transaction response (TAR) DNA-binding protein-43 kDa-immunoreactive inclusions, and progressive multifocal leukoencephalopathy. Patients with combined pathologies, included Alzheimer's disease plus DLB ($n = 2$), dementia with grains plus NFT-predominant form of dementia ($n = 3$), and one patient each of diffuse NFTs with calcification (DNTC)[44] plus DLB and dementia with grains plus Alzheimer's disease. The remaining patients did not fulfil the clinical and/or pathological criteria for neurodegenerative diseases.

Eight out of 105 patients (8/105 = 7.6%) were clinically and neuropathologically diagnosed as having LBD, including Parkinson's disease (2 patients), Parkinson's disease with dementia (2 patients) and DLB (4 patients).

Incidence, distribution and extent of LBAS

BBAR staging

Based on clinical and neuropathologic analyses, the BBAR LB stages were as follows: stage 0 = 66 cases, stage 0.5 = 6 cases, stage 1 = 21 cases, stage 2 = 4 cases, stage 3 = 2 cases, stage 4 = 3 cases and stage 5 = 3 cases. All of the stage 5 cases had DLB, with an LB score corresponding to the value for the neocortical form (DLBN).

LBAS in CNS and peripheral nervous system

We identified 39 (37.1%) out of the 105 individuals with α-synuclein immunopositive LBAS in the CNS or peripheral nervous system (Table 3). Therefore, we focused on these 39 cases in the present study. Here, LBAS was identified by using α-synuclein immunohistochemistry. In LBAS, LBs were confirmed with HE stains and α-synuclein immunohistochemistry. Out of the 39 cases, 33 showed LBAS in the olfactory bulb, 15 in the enteric nerve plexus, 23 in the sympathetic ganglia, and 16 in the pericardial nerve fibers of the left ventricle (Tables 3 and 4).

Olfactory mucosa

The olfactory epithelium is a pseudostratified columnar epithelium lying deep within the recess of the superior nasal cavity; it is composed of a mixture of multipotential stem cells (basal cells), supporting cells and olfactory receptor neurons (Fig. 2). Mature neurons are reported to give rise to fine and unmyelinated axons that ascend through the cribriform plate to synapse at glomeruli in the olfactory bulb.[20,45]

LBAS were found in the olfactory mucosa of seven (17.9%) out of 39 cases (Tables 3 and 4). These seven also had LBAS in the olfactory bulb. LBAS was present in the lamina propria mucosa of the seven cases (Fig. 4a–c). In addition, one case showed LBAS in a bundle of axons in the cribriform plate (Fig. 4d). None of the cases showed LBAS in the olfactory epithelial paraneuron. We summarized the demographic results of these seven individuals with LBAS in the olfactory mucosa in Table 5. Neither phosphorylated tau-positive deposits nor amyloid β immunopositive deposits were detected in the olfactory mucosa.

Table 3 The distribution of α-synuclein deposits in various anatomical regions of 39 cases with Lewy body disease

Age at death/gender	Parietal lobe	Frontal lobe	Temporal lobe	Cingulate gyrus	Entorhinal cortex	Amygdala	Olfactory bulb	Nucleus basalis of Meynert	Substantia nigra	Locus coeruleus	Dorsal motor nucleus of the vagus	Spinal Cord	Gastrointestinal system	Olfactory Mucosa	Sympathetic ganglion	Adrenal gland	Pericardial nerve	Skin	BBAR LB stage	NFT stage	SP stage
104/F																			5	4	C
70/F																			5	4	C
86/F																			5	6	C
84/M																			4	2	A
79/F																			4	2	A
80/F																			4	2	A
81/M																			3	2	A
88/M																			3	3	A
79/M																			2	1	A
68/F																			2	2	B
79/F																			2	6	C
77/F																			2	6	C
78/M																			1	2	A
75/M																			1	2	A
89/F																			1	3	C
93/F																			1	4	C
86/M																			1	4	C
81/M																			1	2	A
90/F																			1	2	A
86/M																			1	2	A
97/F																			1	2	A
78/M																			1	1	A
92/M																			1	3	C
94/M																			1	4	A
85/M																			1	3	A
81/F																			1	5	C
96/F																			1	2	0
87/F																			1	3	A
101/F																			1	4	A
69/F																			1	4	0
83/F																			1	3	A
72/M																			1	1	A
77/M																			1	2	A
83/M																			0.5	4	C
71/M																			0.5	2	A
89/M																			0.5	2	A
85/F																			0.5	3	A
85/F																			0.5	2	A
96/F																			0.5	3	A

Grade 0 = blank, grade 1 = light grey, grade 2 = light blue, grade 3 = blue, grade4 = navy blue. The number in each cell indicates a score based on the semiquantitative scoring system of Lewy-related pathology. 0 = neither Lewy neurites nor bodies detected by using anti-phosphorylated α-synuclein antibody. 1 = sparse phosphorylated α-synuclein immunopositive dots or neurites, neither Lewy bodies nor phosphorylated α-synuclein immunopositive intracytoplasmic aggregations. 2 = one to three Lewy bodies or phosphorylated α-synuclein immunopositive intracytoplasmic aggregations in a low-power field (×10). 3 = more than four Lewy bodies and scattered Lewy neurites in a low-power field (×10). 4 = numerous LBs and neurites with severe immunoreactivity for phosphorylated α-synuclein in the neuropil or background. Individuals of BBAR LB stages 3–5, with clinical Parkinsonism and neuropathologically numerous LBASs in the CNS, showed high incidence (75%, 6/8 individuals) of LBASs in the olfactory mucosa. In contrast, individuals of BBAR LB stages 1–3 without Parkinsonism showed extremely low incidence of Lewy body-related α-synucleinopathy (LBAS) (3%, 1/31) in the olfactory mucosa. LBAS was found in the olfactory mucosa mostly in advanced BBAR LB stages 3–5. BBAR LB Brain Bank for Aging Research Lewy body staging, NFT stage, Braak's stages for neurofibrillary tangles; SP stage, Braak's stages for senile plaques.

Table 4 Regional frequency of Lewy body-related α-synucleinopathy (LBAS) in various anatomical regions

The BBAR LB stage	Olfactory epithelium	Olfactory mucosa	Olfactory bulb	Spinal cord	GI tract	Sympathetic ganglia	Adrenal gland	Pericardial nerve	Skin
0.5	0/6	0/6	2/6	0/6	0/6	2/6	0/6	1/6	0/6
1	0/21	1/21	19/21	7/21	6/27	10/21	1/21	6/21	1/21
2	0/4	0/4	4/4	3/4	1/4	3/4	0/4	2/4	0/4
3	0/2	1/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2
4	0/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
5	0/3	2/3	3/3	3/3	3/3	3/3	1/3	3/3	0/3
All	**0/39**	**7/39**	**33/39**	**18/39**	**15/39**	**23/39**	**7/39**	**16/39**	**5/39**

BBAR LB Brain Bank for Aging Research Lewy body staging.

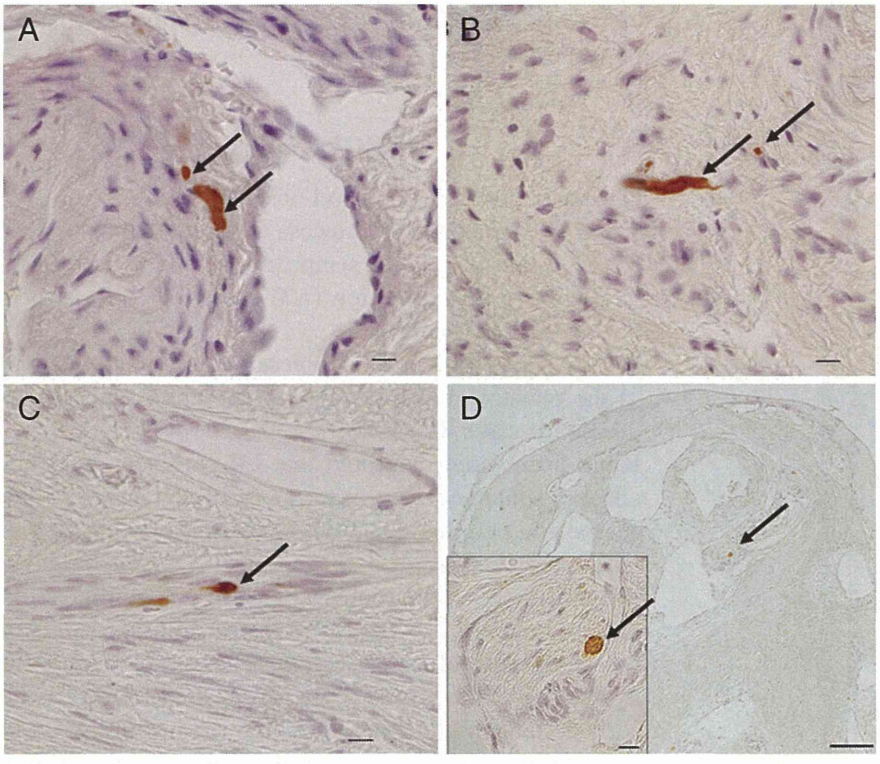

Fig. 4 Photomicrographs show α-synuclein immunopositive deposits (arrows indicate Lewy neurites) in the axonal bundle of the lamina propria (a–c) and cribriform plate (d). The inset in figure (d) shows a higher magnification image of α-synuclein immunopositive deposits in the axonal bundle of the cribriform plate. Immunohistochemistry using monoclonal antibody against phosphorylated α-synuclein (pSyn#64). Photomicrographs (a, b, c and d) were obtained from cases 4, 5, 7 and 3, respectively, in Table 5. (a–c), scale bar = 10 μm; (d) scale bar = 100 μm (inset, 10 μm).

Correlations between α-synuclein immunopositive LBs or LNs in the olfactory mucosa and CNS

Alpha-synuclein immunopositive LBs or LNs in the olfactory mucosa were detected in seven cases, including three with DLB, three with Parkinson's disease or Parkinson's disease with dementia, and one with incidental LBD (Tables 3–5). LBAS in the olfactory mucosa was compared with those in other locations of the CNS (Table 3). Individuals of BBAR LB stages 3–5, clinical and neuropathological diagnosis of LBD, showed a high incidence (75%, 6/8 individuals) of α-synuclein immunopositive LBAS in the olfactory mucosa (Table 6, Fig. 5). Six individuals with Parkinson's disease also showed a high incidence of α-synuclein accumulation (66%, 4/6 individuals) in the olfactory mucosa. In contrast, individuals of BBAR LB stages 0.5–2 (here we classified them into asymptomatic group) showed a low incidence of LBAS (3%, 1/31) in the olfactory mucosa.

Olfactory bulb

There is neural connectivity among olfactory receptor neurons and nuclei in the olfactory bulbs.[45] Hence, we analyzed the frequency of LBAS in the glomeruli, tufted cells, mitral cells and granular cells between LBAS-positive and LBAS-negative groups in the olfactory epithelium.

In individuals of BBAR LB stages 3–5 (symptomatic stage), LBAS was frequently observed in the glomeruli (8/8 cases, 100%), granular cells (8/8, 100%) and tufted cells (7/8, 87.5%). In contrast, there were low numbers of cases with LBAS in the mitral cells (2/8, 25%). Asymptomatic stage cases of LBD, corresponding to BBAR stage 0.5–2,

Table 5 Clinical and neuropathological demography of seven individuals with Lewy body-related α-synucleinopathy (LBAS) identified in the olfactory mucosa

No.	Age at death	Clinically diagnosed as LBD	Cause of death	Neuropathologic diagnosis	BBAR LB stage	LBAS in the olfactory mucosa			NFT stage	SP stage
						Olfactory epithelium	Lamina propria mucosa	Cribriform plate		
1	104/F	None	CHF, MI, Dementia	DLBN, AD	5	0	1	0	1	C
2	70/F	DLB	DLB, pneumonia	DLBN, AD	5	0	1	0	1	C
3	84/M	DLB	Prostate carcinoma, DLB	DLBL	4	0	1	1	2	A
4	79/F	PDD	PDD	PDDN	4	0	1	0	2	A
5	80/F	PD	Pneumonia, PD	PDDL	4	0	1	0	2	A
6	88/M	PD	Pneumonia, PD	PD	3	0	1	0	2	A
7	86/M	None	Pneumonia	AD,Incidental LBD	1	0	1	0	1	C

AD, Alzheimer's disease; BBAR LB stage, Lewy body staging system of the Brain Bank for Aging Research; CHF, congestive heart failure; DLB, dementia with Lewy bodies; DLBL, dementia with Lewy bodies and a Lewy body score corresponding to the limbic form; DLBN, dementia with Lewy bodies and a Lewy body score corresponding to the neocortical form; F, female; LB, Lewy body; LBD, Lewy body disease; M, male; MI, acute myocardial infarction; NFT stage, Braak's stages for neurofibrillary tangles; PD, Parkinson's disease; PDDL, Parkinson's disease with dementia and a Lewy body score corresponding to the limbic form; PDDN, Parkinson's disease with dementia and a Lewy body score corresponding to the neocortical form; SP stage, Braak's stages for senile plaques.

Table 6 Incidence of LBAS in the olfactory mucosa in cases with symptomatic LBD (BBAR stage 3–5)

Clinical and neuropathologic diagnosis of LBD	LBAS in OM		Total
	Present	Absent	
Symptomatic (BBAR 3–5)	6*	2	8
Asymptomatic (BBAR 0.5–2)	1	30	31

*$P < 0.05$. BBAR, Brain Bank for Aging Research; LBAS; Lewy body-related alpha-synucleinopathy; LBD, Lewey body disease; OM; olfactory mucosa.

showed high incidence of LBAS in the glomeruli (23/31, 74.1%) and granular cells (22/31, 70.9%) of the olfactory bulb (Fig. 5).

DISCUSSION

Our study provides two novel observations.

1 LBAS in the olfactory mucosa was frequently observed (6/8 cases, 75%) in the symptomatic patients with LBD, but was a rare condition (1/31 cases, 3.2%) in asymptomatic LBD patients.
2 LBAS was seen in the glomeruli and granular cells in the olfactory bulbs of most symptomatic and asymptomatic cases with LBD.

It has been widely accepted that LB pathology does not develop simultaneously in all anatomical regions of the central and peripheral nervous systems. Hawkes *et al.* proposed that neurotropic pathogens may enter the brain via two routes: (i) a nasal route, with anterograde progression into the temporal lobe; and (ii) a gastric route secondary to the swallowing of nasal secretions in saliva (a dual hit hypothesis).[46] The former route may be associated with the early accumulation of α-synuclein in the human olfactory bulb and cause olfactory dysfunction in sporadic Parkinson's disease. In the present study, there was rare observation of LBAS in the olfactory mucosa in the asymptomatic cases of LBD. Further analysis is important to clarify the possibility of propagation of α-synuclein in the nervous systems.

In the present study, LBAS was frequently observed in the olfactory mucosa (6/8 cases, 75%) in the individuals with clinical LBD. In contrast, LBAS in the olfactory mucosa was a rare observation in asymptomatic patients. It is also important that all seven cases with LBAS in the olfactory mucosa had LBAS in the cerebral cortex and brainstem. Our results have similarities with a previous report concerning Alzheimer's disease.[20] Detection of LBAS in the olfactory mucosa could be hindered by two problems: technical difficulty in obtaining enough nerve fibers and rapid turnover of olfactory receptor neurons.[47,48] In fact, a recent study reported that a biopsy study revealed no α-synuclein immunopositive deposits in the olfactory

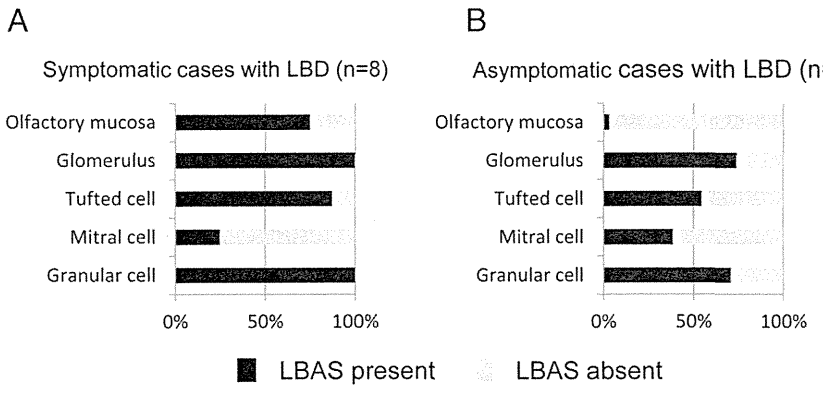

Fig. 5 Frequencies of cases having Lewy-related α-synucleinopathy (LBAS) pathology in the olfactory mucosa and each anatomical region of the olfactory bulb. (a) Cases with symptomatic dementia with Lewy bodies (LBD) ($n = 8$, Brain Bank for Aging Research [BBAR] stages 3–5). Most of the cases show LBAS pathology in the olfactory mucosa (6/8, 75%), glomeruli (8/8, 100%), tufted cells (6/7, 85.7%) and granular cells (8/8, 100%). The number of cases with LBAS in the mitral cells is low (2/6, 33.3%). (b) Cases with asymptomatic LBD ($n = 25$, BBAR stages 0.5–2). Most of the cases show LBAS pathology in the glomeruli (23/31, 74.1%), tufted cells (17/31, 54.8%) and granular cells (22/31, 70.9%). The number of cases with LBAS in the mitral cells (12/31, 38.7%) is low. LBAS pathology of the olfactory mucosa is present in only one case (1/31, 3.2%).

mucosa of patients of Parkinson's disease.[21] Our previous study indicated a high incidence of α-synuclein immunopositive LBs or neurites in aging human olfactory bulbs, and suggested that they extend from the periphery (the second olfactory structure) to the anterior olfactory nucleus (the tertiary olfactory structure).[11] The present study, using 6 μm-thick paraffin embedded sections, revealed that LBAS was most frequently observed in the glomeruli which were composed of axon terminals of olfactory epithelial cells and dendrites of mitral and tufted cells[45] as well as in the glomerular cells which were most numerous in the periphery of the olfactory bulb (Fig. 5). We consider that high incidence of LBAS in glomeruli may represent affected terminal axons of olfactory epithelial neurons. In contrast to our result, a previous study, employing 50 μm-thick floating sections, reported high frequency of LBAS in mitral cells and the internal plexiform layer in individuals with Parkinson's disease but no LBAS in age-matched controls.[49] Further studies are necessary to identify the most vulnerable subset in the periphery of the olfactory bulb.

In conclusion, presence of LBAS in the olfactory mucosa and olfactory glomeruli further supports the importance of olfactory system as an entry zone of LBD. Future studies of LB pathology involving the olfactory system are indicated to understand the pathomechanism of α-synuclein accumulation in individuals with LBD.

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