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^{18}F-APN-1607 Tau PET in Progressive Supranuclear Palsy-Like Symptoms Caused by TBK1 Mutations

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Abstract

Background

Pathogenic mutations in the TANK-binding kinase 1 (*TBK1*) gene have been associated with progressive supranuclear palsy (PSP)-like extrapyramidal symptoms, amyotrophic lateral sclerosis (ALS), as well as cognitive and behavioral alterations. However, the question as to whether *TBK1* mutations may be associated with tau burden remains unanswered.

Methods

To investigate whether patients presenting with PSP-like extrapyramidal symptoms caused by *TBK1* mutations have evidence of tau deposition as reflected by positive ^{18}F-APN-1607 tau PET imaging findings. Four patients who showed PSP-like extrapyramidal symptoms, ALS, and cognitive/behavioral alterations were consecutively enrolled between August 2019 and August 2020. Patients underwent *TBK1* gene sequencing and ^{18}F-APN-1607 tau PET imaging. All PET images were interpreted in a blinded fashion with respect to genetic results. Brain structural changes were investigated with MRI, whereas ^{11}C-CFT or ^{18}F-DTBZ PET imaging was performed to identify dopaminergic degeneration.

Results

Pathogenic *TBK1* mutations were identified in three of the four study patients. The three mutation carriers – but not the case without – showed positive ^{18}F-APN-1607 binding in PSP-related regions, suggesting the presence of tau pathology. Mesencephalic atrophy (hummingbird sign) was observed in all *TBK1* mutation carriers, and two of them also had evidence of frontotemporal atrophy. Dopaminergic degeneration was evident in all cases, regardless of *TBK1* mutations.

Conclusions

Pathogenic *TBK1* mutations in patients with PSP-like extrapyramidal symptoms are associated with positive ^{18}F-APN-1607 tau PET imaging findings. Our data should prompt additional investigations on the potential role of tau accumulation in the pathogenesis of disease conditions associated with *TBK1* mutations.

Introduction

While Amyotrophic Lateral Sclerosis has traditionally been considered as a fatal disorder characterized by progressive degeneration of motor neurons, this condition has been recently reconceptualized as a multisystem disease that is frequently accompanied by extrapyramidal symptoms as well as cognitive

and behavioral alterations.[1] In this regard, 10–15% of all patients with Amyotrophic Lateral Sclerosis also fulfill the clinical criteria for Frontotemporal Lobar Degeneration.[2] Moreover, several studies have documented a concomitant occurrence of Amyotrophic Lateral Sclerosis and Progressive Supranuclear Palsy.[3] Collectively, these clinical observations suggest that Amyotrophic Lateral Sclerosis, Frontotemporal Lobar Degeneration, and Progressive Supranuclear Palsy may share certain pathophysiological underpinnings – including underlying causative genetic defects and/or pathological accumulation of misfolded proteins in the brain.

Genetic counseling along with sequencing of the *C9orf72* and TANK-binding kinase 1 (*TBK1*) genes is recommended for patients who concomitantly show Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration manifestations.[4] We have previously described a pathogenic *TBK1* p.E643del mutation in a patient with Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy-like extrapyramidal symptoms, and cognitive and behavioral alterations.[5] The presence of Progressive Supranuclear Palsy-like symptoms has also been reported in two additional cases harboring *TBK1* mutations.[6] Interestingly, a pathology study revealed the existence of specific Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration patient subgroups characterized by the presence of transactive response DNA-binding protein 43 kDa (TDP-43) inclusions in the brain.[7]

While tau deposition has been shown to occur in at least certain patients with either Amyotrophic Lateral Sclerosis-Frontotemporal Lobar Degeneration or Amyotrophic Lateral Sclerosis -Progressive Supranuclear Palsy syndromes,[8] the question as to whether *TBK1* mutations may result in tau accumulation in the brain remains unanswered. ^{18}F-APN-1607 is a second-generation tau PET tracer which has been extensively applied to detect the presence of cerebral tau deposition in patients with taupathies.[9,10] In this case series, four patients who presented with Progressive Supranuclear Palsy-like extrapyramidal symptoms, Amyotrophic Lateral Sclerosis, as well as cognitive and behavioral alterations underwent both *TBK1* gene sequencing and ^{18}F-APN-1607 tau PET imaging. The presence of tau pathology – as reflected by positive ^{18}F-APN-1607 tau PET imaging findings – was specifically investigated with respect to the carriage of pathogenic *TBK1* mutations.

Methods

Study design and setting

This is a case series of four patients who presented with Progressive Supranuclear Palsy-like extrapyramidal symptoms, Amyotrophic Lateral Sclerosis, as well as cognitive and behavioral alterations between August 2019 and August 2020. All imaging and laboratory procedures and patient visits occurred at the Department of Neurology, Huashan Hospital, Fudan University (Shanghai, China). The study conforms to the ethical guidelines set forth by the Helsinki Declaration and was approved by the Ethics Committee of the Huashan Hospital, Fudan University. Written informed consent was obtained from all participants.

Clinical assessments

Amyotrophic Lateral Sclerosis was clinically diagnosed according to the Awaji criteria.[11] The presence of Progressive Supranuclear Palsy-like extrapyramidal symptoms was assessed according to the recently updated criteria.[12] The evaluation of cognitive and behavioral alternations was carried out in accordance with international consensus criteria for Frontotemporal Lobar Degeneration.[13] The presence of lower motor neuron involvement was confirmed using needle electromyography (EMG). Once diagnosed, signs and symptoms of Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, and Frontotemporal Lobar Degeneration were systematically collected for all patients. The severity of Amyotrophic Lateral Sclerosis was assessed using the Amyotrophic Lateral Sclerosis functional rating scale-revised (ALSFRS-R).[14] Progressive Supranuclear Palsy-like extrapyramidal symptoms were investigated with the Progressive Supranuclear Palsy rating scale (PSPRS).[10] Patients were also administered the Mini-Mental State Examination (MMSE) to assess their cognitive function.

Mutation analysis

Patients' genomic DNA was extracted from peripheral blood leucocytes using a commercially available kit (Qiagen, Hilden, Germany). When necessary, family member DNA samples were also obtained. DNA libraries for whole-exome sequencing (WES) were prepared with the KAPA Library Preparation Kit (KR0453; Kapa Biosystems, Wilmington, MA, USA). The resulting DNA libraries were sequenced on an Illumina NovaSeq system (Illumina Inc., San Diego, CA, USA) using 200-bp, paired-end reads. Multiplex ligation-dependent probe amplification (MLPA) was performed using a SALSA MLPA Probemixes P051-D2/P052-D2 Parkinson kit (MRC Holland, Amsterdam, the Netherlands) according to the manufacturer's protocol. The procedure for identifying genetic alterations in the *TBK1* gene has been previously described in detail.[15] Genetic variants of unknown pathogenicity were ranked according to the 2015 American College of Medical Genetics and Genomics (ACMG) criteria.[16] Whenever feasible and clinically required, familial segregation analysis of selected variants and larger genomic duplications or deletions was performed by Sanger sequencing and MLPA, respectively. The search for *C9orf72* dynamic mutations was undertaken by capillary electrophoresis of repeat-primed PCR amplicons as previously reported.[17]

Acquisition of MRI and PET images

Upon completion of clinical assessment, all patients underwent anatomical MRI in a 3.0-T horizontal magnet (Discovery MR750; GE Medical Systems, Milwaukee, WI, USA). High-resolution T1-weighted images were acquired with the following parameters: TE = 1.95 ms, TR = 2300 ms, TI = 900 ms, flip angle = 12°, acquisition matrix = 256 × 256 × 152, and voxel size = 1 × 1 × 1 mm. After obtaining low-dose CT transmission scans for attenuation correction, PET imaging was conducted using a Siemens mCT Flow PET/CT scanner (Siemens, Erlangen, Germany) in 3D mode. Owing to the presence of extrapyramidal symptoms in all patients, PET molecular imaging of the dopaminergic system was carried out in all participants. One case (patient #3) was imaged using vesicular monoamine transporter, type 2 (VMAT2)

^{18}F-DTBZ PET, whereas the remaining three underwent dopamine transport imaging with ^{11}C-CFT PET. The injected dose of both tracers was 370 MBq. The window scanning time was 60–80 min after injection of ^{11}C-CFT and 90–110 min after injection of ^{18}F-DTBZ, respectively. ^{18}F-APN-1607 tau PET imaging was performed 90–110 min after intravenous administration of the tracer (370 MBq). Images were reconstructed using a 3D ordered-subset expectation (OSEM) maximization algorithm. The detailed procedure has been previously reported.[10,18]

Image processing

Individual PET and corresponding T1-weighted MRI images were coregistered using the SPM12 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) implemented in MATLAB 9.5 (MathWorks, Natick, MA, USA). The transformation matrices of segmented T1-weighted MRI images were applied to matched PET images into the Montreal Neurological Institute standard space. Normalized images were subsequently smoothed with a Gaussian kernel at a half-maximum, with a full-width of either 6 mm for tau PET imaging or 8 mm for dopamine PET imaging. Standardized uptake value ratio (SUVR) maps were obtained using the cerebellar gray matter and the occipital cortex as reference regions for tau and dopaminergic PET imaging, respectively.[10] Alterations of the dopaminergic system were assessed by two experienced nuclear medicine physicians (CZ and JL). Predetermined cortical and subcortical volumes of interest (VOIs) – including the frontal cortex, parietal cortex, occipital cortex, temporal cortex, striatum, caudate, putamen, globus pallidus, thalamus, subthalamic nucleus, midbrain, tegmentum, substantia nigra, red nucleus, pontine base, raphe nuclei, locus coeruleus, and dentate nucleus – were applied for tau PET imaging. Mean values obtained from bilateral examinations were used for all subsequent analyses. SUVR values of ^{18}F-APN-1607 binding were Z-transferred with the normal values we have previously reported.[10,19] The two-region positivity approach – defined by the presence of at least two regions of interests on ^{18}F-APN-1607 tau PET images showing a Z score ≥2 – was used as the criterion to define positive PET findings.[10]

Results

Patients and genetic analysis

Table 1 provides a detailed summary of the observed clinical manifestations. Patients #1–3 harbored *TBK1* mutations, whereas patient #4 did not. According to the 2015 ACMG criteria,[16] two variants were pathogenic and one was likely pathogenic. None of the four patients carried *C9orf72* dynamic mutations. All participants had evidence of lower motor neuron lesions on needle EMG.

Table 1
, Demographic characteristics and clinical information

	Patient #1	Patient #2	Patient #3	Patient #4
Sex	Woman	Man	Man	Man
Age (years)	50	49	69	50
Disease duration (months)	6	12	6	6
Symptoms at onset	Dysarthria	Dysarthria	Dysarthria	Dysarthria
ALS symptoms	Bilateral facial palsy, dysarthria and dysphagia, tongue atrophy, hyperreflexia and positive Babinski's sign	Right facial palsy, dysarthria and dysphagia, tongue atrophy, limb paresis, fasciculations, hyperreflexia and positive Babinski's sign	Dysarthria and dysphagia, tongue atrophy, hyperreflexia and positive Babinski's sign	dysarthria and dysphagia, limb paresis and muscle atrophy, fasciculations, hyperreflexia hyperreflexia and positive Babinski's sign
Bulbar region	LMN+, UMN+	LMN+, UMN+	LMN+, UMN+	LMN+, UMN+
Cervical region	LMN-, UMN+	LMN+, UMN+	LMN-, UMN+	LMN+, UMN+
Thoracic region	LMN-, UMN-	LMN-, UMN-	LMN-, UMN-	LMN+, UMN-
Lumbosacral region	LMN-, UMN+	LMN+, UMN+	LMN-, UMN+	LMN+, UMN+
ALSFRS-R Score	32	34	38	42
Extrapyramidal symptoms	O1, P0, A2, C0	O1, P1, A1, C2	O1, P0, A2, C0 Ataxia Unsteady gait	O1, P2, A2, C0
PSPRS score	20	25	22	21
Behavioral and cognitive alterations	Apathy and memory loss	Apathy, disinhibition, and stereotypies	Apathy, disinhibition, and memory loss	Apathy
MMSE Score	20	26	25	27

	Patient #1	Patient #2	Patient #3	Patient #4
Family history	Father in his eighties with memory loss	-	Mother in her eighties with memory loss.	-
TBK1 sequencing	*TBK1* p.E643del	*TBK1* p.E355K	*TBK1* p.R440X	-
Pathogenic significance	Pathogenic	Likely Pathogenic	Pathogenic	N/A

Structural imaging

On visual inspection of structural MRI images, various degrees of mesencephalic atrophy (hummingbird sign) were observed in *TBK1* mutation carriers (Figure 1A). Severe (patient #3) and mild (patient #2) atrophy of the frontotemporal lobes and hippocampus was also evident in two patients, but not in the remaining two (patients #1 and #4; Figure 1B).

Dopaminergic PET imaging

All of the four patients had reduced dopaminergic binding on PET images (Figure 1C). Specifically, ^{11}C-CFT PET revealed severe lesions in patients #1 and #2 and mild lesions in patient #4. In addition, ^{18}F-DTBZ PET imaging showed mild dopaminergic lesions in the posterior putamen of patient #3.

^{18}F-APN-1607 tau PET imaging

On visual inspection, positive ^{18}F-APN-1607 binding was observed in all of the three *TBK1* mutation carriers (Figure 2A, Patient #1 - 3). Conversely, ^{18}F-APN-1607 tau PET imaging yielded negative findings in patient #4 – who did not harbor pathogenic *TBK1* mutations (Figure 2A, Patient #4). ^{18}F-APN-1607 binding signals were mainly evident in the striatum, midbrain, and dentate nucleus (Figure 2A). All *TBK1* mutation carriers met our previously defined criterion[10] for positivity on ^{18}F-APN-1607 tau PET imaging (Figure 2B). The regional binding patterns and SUVR values are listed in Table 2. Positive regions included the tegmentum, substantia nigra, raphe nuclei, locus coeruleus, and dentate nuclei.

Table 2
Regional standardized uptake value ratio values in the four study patients

	Patient #1	Patient #2	Patient #3	Patient #4	Normal values[a]
Frontal lobe	0.877	0.887	0.928	0.883	0.935 (0.090)
Parietal lobe	0.891	0.901	0.953	0.868	0.945 (0.081)
Temporal lobe	0.913	0.950	1.000	0.934	1.016 (0.075)
Occipital lobe	1.011	1.003	1.021	0.974	1.056 (0.089)
Striatum	1.057	1.035	1.017	0.971	1.035 (0.122)
Caudate	0.939	0.835	0.664	0.881	0.925 (0.103)
Putamen	1.087	1.174	1.326	1.044	1.153 (0.138)
Globus pallidus	1.424	1.385	1.412	1.152	1.255 (0.160)
Thalamus	1.462	1.413	1.738	1.359	1.463 (0.156)
Subthalamic nucleus	1.779	1.770	1.681	1.352	1.366 (0.166)
Midbrain	1.553	1.519	1.420	1.278	1.125 (0.149)
Tegmentum	1.830	1.714	1.491	1.330	1.372 (0.414)
Substantia nigra	1.924	1.757	1.518	1.348	1.284 (0.163)
Red Nucleus	1.467	1.497	1.502	1.320	1.402 (0.140)
Pontine base	1.366	1.398	1.361	1.253	1.128 (0.170)
Raphe nucleus	1.802	1.662	1.512	1.251	1.318 (0.129)
Locus coeruleus	1.473	1.332	1.551	1.254	1.248 (0.140)
Dentate nucleus	1.397	1.425	1.754	1.239	1.359 (0.165)

Data are means (standard deviations) of standardized uptake value ratios calculated on ^{18}F-APN-1607 tau PET images. a, the detailed information of the normal values could be found in our previous work (doi: 10.1002/mds.28672).

Data availability

All the data in the current study are available from the corresponding authors on reasonable request.

Discussion

This is, to our knowledge, the first ^{18}F-APN-1607 tau PET imaging study carried out in patients presenting with Progressive Supranuclear Palsy-like extrapyramidal symptoms, Amyotrophic Lateral Sclerosis, as well as cognitive and behavioral alterations. Tau PET imaging findings were specifically analyzed in

relation to the presence of pathogenic *TBK1* gene mutations. On the one hand, we found evidence of increased ^{18}F-APN-1607 uptake in the subcortical nuclei and brain stem in all of the three carriers of *TBK1* mutations. On the other hand, ^{18}F-APN-1607 tau PET imaging yielded negative findings in the single patient who did not harbor pathogenic *TBK1* gene mutations.

TBK1 gene mutations are the third most frequent cause of Frontotemporal Lobar Degeneration and the second most common cause of Amyotrophic Lateral Sclerosis.[20] In general, the screening for mutations in the *TBK1* gene is recommended for all patients with Amyotrophic Lateral Sclerosis who present with extrapyramidal symptoms.[21] *TBK1* mutations frequently lead to a loss of function and a reduced expression of the functional protein.[22] However, the neuropathological correlates of *TBK1* mutations have not been completely elucidated – especially with respect to the propensity of tau protein to aggregate.

Our findings – obtained using a novel second-generation tau PET tracer (^{18}F-APN-1607) – suggested that *TBK1* mutations present on brain images in a manner consistent with a tauopathy. However, an alternative explanation may lie in the limited specificity of ^{18}F-AV-1451 as a tau tracer which can result in off-target binding to TDP-43. In this regard, Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration have been considered as a part of a continuum that shares TDP-43 as a major disease-associated component in the brain.[23] Conversely, the role of tau pathology in the disease spectrum remains unclear. Previous ^{18}F-AV-1451 tau PET imaging studies have shown that patients with Frontotemporal Lobar Degeneration are characterized by an increased tracer uptake not only in areas of known tau pathology but also in cases with predicted TDP-43 pathology.[24] In addition, the *in vivo* binding patterns of ^{18}F-AV-1451 in a subset of patients with the semantic variant of primary progressive aphasia were in line with the expected distribution of TDP43 pathology.[25] Similar positive ^{18}F-AV-1451 tau PET imaging findings have been reported in a patient with frontotemporal dementia due to *C9orf72* expansion who had TDP-43 pathology but no evidence of tau accumulation.[26]

In our patients with *TBK1* mutations, the ^{18}F-APN-1607 uptake patterns (subcortical nuclei and brainstem) were similar to those we previously reported for Progressive Supranuclear Palsy.[10] Conversely, it was not consistent with the typical binding in the frontotemporal cortex observed for Frontotemporal Lobar Degeneration. It is worth noting that a clinicopathological study on a case with Amyotrophic Lateral Sclerosis and Progressive Supranuclear Palsy manifestations revealed the concomitant presence – but not the colocalization – of both TDP-43 and tau pathology.[27] Specifically, tau and TDP-43 deposits were localized in Progressive Supranuclear Palsy- and Amyotrophic Lateral Sclerosis-related regions, respectively.[28] The current arguments in favor or against an off-target binding of ^{18}F-AV-1451 to TDP-43 are inconclusive. In this scenario, post-mortem studies to analyze ^{18}F-AV-1451 tau PET imaging findings in relation to the presence of tau *versus* TDP-43 deposits will be invaluable to solve this conundrum. Under the assumption that the ^{18}F-APN-1607 binding observed in our patients with Progressive Supranuclear Palsy-like extrapyramidal symptoms was related to tau accumulation, our findings raise interesting questions related to the mechanisms by which *TBK1* mutations may lead to tau pathology.

Interestingly, a recent study found that the TBK1 protein can interact with tau, ultimately promoting its hyperphosphorylation and accumulation in the brain.[29] Further mechanistic studies are needed to shed more light on the link between *TBK1* function and tau protein folding.

Limitations

There are limitations to this study. The first caveat stems from the fact that Progressive Supranuclear Palsy-like extrapyramidal symptoms due to *TBK1* mutations are rare and, consequently, this case series was limited to four patients enrolled from a single center in China. Future collaborative studies with larger, ethnically diverse samples should work to confirm the external validity of our findings. Second, dopaminergic imaging was performed using two different tracers. Finally, a neuropathological examination of the affected brains would also have been interesting; however, due to the study design, we were unable to investigate this aspect. Additional autopsy studies are necessary to further evaluate the presence of tau pathology and/or TDP-43 accumulation in patients with *TBK1*-related disorders and to analyze their relations with clinical manifestations.

Conclusion

Pathogenic *TBK1* mutations in patients with Progressive Supranuclear Palsy-like extrapyramidal symptoms, Amyotrophic Lateral Sclerosis, as well as cognitive and behavioral alterations are associated with positive ^{18}F-APN-1607 tau PET imaging findings. Our data should prompt additional investigations on the potential role of tau accumulation in the pathogenesis of disease conditions associated with *TBK1* mutations.

Abbreviations

ACMG=American College of Medical Genetics and Genomics; ALSFRS-R=Amyotrophic Lateral Sclerosis functional rating scale-revised; MLPA=Multiplex ligation-dependent probe amplification; MMSE=Mini-Mental State Examination; OSEM=ordered-subset expectation; PSPRS=Progressive Supranuclear Palsy rating scale; SUVR=Standardized uptake value ratio; TBK1=TANK-binding kinase 1.

Declarations

Ethics Approval and consent to participate

The study conforms to the ethical guidelines set forth by the Helsinki Declaration and was approved by the Ethics Committee of the Huashan Hospital, Fudan University.

Consent for publication

Written informed consent was obtained from all participants.

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

The authors have no conflicts of interest in relation to this manuscript. Tzu-Chen Yen is an employee to APRINOIA Therapeutics Co., Ltd, Suzhou, China.

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Author's Contributions

FT Liu: Conceptualization (lead); writing-original draft (lead); JY Lu: Formal analysis (lead); validation (lead); YM Sun: Writing-review and editing (Lead); YJ Yang: Writing-review and editing (equal); K Qiao: Data Curation(Lead); Writing-review and editing (equal); QS Chen: Data Curation (equal); L Li: Formal analysis (supporting); software (supporting); XY Li: Data Curation (equal); TC Yen: Supervision (supporting); Writing-review and editing (equal); CT Zuo: Resources (equal); supervision (lead); Y Chen: Resources (equal); supervision (lead); J Wang: Resources (lead); supervision (lead).

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Figures

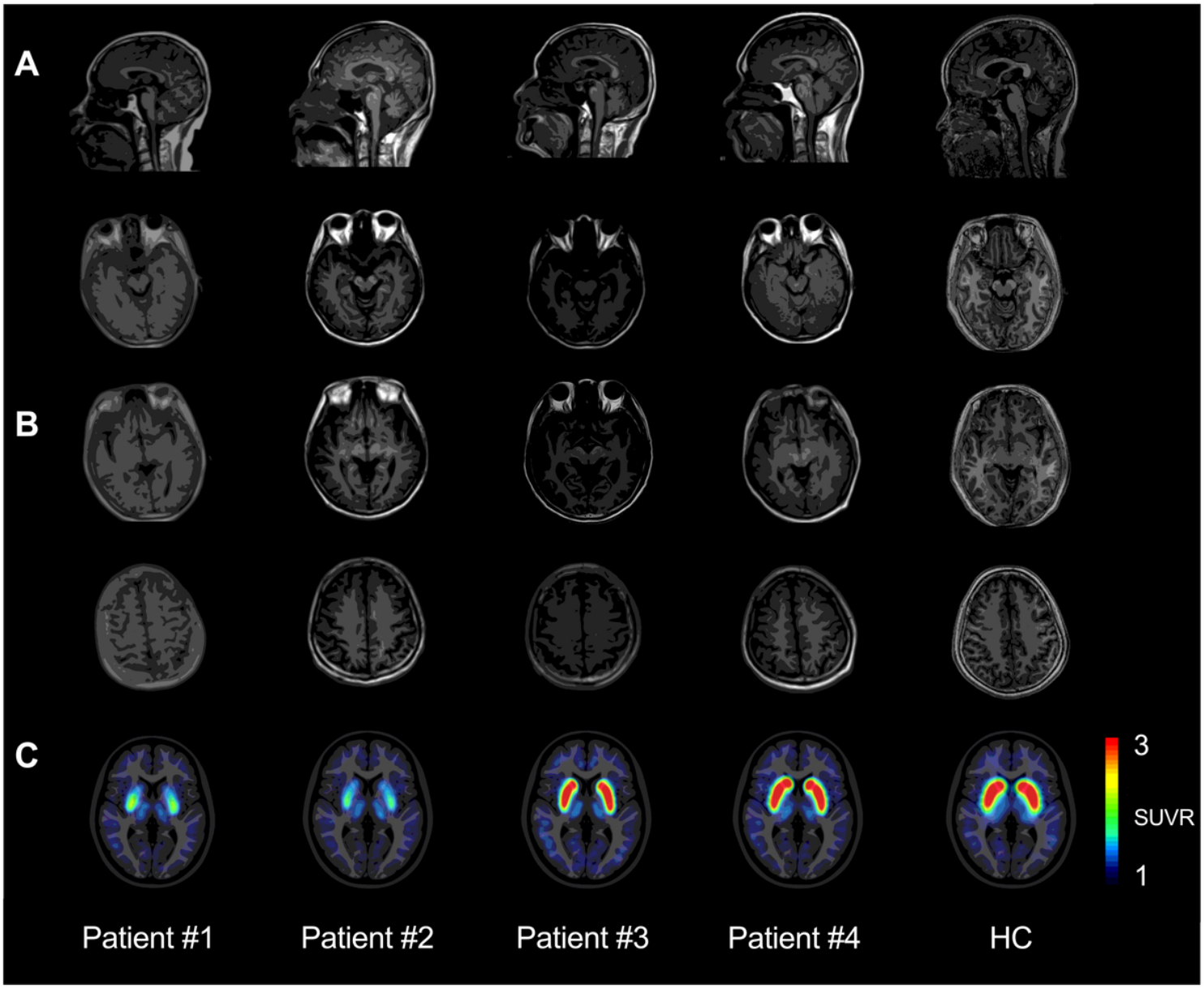

Figure 1

Results of structural and dopaminergic imaging. A. Evidence of mesencephalic atrophy in patients #1–3. B. Atrophy affecting the frontotemporal lobes and the hippocampus in patients #2–3. C. Evidence of severe dopaminergic (11C-CFT) lesions in patients #1–2. Mild dopaminergic lesions were also found in patient #3 (18F-DTBZ) and patient #4 (11C-CFT). HC, healthy control.

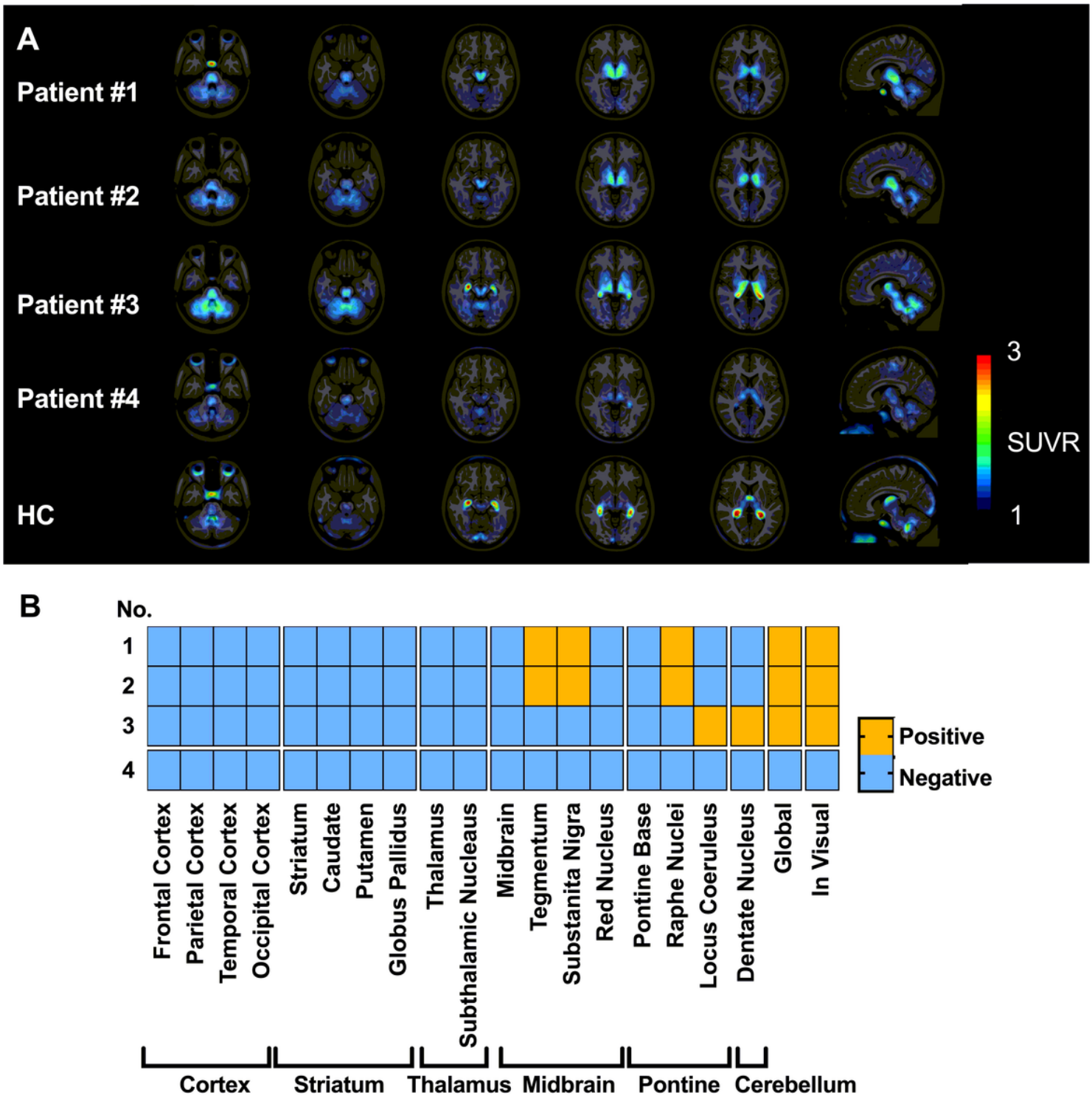

Figure 2

Results of 18F-APN-1607 tau PET imaging. A. Patterns of 18F-APN-1607 binding in the brain. B. Tracer uptake patterns (positive versus negative) are shown in different regions of interest as well as on the global level for the four study patients; the results of visual interpretation are also reported. HC, healthy control.