Aberrent Phosphorylation of α-Synuclein in Human Niemann-Pick Type C1 Disease

YUKO SAITO, MD, PhD, KINUO SUZUKI, MD, CHRISTINE M. HULETTE, MD, AND SHIGEO MURAYAMA, MD, PhD

Abstract. Niemann-Pick type C1 disease (NPC1) is an autosomal recessive neurovisceral storage disease caused by the mutation of NPC1 gene, resulting in perturbed intracellular transport of unesterified cholesterol. In NPC1, early-onset tauopathy is a constant feature. In addition, in NPC1 patients with ApoE e4 homozygosity, deposition of Aβ occurs mimicking Alzheimer disease (AD). Since AD is frequently associated with neuronal expression of α-synuclein, we investigated phosphorylated α-synuclein (psyn) immunoreactivity in the brains of 12 NPC1 patients, ages at death ranging from 9 months to 55 years. Psyn immunoreactivity was demonstrated in the perikarya of storage neurons and oligodendroglia in 10 cases. The immunoreactivity appeared more intense in subjects who had the ApoE e4 allele. Lewy bodies were found in the substantia nigra in 2 of these cases. The psyn immunoreactivity was most intense in the substantia nigra where tauopathy was most severe. Phosphorylated tau and α-synuclein frequently colocalized. This study first documents α-synucleinopathy in NPC1. This observation suggests that the defect in intracellular cholesterol trafficking in NPC1 may provoke aberrant phosphorylation of α-synuclein and tau, and that this phosphorylation is enhanced by the ApoE e4 allele. Thus, elucidation of metabolic pathways in NPC1 could provide clues to common mechanisms associated with neurodegeneration.

Key Words: Apolipoprotein E; β-amyloid; Cholesterol; Diffuse plaque; Lewy body; Neurofibrillary tangle; Phosphorylated tau.

INTRODUCTION

Niemann-Pick type C (NPC) disease is an autosomal recessive neurovisceral storage disorder caused by a perturbation of intracellular cholesterol trafficking (1, 2). Genetically, NPC is classified into type I (NPC1) and type II (NPC2). The former represents the majority of cases and is caused by mutation in NPC1 gene. The latter is caused by mutation in the HEI gene (3).

In addition to abnormal lysosomal storage in neurons and glial cells, neurofibrillary tangles (NFTs) are a constant feature in the brains of patients with both types of NPC (4–7). Furthermore, we have found accelerated tauopathy as well as aberrant deposition of β-amyloid (Aβ) in NPC1 cases homozygous for ApoE e4 (8). Thus, some neuropathological features of NPC mimic Alzheimer disease (AD).

The Lewy body (LB) is another form of an abnormally accumulated, post-translationally modified protein. The LB is the neuropathological hallmark of Parkinson disease and also dementia with Lewy bodies. LBs have also been reported in association with sporadic and familial AD (9–12), as well as with tauopathy in Hallervorden-Spatz syndrome (13), ALS-dementia complex of Guam (14), and diffuse NFTs with calcification (15). A synergistic effect on the assembly of α-synuclein and tau has been reported in a mouse model (16). Recently, LB pathology or accumulation of α-synuclein has been reported in the lipid storage disease, Gaucher disease (17, 18), and in a mouse model of GM2 gangliosidosid (19). Thus, the possible association of α-synuclein with NPC1 is worthy of investigation because of its association with lipid storage disease as well as tauopathy (4, 20).

α-Synuclein is phosphorylated at Ser 129 (psyn) and accumulates in LBs (21). Immunohistochemistry with antibodies raised against the phosphorylation site detects aberrant phosphorylation of α-synuclein in 25% of the aging population (22) and provides much higher specificity and sensitivity for detecting Lewy-associated lesions than anti-ubiquitin immunohistochemistry or routine hematoxylin and eosin (H&E) staining. Thus, we used anti-phosphorylated α-synuclein (psyn) antibodies to examine the accumulation status of α-synuclein in NPC1 human brain tissue.

MATERIALS AND METHODS

Cases

Twelve NPC brains were examined and with the exception of cases 2, 5, and 6, these cases have been reported previously (4, 6, 8). Case profiles are shown in the Table. The ages of the subjects ranged from 9 months to 55 years, with mean age of 25.4 ± 17.8 years, and the male to female ratio was 5:7. Diagnosis was based on filipin stain and cholesterol esterification studies of cultured fibroblasts (2) or with typical clinical and
pathological features in all the cases. Mutation in NPC1 gene was demonstrated in 6 cases.

Neuropathology

Formalin-fixed and paraffin-embedded sections were obtained from the various areas of the central nervous system as specified in our previous report (8). Six-μm-thick serial sections were stained with solochrome cosin, H&E, and Klüver-Barrera. The silver stains included Bodian, Bielschowsky, modified methenamine silver, and the Gallyas-Braak method. For immunohistochemical study we employed antibodies raised against Aβ (12B2, monoclonal, aa. 11–28 and Aβ1–42, polyclonal [IBL, Maebashi, Japan]); phosphorylated τ (ptau) (AT8, monoclonal, Ser/Thr 202/205 [Innogenetics, Temse, Belgium] and AP422, polyclonal, Ser-422, a kind gift from Dr. Y. Ihara); phosphorylated α-synuclein; ApoE, apolipoprotein E; e4–, no e4 allele; e4+, immunoreactive with anti-apoE4 antibody, indicating the presence of at least 1 copy of e4 allele. p-tau: (−), absent; (+), tau-immunoreactivity present in the predilection site; (++), tangles present in the predilection site; (+++), tangles widely distributed in the entire nervous system. Aβ: (+), localized in neocortex; (++) present in hippocampus, entorhinal and transentorhinal cortex, and anterior cingulated gyrus. p-syn: (±), diffuse immunoreactivity among storage with threads and dots; (+), pre-Lewy bodies scattered; (++) pre-Lewy bodies abundant; and (+++), with classic Lewy bodies.

ApoE Genotyping

Genomic DNA was extracted from the frozen brains and ApoE genotype was determined using PCR and confirmed with direct sequencing as previously reported (8).

RESULTS

Neuropathology

Details of the histopathology have been reported in the previous publication (4, 6, 8) and thus, only a brief summary is given here. Microscopic examination of the cerebrum showed numerous neurons with swollen cytoplasm and foamy storage products. Basophilic meshworks, accumulating to form classic NFTs in some neurons, were present in the perikarya of swollen neurons in all cases except case 1. Eosinophilic amorphous masses, focally aggregating into oval or round bodies were also seen in the swollen neurons in all cases except cases 1 and 4. Both brainstorm-type (Fig. 1B) and cortical (Fig. 1E) LBs were present in 2 cases.

Tau immunoreactivity was widely distributed in the entire central nervous system, including the spinal cord, but was the most abundant in the areas where NFTs were observed. Aβ immunohistochemistry detected diffuse plaques in cases 7, 8, and 10, as previously reported (8).

ApoE Genotyping and ApoE4 Immunohistochemistry

ApoE genotyping was done in 7 of the 12 cases, with 4 of the 7 cases having the ApoE ε4 allele (cases 7, 8, 10, and 12). The remaining 5 cases were investigated immunohistochemically with anti-apoE4 antibody. Two cases (cases 2 and 6) showed immunoreactive neurons.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>NPC1 mutation</th>
<th>CC</th>
<th>p-tau</th>
<th>Aβ</th>
<th>p-syn</th>
<th>ApoE</th>
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<tr>
<td>1</td>
<td>0.75</td>
<td>M</td>
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<tr>
<td>2</td>
<td>3.8</td>
<td>F</td>
<td>NA</td>
<td>2.5</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>e4+</td>
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<td>10</td>
<td>F</td>
<td>NA</td>
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<td>+</td>
<td>–</td>
<td>–</td>
<td>e4–</td>
<td>Case 9 (6), Case 2 (8)</td>
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<tr>
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<td>11</td>
<td>F</td>
<td>G insert in 3134b/E1189G</td>
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<td>+</td>
<td>–</td>
<td>–</td>
<td>3/3</td>
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<td>NA</td>
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<td>e4+</td>
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<td>–</td>
<td>+</td>
<td>3/4</td>
<td>Case 10 (6), Case 9 (8)</td>
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Abbreviations: Age, age at death (year); CC, clinical course (year); p-tau, the epitope of phosphorylated tau; Aβ, the epitope of amyloid β; p-syn, the epitope of phosphorylated α-synuclein; ApoE, apolipoprotein E; e4–, no e4 allele; e4+, immunoreactive with anti-apoE4 antibody, indicating the presence of at least 1 copy of e4 allele. p-tau: (−), absent; (+), tau-immunoreactivity present in the predilection site; (++) tangles present in the predilection site; (++++), tangles widely distributed in the entire nervous system. Aβ: (+), localized in neocortex; (++) present in hippocampus, entorhinal and transentorhinal cortex, and anterior cingulated gyrus. p-syn: (±), diffuse immunoreactivity among storage with threads and dots; (+), pre-Lewy bodies scattered; (++) pre-Lewy bodies abundant; and (++++), with classic Lewy bodies.
Fig. 1. Histopathological features of α-synucleinopathy. A: α-Synucleinopathy in the substantia nigra detected immunohistochemically by a monoclonal antibody against phosphorylated α-synuclein (psyn#64) (case 8, bar = 100 μm). B: Lewy body in eosinophilic mass among a storage swollen neuron from substantia nigra (case 8, H&E stain, bar = 10 μm). C: Multiple round aggregation of psyn#64-immunoreactivity (case 8, bar = 10 μm). D: α-Synucleinopathy in the amygdala immunohistochemically visualized by psyn#64. Lewy dots (arrowheads) and threads (arrows) (case 6, bar = 10 μm). E: Cortical Lewy bodies in an anterior cingulate gyrus (case 8, bar = 10 μm). F: Somatic localization with dendritic extension of the epitope of psyn#64 (case 8, bar = 10 μm). G: A pre-Lewy body (case 8, bar = 10 μm). H: Oligodendroglial α-synucleinopathy (case 8, bar = 10 μm), double immunostain with antibodies to psyn#64 (brown) and anti-GFAP (red).

indicating that at least 1 copy of ApoE ε4 allele was present (Saito et al, unpublished observation).

Immunohistochemistry with Anti-Psyn Antibodies

The immunoreactivity with the anti-psyn antibodies was observed diffusely in the perikarya of swollen storage neurons and glial cells in the areas where tauopathy was most severe in 10 of the 12 cases. The youngest case with this change (case 2) had at least 1 copy of the ApoE ε4 allele. Classic LBs were found in the substantia nigra in 2 cases (cases 6 and 8) (Fig. 1B). The amount of psyn immunoreactivity was commensurate with the severity of tauopathy in the background. In the brainstem, psyn immunoreactivity was most intense in the substantia nigra (Fig. 1A), followed by the reticular formation of midbrain and the Edinger-Westphal nucleus. Psyn immunoreactivity was markedly less in the locus ceruleus and nucleus raphe dorsalis of the pons. Immunoreactivity was definitely present but of least intensity in the dorsal motor nucleus of vagus. In 2 cases with LBs in the substantia nigra, cortical LBs and psyn-immunoreactive pre-LBs, dots and threads were seen in the amygdala and the anterior cingulate gyrus (Fig. 1D–H). Psyn-immunoreactive dots and threads were scattered in the entorhinal and
transentorhinal cortex and gyri recti, as well as the frontal and temporal lobes, insular cortex, caudate nucleus, globus pallidus, basal nucleus of Meynert, and hypothalamus. Tauopathy was also found in these areas.

Psyn-immunoreactive, GFAP-negative oligodendroglial cells were scattered in the affected area (Fig. 1H). Immunohistochemistry with anti-ubiquitin or anti-α-synuclein antibodies also detected psyn-immunoreactive neurons, dots, and threads, but its sensitivity and specificity were less.

Confocal Microscopy

Psyn and ptau immunoreactivity was frequently observed in a single neuron or thread. They occasionally colocalized in the neuronal perikarya (Fig. 2A–C), but in the majority of cases were segregated from each other. However, they were frequently colocalized in the threads and dots in the neuropil. There was frequent association with the epitope of ptau and of Aβ, as previously reported (8). However, Aβ and psyn were not found together in the tissues examined. ApoE4 colocalized with ptau and psyn in cases with the ApoE e4 allele. ApoE also colocalized with Aβ in cases with ApoE e4 homozygosity (Fig. 2D–L).

DISCUSSION

This is the first report of α-synucleinopathy in human NPC1 brains. α-Synucleinopathy was observed in the majority of the NPC1 cases. Immunoreactivity colocalized with abnormal storage product. α-Synucleinopathy was associated with tauopathy and the intensity of immunoreactivity for α-synuclein increased in subjects with the ApoE e4 allele.

Previous studies with anti-psyn antibodies indicate 2 types of α-synucleinopathy. The primary type starts in the medulla oblongata, spreads rostrally (24), and is associated with Parkinson disease. The secondary type starts in the amygdala and is associated with AD (9, 10) or other tauopathy (13–15). α-synucleinopathy in NPC1 is accentuated in the midbrain and amygdala where tauopathy is most severe, but is very mild in the medulla oblongata. Although the pattern of cerebral distribution in NPC1 differs from primary or secondary α-synucleinopathy, the colocalization of α-synucleinopathy and tauopathy strongly indicates their mutual interaction. Similar colocalization has been reported in animal models of tauopathy (16). Since tauopathy seems to be a constant feature of NPC, the tauopathy may in turn enhance α-synucleinopathy. In AD, the ApoE e4 allele enhances tauopathy (25). Therefore, the enhancing effect of the ApoE e4 allele on α-synucleinopathy in NPC may also influence the extent of tauopathy. However, there are several reports that ApoE e4 allelic frequency is high in LB disease, especially in dementia with LBs (26, 27). Thus, it is possible that the ApoE e4 allele directly enhances the α-synucleinopathy in NPC1 brains.

The frequency of the ApoE e4 allele in these twelve cases is calculated to be more than 50%, which is much higher than its average allelic frequency among Caucasians in the United States (28). Although the number of cases in our study is small, this accumulation of ApoE
e4 allele among NPC1 cases suggests that ApoE4 may enhance the metabolic defect caused by NPC1 gene mutation and exacerbate clinical symptoms.

Considering the data from other lysosomal storage diseases (17–19), the accumulation of α-synuclein may occur in association with the storage or metabolic defect, eventually leading to its phosphorylation and formation of LBs. The unique feature of α-synucleinopathy in NPC1 is its consistent and anomalously early appearance, as well as its coexistence with tauopathy. Thus, the study of cellular pathology of NPC1 may provide a key to the understanding of the mechanisms underlying tau and α-synuclein deposition in other diseases. It is also interesting that this deposition of phosphorylated tau and α-synuclein is enhanced by the presence of ApoE4 protein. This could produce deposition of Aβ around the storage neurons in association with ApoE e4 homozygosity. The study of neurons carrying the NPC1 mutation and ApoE e4 homozygosity may provide clues to the mechanism common to tauopathy, α-synucleinopathy, and deposition of Aβ. To that end, the creation of double transgenic mice by mating human ApoE4 knock-in mice (29) with NPC1 mutant mice (30) is currently underway in our laboratory.

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