Subcortical Volumetric Reductions in Adult Niemann-Pick Disease Type C: A Cross-Sectional Study


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Subcortical Volumetric Reductions in Adult Niemann-Pick Disease Type C: A Cross-Sectional Study

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ABSTRACT

BACKGROUND AND PURPOSE: Voxel-based analysis has suggested that deep gray matter rather than cortical regions is initially affected in adult Niemann-Pick type C. We sought to examine a range of deep gray matter structures in adults with NPC and relate these to clinical variables.

MATERIALS AND METHODS: Ten adult patients with NPC (18–49 years of age) were compared with 27 age- and sex-matched controls, and subcortical structures were automatically segmented from normalized T1-weighted MR images. Absolute volumes (in cubic millimeters) were generated for a range of deep gray matter structures and were compared between groups and correlated with illness variables.

RESULTS: Most structures were smaller in patients with NPC compared with controls. The thalamus, hippocampus, and striatum showed the greatest and most significant reductions, and left hippocampal volume correlated with symptom score and cognition. Vertex analysis of the thalamus, hippocampus, and caudate implicated regions involved in memory, executive function, and motor control.

CONCLUSIONS: Thalamic and hippocampal reductions may underpin the memory and executive deficits seen in adult NPC. Volume losses in other subcortical regions may also be involved in the characteristic range of motor, psychiatric, and cognitive deficits seen in the disease.

ABBREVIATIONS: ICV = intracranial volume; NFT = neurofibrillary tangle; NPC = Niemann-Pick disease type C; NUCOG = Neuropsychiatry Unit Cognitive Assessment Tool; VBM = voxel-based morphometry

Niemann-Pick disease type C (Online Mendelian Inheritance in Man 257220 and 607625; http://www.ncbi.nlm.nih.gov/omim) is a progressive neurogenetic disorder, resulting from mutations to the genes encoding for the NPC1/NPC2 proteins. Impaired function of NPC1/NPC2 results in altered intracellular sterol trafficking and accumulation of gangliosides and other glycosphingolipids. While most patients present with neurovisceral signs in childhood, in 20% of cases, the illness presents in adults.

Adults demonstrate a range of central nervous system symptoms, including ataxia, dystonia, vertical supranuclear ophthalmoplegia, cognitive impairment, and psychotic illness. Because of the rarity of adult patients, few studies have examined brain changes in the disease at a group level and their relationship with clinical variables.

We previously used VBM to demonstrate that gray matter changes in NPC were most significant in basal and subcortical regions, with reductions in volume in the hippocampus, thalamus, cerebellum, and striatum. These findings matched both animal and human models of NPC, where these regions show the greatest ganglioside accumulation, and where humans develop NFTs. Given that the most significant reductions were in medial and subcortical gray matter regions, we aimed to both validate and extend on our previous findings by using a subcortical segmentation approach. This provided regional volumetric data for a range of subcortical structures, which were then correlated with illness variables. We subsequently used vertex analysis to examine localized changes in brain structures that showed significant volumetric change.
MATERIALS AND METHOD

Subjects

Data were acquired from 10 adult patients with NPC (6 men, 4 women) from the Royal Melbourne Hospital, Melbourne, Australia (Table 1) between 2000 and 2010. This is a larger cohort of patients than our original voxel-based analysis and, given the exceptional rarity of diagnosed adult patients, constitutes the largest published sample size in a neuroimaging study in this disorder. All participants provided written informed consent, and the study was approved by the local research and ethics committee (HREC 2004.042 and 2005.198). Diagnosis was confirmed with biochemical analysis of cultured fibroblasts, by using the cholesterol esterification rate and percentage of cells staining abnormally for perinuclear cholesterol. Age at onset of neurologic symptoms and duration of symptoms were recorded, and symptoms were rated on the NPC-specific rating scale of Iturriaga et al. Nine patients were assessed cognitively by using the NUCOG, a cognitive screening tool that rates cognitive functioning in 5 domains: attention, memory, executive functioning, language, and visuospatial construction function. Patients were matched for age and sex with healthy controls (n = 27; 17 men, 10 women) without a history of major medical, neurologic, or psychiatric illness. NUCOG data were not available for control participants.

MR Imaging Acquisition and Analysis

Participants were scanned on a 1.5T Signa MR imaging scanner (GE Healthcare, Milwaukee, Wisconsin). A volumetric spoiled gradient recalled-echo sequence generated 124 contiguous 1.5-mm coronal sections with TE/TR, 3.3/14.3 ms; flip angle, 30°; matrix size, 256/256; FOV, 24 cm; voxel dimensions, 0.938 mm.

MR Imaging Segmentation

Subcortical structures were segmented with the FMRIB Integrated Registration and Segmentation Tool (FIRST) from the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl). This process provided volumes of the left and right hippocampi, amygdala, nucleus accumbens, putamen, caudate, pallidum, and thalamus (Figs 1 and 2). The SPGR images from both patient and control groups were transformed into Montreal Neurological Institute-152 (MNI-152) standard space via an affine transformation using 12 df at 1-mm resolution. A second registration was applied by using the MNI-152 subcortical mask to exclude voxels outside the subcortical range. Segmentation of subcortical structures was then based on shape models and voxel intensities, in which surface meshes of each subcortical structure were extracted, transformed to original MR imaging space, and then filled and boundary-corrected. Absolute volumes of subcortical structures were calculated in cubic millimeters. All segmentations were then visually checked for errors in registration and segmentation.

The ICV was determined in a semiautomated fashion by using FSL software. First, brains were skull-stripped with the Brain Extraction Tool, carefully checked for accuracy, and then aligned linearly to the MNI-152 one-millimeter T1-weighted template. The inverse of the determinant of the affine transformation matrix was multiplied by the ICV of the MNI-152 template to produce a measure of ICV for use as a scaling factor, measured in cubic centimeters.

Table 1: Clinical, biochemical, and ocular-motor variables for the NPC patient group

<table>
<thead>
<tr>
<th>Patient</th>
<th>Demographic Variables</th>
<th>Illness Variables</th>
<th>Biochemical Variables</th>
<th>Cognitive Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (yr)</td>
<td>Sex</td>
<td>AOO (yr)</td>
<td>DON (yr)</td>
</tr>
<tr>
<td>1</td>
<td>49</td>
<td>M</td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>M</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>F</td>
<td>29</td>
<td>4</td>
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<td>4</td>
<td>32</td>
<td>F</td>
<td>26</td>
<td>6</td>
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<td>5</td>
<td>43</td>
<td>F</td>
<td>41</td>
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<td>20</td>
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<td>19</td>
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<td>7</td>
<td>32</td>
<td>M</td>
<td>25</td>
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<td>M</td>
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<td>9</td>
<td>18</td>
<td>M</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>F</td>
<td>31</td>
<td>2</td>
</tr>
</tbody>
</table>

Note: AOO indicates age at onset of neurologic symptoms; DON, duration of neurologic symptoms; NA, not applicable; Filipin, a complex of polyene antibiotics obtained from Streptomyces filipinensis.

FIG 1. Axial, coronal, and sagittal sections of a T1-weighted MR image, indicating the deep gray matter regions segmented for later analysis. Top, healthy control; bottom, NPC patient.
performing multiple Bonferroni tests at each intersection, 12

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enaude et al10 was used to assess localized size and shape differ-

ces. We elected to undertake this on structures that showed

significant between-group differences and on which shape analy-
sis may allow localization of changes to regions approximating
subregions or nuclei of the structures involved.13 For each struc-
ture, all subject surfaces are aligned to the mean surface from the
model by using a 6-df transformation. Rather than using a multi-

Statistical Analysis
Comparison of the brain stem neuroimaging variables was un-
dertaken with analysis of covariance, controlling for ICV. Effect
size for each analysis of covariance was calculated by using Cohen
$\eta^2$, with an $\eta^2 > 0.14$ constituting a large effect.11 Correlations
between oculomotor, illness, and brain stem variables were un-
dertaken by using the Spearman correlation coefficient. Correc-
tion for multiple comparisons was undertaken by using the Holm-Bonferroni correction, which controls the family-wise
error rate at a given level of $\alpha$ to minimize type I errors while
performing multiple Bonferroni tests at each intersection,12
while minimizing the type II errors inherent in a simple Bon-
ferroni correction. In this method, uncorrected $P$ values are or-
dered, with the smallest value compared with $0.05/n$ (where
$n =$ number of comparisons). If this value is lower, the null hy-
thesis is rejected, and the next smallest is compared with $0.05/
(n–1)$; this process continues until the null hypothesis cannot
be rejected and all $P$ values above this level are considered non-
significant. For between-group comparisons, $n = 14$ (7 sub-
cortical structures in each hemisphere; for correlational analyses)
and $n = 18$ (3 subcortical structures in each hemisphere cor-
related with 3 illness variables). All analyses between total volume
and clinical variables were undertaken with PASW Statistics,

Vertex Analysis
For local shape analysis on subcortical structures generated by
FIRST, a modified approach to vertex analysis proposed in Pat-
enaude et al10 was used to assess localized size and shape differ-
ces. We elected to undertake this on structures that showed
significant between-group differences and on which shape analy-
sis may allow localization of changes to regions approximating
subregions or nuclei of the structures involved.13 For each struc-
ture, all subject surfaces are aligned to the mean surface from the
model by using a 6-df transformation. Rather than using a multi-

RESULTS

Demographic Data
Demographic data are presented in Table 2. There was no significant differ-
ence between the control and NPC groups on measures of age ($t = 0.348, P = .730$),
sex ($\chi^2 = 0.024, P = .869$), and intra-
cranial volume ($t = 0.642, P = .525$). The mean duration of ill-
ess for the sample was $4.50 \pm 3.41$ years (range, 1–12 years),
and the mean illness scale score was $8.20 \pm 3.71$ years (range,
4–15 years; Table 1).

Between-Group Analyses
Most subcortical structures, corrected for head size, were smaller
in the NPC group compared with controls (Table 2). Differences
in bilateral hippocampus, thalamus, putamen, right amygdala,
and caudate survived Holm-Bonferroni correction. The greatest
effect size was seen in the thalamus, where $\eta^2$ was $>0.4$ for both
left and right thalami. The hippocampus and striatum (caudate
and putamen) showed effect sizes of 0.15–0.25, with the smallest
effect sizes seen in the amygdala and accumbens.

Relationship between Subcortical Volumes and
Illness Variables
There was a correlation between total gray but not white matter
volume with duration of illness and symptoms and a correlation
with global cognitive function (Table 3). When subcortical vol-
umes were examined, the strongest correlations were seen for the
thalamus and hippocampus respectively, more so with left-sided
structures. The correlations between left hippocampal volume and
illness scale score and cognition survived multiple-comparison test-
ing. No structure correlated with illness biochemical variables.

Vertex Analysis
Vertex analysis was undertaken on the hippocampus, thalamus,
and caudate, shown in Fig 3. In the hippocampus, regional reduc-
tions were shown bilaterally in the laterally located CA1 region of
the hippocampus, more so on the left, and in the inferiorly located
left subiculum. In the thalamus, reductions were seen bilaterally
in regions corresponding to the location of the anterior and lateral
dorsal nuclei and the pulvinar. The caudate showed reductions in
both dorsolateral and dorsomedial regions through the head,
body, and tail.
DISCUSSION

These findings in gray matter nuclei are broadly in accordance with but expand the limited findings from the neuroimaging literature in NPC. The initial case series in which MR imaging was included had based analyses on visual inspection and generally reported anterior cortical and cerebellar atrophy and cortical thinning, though it did not implicate all striatal nuclei. Tedeschi et al. described proton MR spectroscopy parameters in 10 (mostly adult) patients compared with controls and showed reduced NAA/creatinine ratios in the caudate. Battisti et al. described significant frontal cortex, thalamic, and cerebellar hypoperfusion on FDG-PET associated with illness progression. Zaaraoui et al. compared 2 patients individually against a group of controls by using magnetization transfer ratio and found reductions in the thalamus, putamen and globus pallidus, and cerebellum. The current study confirms the findings from these small series that volumetric changes occur in thalamic, hippocampal, and striatal regions in NPC and suggests that these changes progress with illness.

These findings also correlate with changes described in animal models of NPC and the limited number of human neuropathologic studies. In feline NPC models, the formation of axonal spheroids occurs in gamma-aminobutyric acid–ergic neuronal populations in the cerebellum, brain stem, hippocampus, and basal ganglia. In murine NPC models, intracellular storage of gangliosides occurs maximally in large pyramidal neurons, Purkinje cells in the cerebellum, and neurons in the lateral thalamus, hippocampus, and brain stem; and maximal neuronal loss occurs in the thalamus and cerebellum. The earliest human neuropathologic studies showed that neuroaxonal dystrophy was maximal in the thalamus and cerebellum. In humans, neuropathologic studies demonstrated that noncerebellar brain regions develop NFTs in the basal ganglia, thalamus, brain stem, cerebral cortex, and hippocampus. The convergent animal and human data suggest that in addition to notable cerebellar Purkinje cell loss, significant changes also occur in the thalamus and hippocampus. While most visually rated MR imaging re-

| Table 2: Demographic and subcortical variables across control and NPC patient groupsa |
|---------------------------------|-----------------|-----------------|-----------------|
| Age (yr) | 31.50 (± 9.62) | 30.24 (± 9.78) | t = 0.348 | .730 | – |
| Sex (M/F) | 6:4 | 17:10 | χ² = 0.024 | .869 | – |
| Intracranial volume (mm³) | 14.65 (± 2.30) × 10⁵ | 15.07 (± 1.59) × 10⁵ | t = 0.642 | .525 | – |
| Total gray matter (mm³) | 7.84 (± 0.69) × 10⁵ | 8.44 (± 0.39) × 10⁵ | F = 5.951 | .006 | 0.221 |
| Total white matter (mm³) | 6.36 (± 0.35) × 10⁵ | 6.76 (± 0.35) × 10⁵ | F = 5.327 | .01 | 0.198 |

Note: — indicates no statistical calculation.

Table 3: Correlational matrix between volume of subcortical structures in patients with NPC and illness variablesa

<table>
<thead>
<tr>
<th>Duration of illness (yr)</th>
<th>Illness Scale Score</th>
<th>NUCOG (/100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Left</td>
<td>Hippocampus</td>
<td>−0.670</td>
</tr>
<tr>
<td>Thalamus</td>
<td>−0.623</td>
<td>.027</td>
</tr>
<tr>
<td>Putamen</td>
<td>−0.226</td>
<td>.265</td>
</tr>
<tr>
<td>Right</td>
<td>Hippocampus</td>
<td>−0.566</td>
</tr>
<tr>
<td>Thalamus</td>
<td>−0.597</td>
<td>.034</td>
</tr>
<tr>
<td>Putamen</td>
<td>−0.332</td>
<td>.182</td>
</tr>
</tbody>
</table>

Note: a Volumes are presented as means.
b Effect sizes (Cohen d) presented for significant between-group comparisons.
c P value surviving Holm-Bonferroni correction.

ports in NPC disease focus on atrophy in surface regions such as the cerebral cortex and cerebellum, which are more obvious on T1-weighted imaging, the atrophy of deeper nuclei may be less obvious.

Neuronal loss in the hippocampus and thalamus, the subcortical regions of greatest and most significant volume loss in this study, would be expected to cause marked neuropsychological impairment in NPC. The hippocampus plays a key role in episodic and declarative memory, and because the hippocampus projects to the anterior thalamus via the fornix (to the mammillary bodies) and then via the mamillothalamic tract, pathology in the thalamus is known to cause significant impairment in both new memory encoding and executive function. Cognitive impairment is a core symptom cluster in adult NPC, in addition to movement disturbance, gaze palsy, and psychiatric illness, and is present, to some degree, in all adult patients at diagnosis and progresses with time. Significant memory and executive impairment have been described in a number of adult patients in case reports, however, few studies have systematically examined cognitive function in adult patients with NPC. Klerner et al demonstrated that verbal memory impairment was present in most adult patients with moderate progression, whereas executive impairment occurred early. Hinton et al also showed that verbal memory impairment was present in 13/14 adolescent and adult patients, and all patients had demonstrably impaired executive function. These findings suggest that the memory impairment in adult NPC is related to the primacy of pathology affecting the hippocampus in particular and the thalamus.

Results of the vertex analyses for the hippocampus showed significant reductions in the CA1 region, a zone vulnerable to metabolic stress and where NFT formation is regionally concentrated in human patients with NPC. The CA1 region is crucial for autobiographic memory retrieval and spatial learning. The subiculum, where particular reductions were seen on the left (and where NFTs are also found but are less numerous than in CA1), is principally concerned with spatial navigation and mnemonic processing. These findings consolidate the assertion that memory impairment in adult NPC is driven by hippocampal pathology.

In addition, the regional reductions seen in the thalamus implicated the anterior and lateral dorsal nuclei and the pulvinar. The anterior nucleus receives input from the hippocampus, cingulate, and hypothalamus, projecting back to these regions, and is involved in recognition memory; pathology in this nucleus results in varying levels of verbal and spatial memory impairment. The lateral dorsal nucleus has similar connections and appears to act in concert with it in spatial learning and memory. The pulvinar plays a key role in stimulus-driven visual attention and has reciprocal connections throughout the cortex, particularly the frontal and visual cortices. It may also integrate cortical control of voluntary eye movements from the frontal cortex with reflexive movements that originate in the superior colliculus, and pathology may thus contribute to the deficits seen in self-generated saccades and antisaccade performance in adult NPC. It is possible that the thalamic volume loss is primary, related to both altered neuronal ultrastructure and NFT formation in this region, but given the close functional relationship with the hippocampus, there may be a contribution of trans-synaptic degeneration secondary to deafferentation, which is known to affect the anterior thalamic nuclei in hippocampal sclerosis.

The volumetric reductions in the basal ganglia, though not significant when corrected for multiple comparisons, are consistent with the motor findings in adult NPC. Whereas ataxia follows early loss of cerebellar Purkinje neurons, dystonia is also common, with Parkinsonism and chorea occurring in a small group of patients. Only a small group of studies has specifically examined dystonia in NPC. Floyd et al examined 15 adult patients with NPC by using accelerometry and surface electromyography; 13/15 showed physiologic evidence of action tremor; 6/15, of dystonia; and 2/15, of myoclonus. In 14 adolescent/adult patients
and 14 matched controls, spiral analysis of upper limb kinematics found tremor in most patients but also showed abnormal pressure-time relationships consistent with focal dystonia. Dystonia occurs in concert with the pathology of the basal ganglia, the putamen in particular. This study showed the greatest volumetric loss in the putamen, followed by caudate and pallidal volumes. The basal ganglia is a known site of neurofibrillary tangle formation in human patients with NPC \(^7,23\) and of marked neuroaxonal dystrophy in the feline NPC model. \(^37\)

Cerebellar dysfunction may also play a role in contributing to dystonia, via cerebellar-basal ganglia connections through the thalamus, red nucleus, and zona incerta\(^{38}\), the interaction of both cerebellar and basal ganglia pathology may thus underpin the high rate of dystonia in adult patients with NPC. The vertex analysis of the caudate showed that dorsolateral regions were particularly affected; this region of the caudate is a central node in the dorsolateral prefrontal cortical loop, which reciprocally connects this region of the caudate to the dorsolateral prefrontal cortex. This circuit subserves executive function, such as problem-solving, self-direction and judgment, and shifting behavioral set. The executive impairment frequently described in the illness \(^{26,27}\) may thus also be increased by dysfunction in this frontot striatal circuitry. The caudate also plays a role in inhibiting saccade neurons in the superior colliculus\(^{39}\), damage to this pathway leads to errors on the antisaccade task, a task that patients with NPC perform poorly if at all. \(^40\)

The nucleus accumbens showed modest nonsignificant volumetric reductions in our study. The accumbens is thought to function as the interface between limbic and motor systems and to play a significant role in goal-directed behavior, the reward system, and addiction. \(^41\) Abnormalities in the accumbens have also been implicated in schizophrenia, \(^42\) and if function of the accumbens is impaired in NPC, it may be related to the high prevalence of schizophrenia-like psychosis in the disorder. \(^2,3\) The only study to examine the accumbens in NPC, however, found no reductions in number but did find abnormal morphology in accumbal cholinergic neurons. \(^19\)

Finally, modest reductions were seen in amygdala size in our adult NPC cohort. The amygdala is known to play a role in emotional regulation and social cognition; bilateral amygdala lesions are known to result in the lack of awareness of threat and in social disinhibition. \(^43\) Social disinhibition and a lack of interpersonal “distance” have frequently been described in adult patients with NPC. \(^3,14\) Neurofibrillary tangles and distended neur-\[\]

- **CONCLUSIONS**

We have demonstrated that patients with adult NPC show key volume reduction in a number of subcortical regions compared with healthy individuals, occurring in sites where neuropathologic abnormalities have been shown to occur. These alterations may underpin some of the key motor, cognitive, and emotional features of adult NPC. Larger cohorts and longitudinal studies will allow a clearer understanding of how these structural changes correlate to specific clinical features and how the timing of changes in brain structure and function relate to the onset of symptoms of this disease.

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