



Research article

In vivo evidence of cortical amyloid deposition in the adult form of Niemann Pick type C



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ABSTRACT

Background: Niemann Pick disease type C (NPC) is a lysosomal lipid storage disorder presenting visceral and neurological impairment with cognitive decline. Neurodegeneration in NPC is associated to deposition of amyloid- β and abnormal tau aggregations likewise Alzheimer disease (AD). Dementia is also related to intracortical circuiting abnormalities that can be detected by neurophysiological procedures both in NPC and in AD. Aim of this study is to find the in vivo evidence of amyloid deposition in NPC patients with cognitive impairment and to investigate the pathophysiology of dementia according to similarities with AD.

Methods: Two sisters affected by NPC and cognitive decline underwent neuropsychological tests, PET scans with ¹⁸F-Florbetaben and neurophysiological protocols to assess cortex excitability by means of transcranial magnetic stimulation (TMS), such as short-latency afferent inhibition (SAI), short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF).

Results: Both patients presented a multidomain cognitive impairment. ¹⁸F-Florbetaben uptake was detected in brain frontal areas, while SAI and SICI were abnormal in both patients.

Discussion: Cognitive impairment in NPC is associated to cortical amyloid deposition as revealed by ¹⁸F-Florbetaben PET scan. Amyloid imaging data, together with specific abnormalities found at TMS studies, suggest similar mechanisms underlying NPC and AD dementia.

1. Introduction

Niemann Pick disease type C (NPC) is a lipid storage disorder caused by mutation within the *NPC1* (95% of patients) or *NPC2* (5% of patients) gene. The clinical spectrum of the disease ranges from a neonatal rapidly fatal disorder to an adult-onset chronic neurodegenerative disease. In adult forms of NPC patients present a severe and complex neurological impairment with invariable cognitive decline in addition to visceral disorders [1, 2, 3, 4]. The mechanism of neurodegeneration is not yet fully understood although there are few studies that could suggest similarities with Alzheimer's disease (AD) pathophysiology [5, 6]. Abnormal cholesterol and ganglioside metabolism may influence the generation and aggregation of amyloidogenic fragments, such as amyloid- β (A β) peptides, from amyloid-precursor protein (APP), crucial factors causing neurodegeneration [7, 8]. Based on the link between cholesterol and A β deposition, NPC has been considered as a model of "juvenile Alzheimer Disease (AD)" [9]. Actually, unesterified cholesterol and GM2

gangliosides lysosomal accumulates in cerebral cortex and cerebellum of NPC patients causing neuronal death through a molecular process that may resemble AD progressive degeneration. Both NPC and AD develop neurodegeneration with accumulation of A β peptides, hyperphosphorylation of tau protein and abnormal cholesterol metabolites [10].

The examination of the brain of demented NPC patients revealed diffuse cortical A β deposits with a higher load at hippocampus similar to amyloid distribution of AD [11]. The evidence of A β cortical load can be detected by PET imaging using different tracers since the early stage of AD [12].

In a recent study of Villemagne [13], a group of patients with NPC were investigated by multimodal PET scans to find deposits of A β and tau tangles: half of patients showed tau burden but none of them had amyloid deposition. Interestingly, tau deposits were associated with measures of disease onset and progression, but not with cognitive performance [13].

Recently, the link between NPC and AD has been also substantiated

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by evidence of central cholinergic dysfunction demonstrated in vivo by means of transcranial magnetic stimulation (TMS). Specifically, an impairment in short-latency afferent inhibition (SAI), a measurement of sensorimotor integration obtained from the combination of TMS with peripheral electrical stimulation, has been recently observed in NPC [14, 15]. SAI is considered a biomarker of cognitive dysfunction [16], since it seems to be altered already in the early phase of AD [17]. Interestingly, SAI parallels with cognitive impairment in NPC at long term follow-up [18]. Here we aim to prove the presence in vivo of brain amyloid burden by positron emission topography (PET) in two siblings with NPC and cognitive impairment. Moreover, we also investigated cortical excitability in NPC by means of specific TMS protocols to further demonstrate similarities between AD and NPC.

2. Materials & methods

At the current assessment patient 1 (P1) was a 31 years old woman with 5 years of disease duration whereas her sister, patient 2 (P2), was 28 years old with a disease duration of 12 years. They both presented compound heterozygous mutations of the *NPC1* gene: c.2974GNC (p.G992R) and c.2130dupG (p.R711EfsX3). The younger sister developed neurological disorders 7 years before receiving the diagnosis of NPC that was at the same time confirmed also in her sister who presented only slight signs of brain impairment. P1 and P2 were extensively studied on their cognitive and neurophysiological profile by our research group and results were detailed in previous studies [14, 18, 19].

The two patients underwent amyloid imaging with PET. Scans were obtained 90 min after injections of 365 MBq of ¹⁸F-Florbetaben on a discovery 710 (General Electric) PET/CT scanner.

Tracer uptake was visually assessed and quantified using regional cortical tracer uptake (RCTU) score determined from pre-established VOIs on the individual gray-matter-segmented PET data.

Moreover, both patients underwent neurological evaluation, standard neuropsychological test battery [20, 21, 22], and neurophysiological assessment of cortex excitability by applying ad hoc TMS protocols, such as SAI and short-intracortical inhibition (SICI) and intracortical facilitation (ICF) paradigms. SAI and SICI-ICF protocols were studied at rest via a paired pulse paradigm, delivered in a conditioning-test design, as previously reported [23, 24, 25, 26]. Twelve healthy subjects, not affected by any neurological, psychiatric or other relevant clinical condition (7 females; mean age 33.1 ± 12.7 years, age range 25–44) were evaluated as the control group for the neurophysiological study.

The experiments conformed to the Declaration of Helsinki and were approved by the ethics committee of the University of Naples Federico II, Italy (N. 100/17). Lastly, informed consent was obtained from all participants.

3. Results

At the present time P1 displayed cognitive impairment in memory functions and cognitive flexibility, whereas P2 showed a multidomain dementia. P2 showed lower scores in comparison to P1 in all neuropsychological tests (Table 1). Both patients did not exhibit any psychiatric symptom or were on central nervous system-active drugs at the time of the study.

Mild ¹⁸F-Florbetaben uptake was detected in cortical frontal areas of both patients consistent with the presence of cortical A β plaques in those areas (Fig. 1).

Neurophysiological analysis with TMS revealed for both patients a clear impairment in mean SAI (P1 = 114.96% of basal MEP; P2 = 146.09%, normal value <72 %) and mean SICI at ISIs of +2 and +3 ms (P1 = 85.47 % of basal MEP; P2 = 102.60%, normal value <66 %) compared with healthy controls (see Fig. 2). On the contrary the mean ICF of both patients (P1 = 138.97 % of basal MEP; P2 = 198.05 %, normal value <213%) displayed no difference compared to healthy controls (see Fig. 2). Overall these results put forward an abnormal motor

Table 1
Neuropsychological follow-up.

	P1	P2	Normal value
Age at examination (years)	32	29	
Educational level (years)	18	13	
Neuropsychological evaluation			
<i>Screening tests</i>			
MMSE	28.10	22.59	>24.1
FAB	14.19	7.7	>12.19
<i>Spatial and verbal working memories</i>			
Corsi's test	3.32	1.49	>4.29
Verbal span	3	4.25	>3.40
<i>Long-term memory</i>			
15-word immediate recall	28.5	23.3	>28.6
15-word delayed recall	4.4	1.2	>5.4
Story recall test	7.5	7	>7.75
<i>Non-verbal intelligence</i>			
RCPM	24.3	16	>18.96
<i>Cognitive flexibility</i>			
Phonological fluency	9.6	10.8	>17.35
<i>Visuospatial skill</i>			
Copying task	8.25	7.10	>7.3

MMSE = mini mental state examination; FAB = frontal assessment battery, RCPM = Raven's 47 colored progressive matrices. In bold are reported abnormal findings. Bold values are reported abnormal findings.

cortex excitability involving mainly GABA-A receptors [27] and further support the hypothesis of alteration of central cholinergic circuits explored by SAI.

4. Discussion

This is the first evidence in vivo of amyloid cortical burden in patients affected by NPC. Villemagne already studied in vivo protein cerebral deposition in NPC and found the presence of tau pathology but could not detect amyloid with PET imaging using ¹¹C-PiB and ¹⁸F-florbetapir as tracers. In the present study amyloid deposition in NPC patients was identified by ¹⁸F-Florbetaben PET scans. Actually ¹⁸F-Florbetaben, ¹¹C-PiB and ¹⁸F-florbetapir have comparable efficacy to detect amyloid plaques [28] therefore our findings are likely not related to tracer specificity. The evidence of cortical amyloid burden by PET imaging in our patient may be associated to their specific clinical features with severe cognitive impairment. Moreover siblings of the present report have a different mutation in the *NPC1* gene in comparison to patients of Villemagne study and we could also speculate that genetic features may influence the timing of amyloid deposition in the cortex.

In our patients amyloid burden was found in frontal areas like the early stages of AD. This may be a further element of similarity between NPC and AD. Moreover, the two patients are presenting progressive cognitive impairment despite of the treatment that is indeed helpful for visceral damage and motor disorders. Indeed, Miglustat seems to interfere with tau deposition and not with amyloid accumulation therefore cognitive preservation would not be expectable with this treatment [29].

Neurophysiological assessment also provided evidence of alterations similar to those already described in AD. Our patients showed SAI and SICI impairment consistent with motor cortex hyperexcitability and impaired sensory-motor integration confirming data reported by another group that showed consistently reduction of SAI and a trend for reduced SICI in their NPC patients [15].

These results put forward an abnormal motor cortex excitability involving mainly GABA-A receptors [27] and further support the hypothesis of alteration of central cholinergic circuits explored by SAI. Interestingly, SAI impairment scaled with cognitive deficit, indeed neuropsychological evaluation in P2 showed lower scores in all cognitive domains compared to P1. Finally our patients present TMS findings similar to those reported in AD patients [30, 31] supporting the thesis of common mechanism producing dementia.

More information on pathophysiology of neurodegeneration in NPC

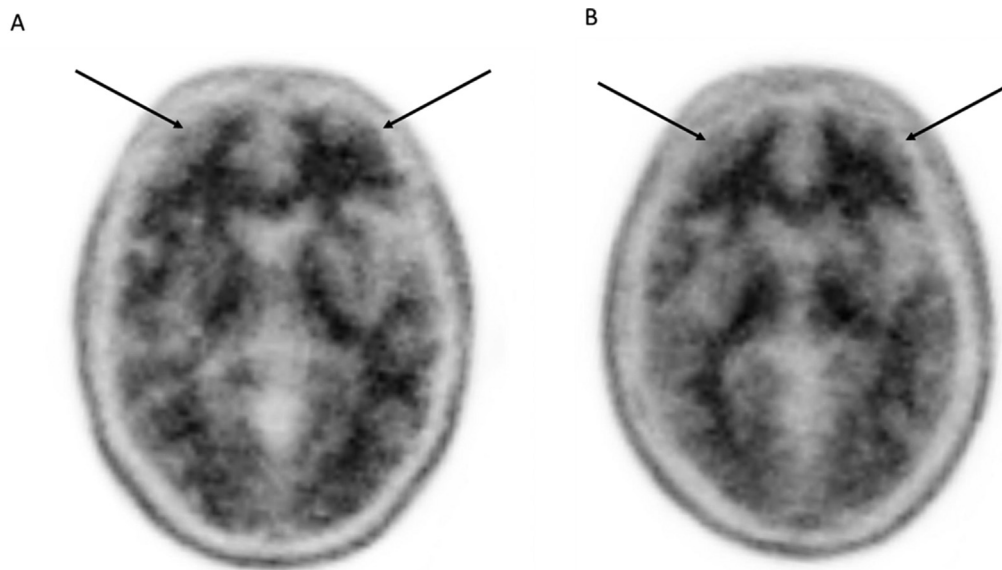


Fig. 1. Axial view of ^{18}F -Florbetaben PET scans comparing P1(A) and P2 (B); to enable a direct visual comparison, both volumes have been automatically spatially coregistered by the software Syngo.via (Siemens Healthcare). Arrows indicate cortical uptake of amyloid deposition at the frontal lobes.

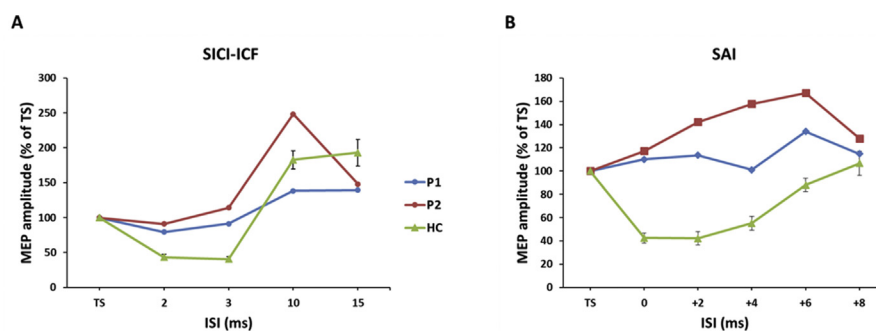


Fig. 2. Neurophysiological assessment of cortical excitability and sensorimotor integration in Niemann Pick type C (A). Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) of P1 and P2 vs healthy controls (HC) (B). Short-latency afferent inhibition (SAI) of P1 and P2 vs HC. ISI = interstimulus interval; TS = test stimulus.

could be obtained investigating our patients also with tau imaging through PET scans, unfortunately tracers for tau are not yet approved for clinical use in Italy where these patients are living. We are also aware that measuring amyloid increase in biological samples, such as cerebrospinal fluid or peripheral blood, would have added crucial information to further demonstrate amyloid loading.

A follow up ^{18}F -Florbetaben PET scan of our patients could be of interest in the next few years to study the correlation between cognitive functions and cerebral amyloid load and to better understand if amyloid deposition is not a merely consequence of a long-lasting neurodegenerative condition. These features could help to understand how neurodegeneration proceeds in NPC in comparison to AD, providing new insights for understanding the pathological mechanisms underlying both diseases.

Declarations

Author contribution statement

M. Esposito, R. Dubbioso, F. Manganelli, S. Tozza: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M. Aiello, C. Cavaliere, E. Nicolai, M. Salvatore: Performed the

experiments.

R. Iodice, L. Santoro: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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