CASE REPORT

Niemann-Pick's disease type B and brain iron accumulation

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ABSTRACT

Background: Niemann-Pick's type B (NP-B) disease is a rare, autosomal recessive visceral storage disorder related to a lysosomal accumulation of sphingomyelin, which is caused by mutations in the sphingomyelinase gene, *SMPD1*.
Case report: We present a boy who had normal early development, but from one year of age, he showed progressive manifestations of hepatosplenomegaly, somatomotor retardation and cardiopulmonary dysfunction. The activity of the sphingomyelinase enzyme was very low in his fibroblasts. He died at 17 years of age from cardio-respiratory insufficiency. Gross pathology and histology of the internal organs were compatible with Niemann-Pick's disease. His brain and spinal cord displayed no signs of storage disease, confirming the subtype of NP-B. Unexpectedly, however, significant accumulation of iron was seen in the substantia nigra, subthalamic nuclei, putamen, globus pallidus and some cortical regions accompanied by axonal spheroids. Brain iron accumulation is the hallmark of a disease group termed neurodegeneration with brain iron accumulation (NBIA). Sequencing of the known NBIA disease genes was unsuccessful in the proband's DNA isolated from formalin-fixed, paraffin-embedded blocks, but both asymptomatic parents were heterozygous carriers of the same *c19orf12* deletion.
Conclusions: This case initially raised the question as to whether two rare autosomal recessive disorders, NP-B and a subtype of NBIA could have co-occurred in our patient, or the lipid dysmetabolism due to sphingomyelinase deficiency caused secondary brain iron accumulation. Genetic analyses in the parents suggested the former possibility by identifying a *c19orf12* gene deletion known to underlie in homozygous state Mitochondrial Membrane Protein Associated Neurodegeneration.

Key Words: Niemann-Pick's type B, Lipid metabolism, Brain iron accumulation

1. BACKGROUND

In small amounts, iron is normally present in the brain with preferential distribution in the globus pallidus, substantia nigra, brainstem and dentate nuclei. A moderate increase in brain iron is noted in inflammatory and neurodegenerative disorders. A significant accumulation of brain iron was first described in Hallevorden-Spatz disease (HSD) that has been renamed to Pantothenate Kinase-Associated Neurodegeneration (PKAN), following the discovery of the mutated *PANK2* gene.[1,2] New entities of neurodegeneration with brain iron accumulation (NBIA) have subsequently been identified along with mutations in the causative genes.[3,4]

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In the Hungarian population, the most frequent pathogenic NBIA-causing mutations have been detected in the *c19orf12* gene underlying Mitochondrial Membrane Protein Associated Neurodegeneration (MPAN), followed by *PANK2* and *PLA2G6* mutations underlying PKAN and phospholipase A - related neurodegeneration (PLAN), respectively.[5] While most affected NBIA genes result in abnormalities in mitochondrial lipid metabolism, the question of how this biochemical pathway alterations relate to brain iron accumulation is still debated.[4] Here we present a case of NP-B characterized by severe deficiency in the sphingomyelinase enzyme activity, hepatosplenomegaly and pulmonary fibrosis, without clinical signs of neurological impairment,[6] but with marked accumulation of iron in the autopsied brain. The question arose whether this significant cerebral iron deposition was secondary to the lipid dysmetabolism involving sphingomyeline and ceramide in NP-B, or it was rather part of a second rare disease caused by an inherited NBIA gene mutation. To our knowledge, iron accumulation in the brain has not been reported in NP-B.[6]

2. CASE REPORT

2.1 Clinical course

The proband was a Caucasian boy born in 1977 to healthy, Hungarian parents who were distantly related (their great grandmothers were sisters). He had normal birth, an uneventful perinatal period and normal early development, but hepatosplenomegaly and somatomotor retardation were noted at one year of age, raising the suspicion for Niemann-Pick's disease. Bone marrow and skin biopsies were taken in 1984. The analyses of sphingomyelinase in the fibroblasts showed extremely low enzyme activity (0.033 μmol/min/g protein). There were no clinical signs of central nervous system involvement. The clinical features and decreased sphingomyelinase activity suggested the diagnosis of NP-B. Over time, the patient developed severe lung fibrosis with progressive cardio - respiratory difficulties, which required repeated hospitalization, cardio-pulmonary support and antibiotic treatment. In 1989, he fell off the steps of a bus, hit his head and lost consciousness due to a subdural hematoma with occipital fracture, cerebral contusion and liquorrhea. Craniotomy, hematoma evacuation and osteoplasty were performed with a good subsequent recovery. He was evaluated for liver transplantation in 1991, but did not qualify for it due to the severe pulmonary fibrosis. In 1994, the patient developed cardio-respiratory failure and passed away in the hospital.

The proband's archived clinico-pathological documentation was brought to our attention by his sister in the spring of 2016. She was born in 1979, clinically healthy and bearing her first pregnancy at the time. She came to us seeking counseling because of her sibling's (proband) NP-B disease. That prompted us to reinvestigate and see in a new perspective the proband's histopathological documentation from 1994, as summarized below.

2.2 Gross pathology

At gross pathological examination the proband's corpse was small with very thin extremities, while the abdomen significantly emanated out of the thoracic level. The heart had hypertrophic muscles and displayed dilatation of the atria as well as of the right ventricule. The lungs appeared with diffusely dense consistence and grayish-brownish color on the sectioned surfaces. Scattered underneath the pleura, numerous 0.5 cm - 1 cm fibrotic foci with cartilaginous texture were detected. The spleen weighed 630 g (much beyond that of an age-matched healthy control), looked "sugar-coated" and its normal follicular structure was unrecognizable on the sectioned surfaces. The liver weighed 1,650 g (also much too heavy for the patient's age) and was described with uneven surface and hardened consistence. The sectioned surfaces were grayish-brownish. The other internal organs appeared grossly normal.

The brain weighed 1,290 g. The sulci were narrow, the gyri were wide and flat, and the cerebellar tonsils bore signs of herniation. Remains of the old contusion were seen in the left frontobasal, temporopolar and occipital regions. The meninges were hyperemic and the blood vessels appeared normal. On coronal sections, the ventricules appeared narrow. The pallidum and caudal nuclei were notable for a dark brownish discoloration compared to the adjacent and cortical gray matter (see Figure 1A). The spinal cord appeared normal.

Gross pathological diagnosis: Bronchopneumonia, pulmonary fibrosis, hepato-splenomegaly, Niemann's-Pick disease.

2.3 Histopathology

The histopathological examination revealed high cellularity in the bone marrow. The spleen was infiltrated by cells with granulation, swollen cytoplasm and scrambled cell membrane, which replaced the normal follicular structure (see Figure 1B). A similar infiltration confounded the original structure of the liver. Iron accumulation was not detected in the liver and spleen. The histological appearance of the heart, pancreas, and the suprarenal and thyroid glands were normal. The kidneys were swollen. The enlarged lymph nodes were infiltrated by histiocytes. In the edematous lungs, severe fibrosis was seen throughout, and the alveoli were infiltrated by macrophages filled with fine granular material.

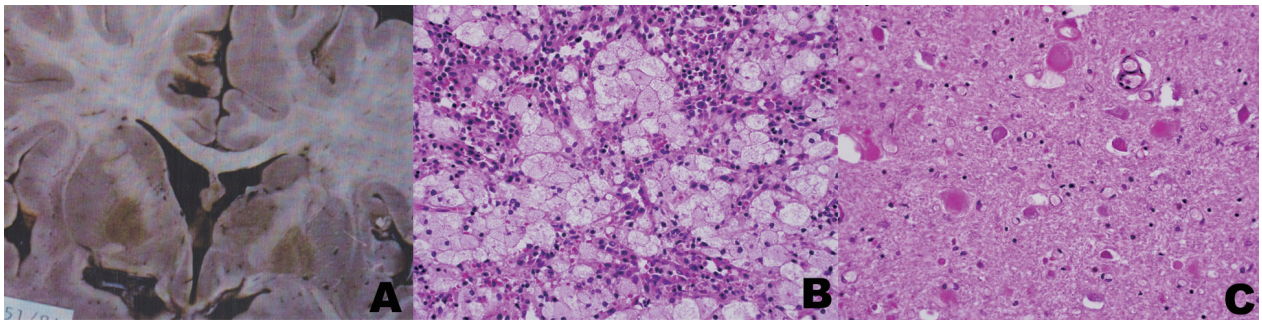

Figure 1. Iron accumulation in the brain associated with a visceral storage disorder

Figure 1A features a coronal brain section with symmetric distribution of parenchymal and perivascular brownish discoloration caused by iron predominantly within the globus pallidus, less strikingly in the caudate nuclei, thalami, external capsule and cortical regions. Figure 1B shows macrophages swollen by stored material in the spleen. Hematoxylin-Eosin staining. Figure 1C illustrates neuroaxonal dystrophy with spheroids in the brain. Hematoxylin-Eosin staining.

The neurohistological examination was carried out on sections from formalin-fixed and paraffin-embedded (FFPE) blocks, and involved the frontal lobes from the cerebral hemispheres, and the cerebellum, brainstem, basal ganglia and segments of the spinal cord. No signs of a storage disorder were detected in the cells of brain or spinal cord. However, severe extracellular and intracellular pigment deposition corresponding to iron, and axonal spheroids were noted bilaterally in a symmetric distribution within the substantia nigra, subthalamic nuclei, thalami, nuclei of putamen and globus pallidus, caudates and some cortical regions. A great proportion of Purkinje cells were lost (see Figure 1C). Myelin staining revealed no abnormality.

Histological diagnosis: neuroaxonal dystrophy (Hallevorden-Spatz disease) in combination with Niemann-Pick's disease.

2.4 Genetics

Molecular genetic studies were performed within the *PANK2, C19orf12, PLA2G6, WDR45* and *Coasy* genes in 2016 at the Center for Genomic Medicine and Rare Diseases, Semmelweis University, Budapest. Unfortunately, even multiple sequencing attempts yielded no results in these NBIA genes of the proband due to the poor quality of the only available DNA from the old FFPE blocks. Therefore, we have the above genes sequenced in the DNA specimens of the asymptomatic live parents, and learnt that they are both heterozygous carriers of the same deletion (c. 204_214del11) in exon 3 of the *c19orf12* gene. This deletion results in an early stop codon (p. Gly69Arg fsTer10) and has been previously reported.[7–9] Brain magnetic resonance imaging (MRI) of the parents was subsequently ordered on a 3 Tesla Philips scanner, which revealed increased iron in the globus pallidus on T2-Fast Field Echo (FFE) scans in the 62 years old mother's but not in the 64 years old father's brain (see Figure 2).

3. Conclusions

The most striking feature of this case involves typical presentation of NP-B in combination with an unusual accumulation of iron in the brain, without clinical signs of neurological involvement. The question arose as to whether the patient had two diseases, namely NP-B and one of the NBIAs, or the brain iron accumulation was related to the biochemical abnormality underlying NP-B. A third possibility, namely that an NBIA caused a NP-B-like clinicopathological manifestation was also considered, but found the least likely. As the prevalence of both NP-B and MPAN is 1-9/10^6 (`http://www.orpha.net/consor/cgi-bin/OC_Exp.php?Expert=77293`, `http://www.orpha.net/consor/cgi-bin/OC_Exp.php?Expert=385`), the co-occurrence of these entities must be exceptionally rare and to our knowledge has not been reported.

NBIA is a heterogeneous group of genetically inherited diseases typically presenting before five years of age with signs of movement disorders (dystonia, chorea, tremor, tics), corticospinal involvement (spasticity, paresis), ataxia, speech abnormalities, cognitive and behavioral problems, and in some forms also with optic neuropathy and peripheral neuropathy. Atypical clinical presentation with slower progression and less severe neurological impairment usually occurs in later years, when parkinsonism, dystonia, psychiatric and behavioral problems may predominantly cause disability. Most (but not all) of these disorders follow autosomal recessive inheritance. Globally, PKAN is the most common subtype, representing 50% of all NBIAs, but it is only the second most frequent subtype after MPAN in Hungary. The list of NBIAs is continuously expanding. Besides PKAN caused by mutations in the *PANK2* gene,[1,2] the best characterized subtypes include MPAN with mutations/deletions in the *c19orf12* gene,[7–9] the PLA2G6 Associated Neurodegeneration (PLAN) with mutations in the *PLA2G6* gene,[10] the

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Coasy Protein Associated Neurodegeneration (CoPAN) with mutations in the *Coasy* gene,[11] and the Beta-Propeller Protein Associated Neurodegeneration (BPAN) with mutations in the *WDR45* gene.[12] A shared feature of these diseases is brain iron accumulation that is likely secondary to abnormalities in lipid metabolism and autophagy.[3,4]

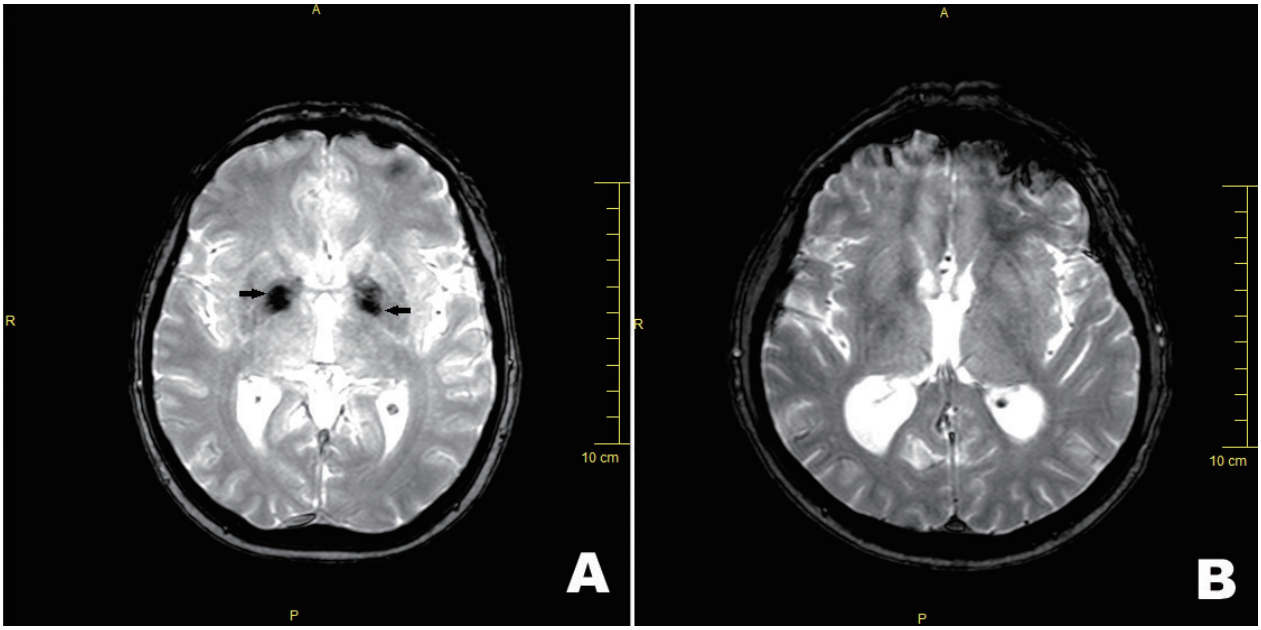

Figure 2. Iron accumulation in the brain of the proband's mother but not of the father, who were both heterozygous carriers of the same c19orf12 gene deletion

Figure 2A and B are T2 FFE (corresponding to T2) weighted axial brain images taken by a 3 Tesla Philips scanner. Figure 2A depicts the mother's brain with marked iron accumulation (arrows) in the globus pallidus, while Figure 2B shows no iron accumulation in the father's brain.*

When we reopened the old documentation of our case in 2016 and noted the co-occurrence of NP-B and iron in the patient's brain, first we considered the biochemical link between lipid dysmetabolism and cerebral iron deposition, since the co-occurrence of the two very rare diseases (NP-B and an NBIA) appeared less likely. This biochemical link has been well described (though only partially understood) in NBIAs. In PKAN, *PANK2* mutations cause defective pantothenate kinase activity, which negatively affect the phosphorylation of pantothenate in the first step of the acetyl-coenzyme A (CoA) synthesis. Low CoA levels lead to abnormalities in the complex pathway of mitochondrial lipid metabolism and eventually also affect levels of ceramide. Similarly, decreased CoA and ceramide levels are features of CoPAN, another NBIA, caused by mutations in the coenzyme A synthase gene (*Coasy*).[3,4]

In addition to the downstream lipid metabolism pathway exemplified in PKAN and CoPAN, ceramide may also derive from membrane-bound sphingomyelin by hydrolysis, or from *de novo* synthesis from palmytoyl CoA and serine.[3] Sphingomyelin is a cell membrane lipid that is directed to the lysosomal compartment for degradation by sphingomyelin-phosphodiestherase 1 (SMPD-1) to form ceramide. In NP-B, inactivating mutations in the *SMPD-1* gene cause decreased activity of the sphingomyelinase, leading to the accumulation of sphingomyelin and reduced amount of ceramide in various organs, as seen in our case. This biochemical pathway is also affected in PLAN that is caused by inactivating mutations in the *PLA2G6* gene. Altogether, ceramide is likely a shared biochemical crossing point where several NBIA disorders (PKAN, CoPAN, PLAN and MPAN) and NP-B may converge.[3] The relationship between metabolic disorders and iron accumulation is not only suggested in NBIA, but also in Gauchet's disease related to deficiency of the lysosomal glucocerebrosidase, which is associated with hyperferritinemia and peripheral (e.g. liver) iron overload.[13] Expanded data mining and ontological analyses establish even a broader inter-relationship between abnormalities in myelin-related, lipid rich structures and iron.[4,14] At first sight, these observations prompted us to postulate that iron deposition in the brain might have been secondary to sphingomyelin dysmetabolism in our case, even though such a consequence of NP-B has not previously been reported.

The detection of excessive brain iron accumulation during au-

topsy of the neurologically unaffected but viscerally severely compromised proband with NP-B in 1994 preceded the gene discovery and renaming of HSD to PKAN[1,2] and also of the newer subtypes of NBIA. Most NBIAs (and particularly those ones discussed here) are not associated with visceral diseases such as hepatosplenomegaly, cardiomyopathy or lung fibrosis seen in our patient with biochemically supported NP-B. The pathology of most NBIAs such as PKAN and MPAN is restricted to the central and peripheral nervous system, and involves neuronal and neuroaxonal loss with spheroids, astrogliosis and significant accumulation of (both intra and extracellular) iron in the basal ganglia, particularly in the globus pallidus and the brainstem. While prominent ubiquitin deposition and neurofibrillary tangles may be seen, only faint tau staining has been reported in PKAN.[15,16] In contrast, α-synuclein/Lewy bodies and tau are part of the pathology in MPAN.[16] In regards to axonal spheroids and neuronal loss, astrogliosis and iron deposition, the cerebral pathology of our case shared similarities with those of PKAN and MPAN, but without the clinical phenotype of either one.

Therefore, when in 2016 we reopened the archived case with NP-B and iron in the brain, we were compelled to test the second potential explanation of the dual-pathology, mutations in the recently discovered NBIA-related genes. However, only FFPE blocks were available from the patient for DNA isolation and candidate gene sequencing that resulted in no outcome. Fortunately, analyses of the buffy coat – derived DNA revealed an identical heterozygous deletion in the *c19orf12* gene of the neurologically asymptomatic live parents, which suggested that the proband also might have been carrier of this deletion, although without clinical symptoms of MPAN. Thus, the diagnosis of NP-B was established based on the clinical presentation, pathological findings and the severe reduction of the sphingomyelinase activity in the patient, while the genetic cause of the increased brain iron, the *c19orf12* deletion could only be inferred from his parents' genetic status, but without the determination of whether the deletion was homozygous or heterozygous in the proband. This latter question became even more intriguing, when after the genetic study we have the parents' brain-MRI taken and learnt that the mother (but not the father) had significantly increased iron in the globus pallidus on the T2 FFE (corresponding to T2*) scans (see Figure 2). This observation raised the possibility that occasionally brain iron accumulation may be detected in heterozygous (clinically asymptomatic) carriers of the *MPAN* gene. Thus our patient, who died at 17 years of age, could have been not only a homozygous carrier of the *c19orf12* deletion with a preclinical presentation, but also a heterozygous carrier with incomplete phenotype of MPAN.

Finally, based on the genetic finding, we also considered a third possibility to explain the co-occurrence of NP-B and MPAN in our patient, namely that the *c19orf12* deletion(s) could have led to a phenotype mimicking NP-B. This potential explanation would put a single genetic cause to account for all the pathological findings. Unfortunately, this possibility could not be tested in the patient's little remaining post mortem material, and overall this interpretation appeared to us the least likely too, as the visceral storage disorder and the extremely low sphingomyelinase enzyme activity detected in our patient, are not reported features of MPAN. Nevertheless, determination of the full phenotypic spectrum associated with *c19orf12* deletion(s) merits further studies.

In summary, the case presented here is of interest because of the co-occurrence of two very rare autosomal recessive disorders, NP-B and MPAN, an observation compatible with the consanguineous marriage of the parents. While the lipid dysmetabolism in NP-B itself could theoretically be considered as a cause for brain iron accumulation, no such biochemical link between sphingomyelinase deficiency and brain iron accumulation has thus far been reported. *Vice versa*, no report available to support that MPAN may mimic biochemical and clinico-pathological features of NP-B. The detection of severe sphingomyelinase activity loss causing NP-B and the accumulation of iron in the brain of the proband along with the identification of a *c19orf12* heterozygous deletion in both clinically asymptomatic parents point to two independent genetic causes underlying the simultaneous occurrence of NP-B and preclinical or incomplete MPAN in our patient.

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CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

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