Niemann-Pick Disease Type C: New Aspects in a Long Published Family – Partial Manifestations in Heterozygotes

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Abstract Decades ago, a family with three children with a neurovisceral lysosomal storage disease was described. The patient siblings died at ages 7, 9, and 11 years, respectively, and according to the current concept had the late-infantile neurologic form of Niemann-Pick type C1 (NPC) disease, given by the present molecular study that there were severe *NPC1* gene variants: Blood samples preserved since that time from one patient sibling and his presently 55-year-old essentially healthy sister have now been studied, revealing the variants p.I1061T and p.G1162V in the *NPC1* gene, the first long known, the second newly found but predicted to be pathogenic and similar to the known G1162A. Now, with the molecular diagnosis, that initial description warrants new interest for the following reasons. The mentioned sister carries only the I1061T variant. She had storage macrophages (“Niemann-Pick cells”) in her bone marrow, but also displayed distinct splenomegaly with indurated consistency of the organ, proven in childhood and confirmed several times up to age 13, but disappeared at age 55 years. She shares the I1061T variant with her still healthy mother, and the bone marrow finding with both parents, her father having died at 66 years from a carcinoma. The present study is one of the first describing hematological and relevant clinical symptomatology, even

in heterozygotes, of molecularly diagnosed human NPC. Feline NPC is known to model such a situation. For human diagnostic and clinical NPC management, the possibility of “heterozygous disease” should be kept in mind.

Abbreviations

BMP	bis(monoacylglycero)phosphate
NPC	Niemann-Pick type C disease
NPC1 and NPC2	subtypes of NPC, and related proteins

Introduction

Niemann-Pick disease type C (NPC; OMIM 257220; OMIM 607625) is a neurovisceral lipidosis resulting from a generalized lipid trafficking defect, with lysosomal accumulation of cholesterol, sphingomyelin, glycolipids, and other lipids; defects in the *NPC1* (about 95 % of patients have NPC1 disease) or *NPC2* (5 % NPC2 disease) genes that encode the multifunctional membrane and/or transport proteins, NPC1 and NPC2, are responsible (for reviews, see Vanier 2010; Patterson et al. 2013; Stampfer et al. 2013). According to Vanier (2010), the degree of neurological involvement defines disease severity in most patients but may be preceded by cholestatic jaundice in the neonatal period, and spleno- or hepatosplenomegaly in infancy or childhood. Neurologically, single or combined signs in the following spectrum may be observed, also depending on the age of clinical onset: delay in developmental milestones, gait problems, clumsiness, cataplexy, seizures, dystonia, school problems, supranuclear gaze palsy, dysarthria, dysphagia, (cerebellar) ataxia, initial psychiatric problems, or progressive dementia. According

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to Patterson et al. (2013), about two-thirds of patients have infantile or juvenile onset of neurological manifestations, while one third present in adolescence or adulthood. The most frequent neurological signs, through all onset groups and in decreasing order, are ataxia, vertical supranuclear gaze paresis, dysarthria, cognitive impairment, and dysphagia. The laboratory diagnosis of NPC is usually preceded by the nonspecific, though usually helpful, screening for increased plasma chitotriosidase activity, and then made by cytochemical demonstration of lysosomally accumulated free cholesterol in skin fibroblast cultures using the staining method with filipin (Vanier 2010) and by genetic testing. However, the combined methods also leave some patients without a certain laboratory diagnosis.

In 1972, a family with “infantile-juvenile, slowly progressive lipidosis belonging to the sphingomyelinoses (Niemann-Pick – a new type?)” was described (Wiedemann et al. 1972). There were four siblings, three of whom died at ages ranging from 7 to 11 years from a severe neurodegenerative disease. Clinical signs included prolonged neonatal jaundice, early postnatal splenomegaly, foam cells in the bone marrow, and many of the above-mentioned neurological symptoms. Wiedemann et al. (1972) hesitated to classify their patients as “Crocker’s group D” or juvenile/subacute Niemann-Pick disease, but they spoke of some similarity of the patients with these groups. In fact, Crocker’s groups C and D and juvenile/subacute or dystonic Niemann-Pick disease (for review, see Frank and Lasson 1985) were defined at that time by criteria that are rather congruent, still, with a large portion of the spectrum of clinical symptoms recorded today in NPC patients diagnosed by specific laboratory methods. Wiedemann et al. (1972) could possibly have assigned their patients to those long-defined groups that are rather equivalent to NPC if they had regarded, e.g., the vertical gaze palsy which was certainly present (see below), though not described in their patients. In the early 1970s, there were already many reports describing the association of typical eye movement problems, defined as vertical gaze paresis or supranuclear ophthalmoplegia, with the long-defined NPC equivalents. These equivalents were partially substituted somewhat later for another, relatively specific NPC equivalent, termed “ophthalmoplegic neuropilidosis” (for review, see Frank and Lasson 1985).

In this study, the diagnosis of NPC was confirmed in that family (Wiedemann et al. 1972) by the demonstration of one long known and one newly recognized point mutation on either allele of the *NPC1* gene (NPC1 disease). This was made possible by the availability of blood samples frozen for 40 years from one patient and his sister, and by generous help, including an additional blood sample and clarifying information provided by family members who are still alive.

With the diagnosis known, the report by Wiedemann et al. (1972) and also that by Seng et al. (1971) deserve renewed interest for the following features: (i) This is one of the first genetically proven NPC families in which heterozygous carriers were unambiguously found to have clinical and/or hematological symptoms of the NPC spectrum. (ii) The reports have very early added to the first description of the accumulation of a key lysosomal lipid, bis(monoacylglycerol)phosphate (BMP), in a clearly diagnosed lysosomal storage disorder (Niemann-Pick disease type A) (Rouser et al. 1968), a second description of such an accumulation. Although Seng et al. (1971) and Wiedemann et al. (1972) were unsure about the disease subtype, they spoke of Niemann-Pick disease with BMP accumulation in that family and prepared the way to the present molecular definition of NPC, subgroup NPC1, for those patients. Very recently, the anionic phospholipid BMP has attracted attention for its indispensable role in lysosomal lipid homeostasis (Sandhoff and Harzer 2013) and for its marker properties in lysosomal affections including NPC disease (Scherer and Schmitz 2011). (iii) Wiedemann et al. (1972) underscored the great clinical variability in NPC disease when they described the almost directly postnatal appearance of splenomegaly versus the only juvenile manifestation of severe neurologic symptoms and the increased, instead of the usually reported reduced, growth in body length in their patients.

Case Report

The sister of the three described siblings with NPC disease (Wiedemann et al. 1972) (see pedigree in Fig. 1) is now 55 years old, essentially healthy, practicing sport and works as a hospital nurse. She was referred to as Case 1 in the original paper and is II/1 (Fig. 1) in this study. One (II/3) of the diseased siblings (II/2 to 4) was originally described as Case 3. In II/1 (sister), an enlarged spleen was noticed at the age of 8 years and was confirmed 1, 4, and 5 years later to be enlarged by 3–4 cm below the costal margin and of indurated consistency (Wiedemann et al. 1972); recently, at age 55 years, the spleen had a size of 10.7×5.9 cm on sonography, confirmed to be normal by the examiner. In the smear of her bone marrow studied when she was 6 years old, there were a number of foamy storage cells (Fig. 2). Patient II/3 (Fig. 1) was first remarkable by splenomegaly and slight hepatomegaly at the age of 6 weeks. When this boy was 3 months old he was found to have large numbers of foam cells in his bone marrow (Gerken and Wiedemann 1964), and with 16 months he had thrombocytopenia with lower leg subcutaneous bleedings (Wiedemann et al. 1972). It should be noted that the bone marrow foam cells were depicted at the time (Gerken and Wiedemann 1964) when

Fig. 1 Pedigree of the Niemann-Pick disease type C (NPC) family. Family members in generation I, 1 and 2, and II, 1 to 4. Squares, male; circles, female. Black/white, heterozygous; diagonal line, died;

black, NPC siblings. Letter **b.**, year of birth; **d.**, year of death. Molecular study was in I/2 (*NPC1* p.I1061T/wildtype), II/1 (same genotype) and II/3 (I1061T/G1162V)

the diagnosis in this family was erroneously suggested to be Gaucher disease, but, in fact, these storage cells resemble storage macrophages of the “Niemann-Pick type” (Fig. 2). Patient II/3 started at the age of 2 years a very insidious neurological downhill course which slowly progressed, including epileptic and cataplexic fits (for details, see Wiedemann et al. 1972), and led to a fatal end at the age of 11 years. Importantly, different caregivers who were later found and interviewed by the sister lady (II/1) clearly recalled the distinct problems the boy had when he tried to look upward and was unable to do so, but tried to compensate by extreme flexing of the head into the neck (vertical gaze palsy). The histories of patient siblings II/2 and II/4 were similar but with some variations (Wiedemann et al. 1972); II/2 died at age 9 and II/4 at 7 years (see Fig. 1). The mother, I/2, is now 78 years old and healthy. Her spleen was recently observed as being of normal size of 11.2×3.9 cm on sonography. But when she was 28 years old, a number of foam cells were found in her bone marrow (Fig. 2). The father (I/1) had similar foam cells at the age of 29 years (Fig. 2); his daughter (II/1) recently reported that he had suffered from myasthenia gravis with the inability to lift the upper eyelids, but the symptoms improved upon mestinon administration and thymectomy, and that he died from a lung carcinoma at the age of 66 years. No splenomegaly was reported for him.

Genetic Testing

Leukocyte pellets from peripheral blood were available from II/1 and II/3 (Fig. 1), the samples of which had been frozen in the laboratory of one of the authors (K.H.) for 40 years at -20°C . A blood sample was also taken recently from the mother (I/2) with informed consent. Blood leukocytes were isolated using standard procedures. Genomic DNA was

isolated from the leukocyte pellets using the DNA isolation Kit from Roche®. Molecular genetic analysis of the 25 coding exons of *NPC1* and the flanking intronic regions was performed by Sanger sequencing. PCR conditions and primer sequences are available on request.

Results and Discussion

Forty-nine years after the first report (Gerken and Wiedemann 1964), and 41 years after the comprehensive, nosologically more adequate description (Wiedemann et al. 1972) of three siblings with a neurovisceral lysosomal storage disease, the final confirmation of the diagnosis was achieved. However, not only those careful clinical, morphological, and biochemical descriptions but also the excellent lipid chemical study on the brain of patient II/2 (Fig. 1) by Tjiong et al. (1973) have long been highly suggestive of classical NPC disease. In fact, genetic testing of patient II/3 revealed the most frequent mutation in the *NPC1* gene (Millat et al. 1999; Vanier 2010), p.I1061T (c.3182T>C), and an as yet not described mutation, p.G1162V (c.3485G>T). Both the sister (II/1 in Fig. 1) and her mother (I/2) were found to carry only I1061T and no other serious *NPC1* variants. This constellation assigned the mutations in patient II/3 to different alleles. The pathogenicity of G1162V was predicted ex silico and confirmed by practical experience (Heiko Runz, Heidelberg, personal communication, 2012). This mutation is similar to G1162A (c.3485G>A), known to be pathogenic (Stampfer et al. 2013).

The heterozygous NPC carriers, II/1 and I/2, and the father I/1 (Fig. 1) of II/1 and II/3, were remarkable for the findings of foamy storage cells in their bone marrow smears (Gerken and Wiedemann 1964; see Fig. 2). These bone marrow findings certainly repeat very similar, much earlier observations in heterozygotes of “ophthalmoplegic neuro-

lipidosis” (Frank and Lasson 1985); this archaic term being a very likely equivalent for NPC disease. Still, the present family is only the second in which such findings in heterozygotes can be attributed to a demonstrable pathogenic *NPC1* gene variant, i.e., I1061T in I/2 and II/1, and G1162V, presumably, in the deceased father I/1. The first report was on a family with four NPC patients in which the patients’ father had been known to have storage cells in the bone marrow (Ceuterick et al. 1986) – and also lysosomal storage inclusions in his skin biopsy – and who was studied molecularly many years later with the result of his *NPC1* heterozygous I1061T genotype (Millat et al. 1999).

Even more interesting, the present sister lady II/1 is remarkable for her distinct splenomegaly observed at age 8 and confirmed three times up to age 13 years, with an indurated consistency of the organ (Wiedemann et al. 1972), but at age 55 years no longer provable. This is one of the first observations of a relevant clinical symptom attributable to a known pathogenic *NPC1* variant (*NPC1*[−]), but in combination with a wildtype allele (*NPC1*⁺). Possible explanations remain speculative: Is there a gain of pathologic function by the mutant *NPC1*[−] protein? Are there influences of the total genetic background or certain epigenetic constellations? Is there still a third mutant allele in this “heterozygote” and her family, given that sensitivity of *NPC1* sequencing does not reach 100%? Might an imbalance in *NPC1*[−] and *NPC1*⁺ expression in different cells and tissues play a role? An observation of a neurological picture in an NPC heterozygote was reported by Josephs et al. (2004). They described a 75-year-old woman with continuous tremor of the head and limbs unresponsive to L-dopa/carbidopa who carried the pathogenic p.G992A (c.2974G>C) variant (Sévin et al. 2007), but her brothers with childhood onset NPC, upon examination many years before, had no tremor except when they were in stressful situations.

The present observations correlate well with a feature long known for cytochemical NPC diagnostics: In filipin-testing of skin fibroblasts cultured from NPC heterozygotes, a partial filipin positivity, i.e., NPC-typical cellular cholesterol accumulation, is not infrequently found (Vanier 2010). As to animal models, feline NPC has been described with clinical, cytological, and cytochemical disease signs in heterozygotes (Brown et al. 1994 and 1996).

Fig. 2 Foamy storage macrophages (“Niemann-Pick cells”) in bone marrow smears from heterozygotes with Niemann-Pick disease type C and a patient. Pappenheim staining; the largest diameters of the storage cells are 25 to 50 μm . (a–c) heterozygotes: (a) father, I/1, of II/1 (see Fig. 1); (b) mother, I/2; (c) II/1 (note the nucleus at the right edge of the light storage cells but beneath the dark lymphocyte); (d) a 10-year-old patient with late-infantile neurologic Niemann-Pick disease type C (independent observation). a–c are reprinted from Gerken and Wiedemann (1964), with consent of the Schweizerische Gesellschaft für Pädiatrie, and S. Karger, Basel

For the future, it should be kept in mind that even heterozygotes of NPC, at least NPC1 disease, can in some instances express not only bone marrow cytological or fibroblast-cytochemical signs typical of NPC, but can sometimes even develop relevant clinical symptoms such as splenomegaly (Wiedemann et al. 1972) and possibly tremor (Josephs et al. 2004). The generally accepted “recessive inheritance” of NPC, therefore, gains a new aspect.

The report on the NPC family by Wiedemann et al. (1972), with the genetic diagnosis in hand, also deserves renewed attention with respect to the achieved confirmation of BMP as a very essential lysosomal lipid (see [Introduction](#)).

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Synopsis

Molecularly proven human heterozygous carriers of Niemann-Pick disease type C can develop relevant clinical and/or hematological (bone marrow storage cell) symptoms.

Compliance with Ethics Guidelines

Conflict of Interest: Klaus Harzer, Stefanie Beck-Wödl, and Peter Bauer declare that they have no conflict of interest.

Informed Consent: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

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