Distinct Niemann-Pick Disease Type C Clinical, Cytological, and Biochemical Phenotype in an Adult Patient With 1 Mutated, Overexpressed *NPC1* Allele

Julia Jecel, MD[1], Klaus Harzer, MD[2], Eduard Paschke, MD[3], Stefanie Beck-Wödl, PhD[4], Peter Bauer, MD[4], Milos Hejtman, MD[5], and Regina Katzenschlager, MD[1]

Abstract

Niemann-Pick disease type C (NP-C) is a rare autosomal-recessive neurovisceral lysosomal storage disease. We report on a juvenile onset, now 25-year-old female patient with typical neurologic symptoms, including vertical gaze palsy, of NP-C. The diagnosis was supported by a positive filipin test ("variant biochemical phenotype" of cholesterol accumulation) in cultured fibroblasts, high numbers of "Niemann-Pick cells" in the bone marrow, and 1 positive out of 3 NP-C biomarkers tested, but NP-C was not definitely confirmed genetically. She showed only 1 known *NPC1* variant (3 bp deletion in exon 18; p.N916del); this allele, however, being distinctly overexpressed at the messenger RNA level as compared to the wild-type allele, as a not as yet clarified (copathogenic?) phenomenon. The patient's mother, also carrying the p.N916del allele but without overexpression, has a chronic inflammatory disease of the central nervous system classified as multiple sclerosis. However, her severe clinical phenotype includes some signs also consistent with NP-C. The laboratory diagnosis of NP-C can be challenging in detecting novel disease constellations.

Introduction

Niemann-Pick disease type C (NP-C) is a rare neurovisceral lysosomal lipid storage disorder caused by mutations of either the *NPC1* (95% of cases) or the *NPC2* gene, both of which encode proteins involved in the late endosome-/lysosome-mediated cellular cholesterol transport. Clinical presentation is extremely heterogeneous, ranging from a severe, rapidly fatal systemic disease with neonatal onset to a chronic neurodegenerative disorder in adult age. Initial neurological symptoms vary with age of onset and may be preceded by systemic signs such as neonatal cholestatic jaundice or isolated spleno- or hepatosplenomegaly.[1,2] Characteristic neurological manifestations include saccadic eye movement abnormalities or vertical supranuclear gaze palsy (VSGP), cerebellar signs (ataxia, dysmetria, dysarthria, and dysphagia), dystonia, and gelastic cataplexy (loss of nuchal muscle tone induced by laughing) as well as epileptic seizures.[3,4] Psychiatric disturbances and cognitive troubles may be the first disease signs in adults.[5] While suspecting NP-C is relatively straightforward in patients with typical clinical symptoms, confirmation of the diagnosis can be challenging. Biochemical testing as well as genetic testing for NP-C are

[1] Department of Neurology and Karl Landsteiner Insitute for neuroimmunological and neurodegenerative disorders, Danube Hospital/Donauspital, Langobardenstr. 122, 1220 Vienna, Austria
[2] Neurometabolic Laboratory, Klinik für Kinder-und Jugendmedizin, University of Tübingen, Hoppe-Seyler-Str.1, 72076 Tübingen, Germany
[3] Department of Pediatrics, University Hospital Graz, Auenbruggerplatz 34, 8036 Graz, Austria
[4] Department of Medical Genetics, University Hospital Tübingen, Calwerstr. 7, 72076 Tübingen, Germany
[5] Department of Laboratory Medicine and Hematology, Danube Hospital/Donauspital, Langobardenstr. 122, 1220 Vienna, Austria

Corresponding Author:
Klaus Harzer, Neurometabolic Laboratory, Klinik für Kinder-und Jugendmedizin, University of Tübingen, Hoppe-Seyler-Str.1, 72076 Tübingen, Germany.
Email: harzer-rottenburg@t-online.de

complex and can be difficult to interpret due to a variety of methodological factors.[3]

Biochemical testing includes the demonstration of an impaired intracellular cholesterol transport by filipin staining of cultured skin fibroblasts. NP-C fibroblasts typically reveal strongly fluorescent (cholesterol storing) perinuclear vesicles. Cholesterol storage in high numbers of such lysosomal vesicles, referred to as "classical biochemical phenotype," can be observed in 80% to 85% of cases. Lower, still variable numbers of cholesterol-positive vesicles are seen in the remaining 15% to 20% of patients and referred to as "variant biochemical phenotype."[1,3,6,7] These attenuated storage patterns cannot clearly be distinguished from the mildly abnormal patterns found in NP-C heterozygotes or patients with sphingomyelinase deficiencies.[1,8,9] Moreover, filipin staining patterns can be apparently negative in about 3% to 5% of patients with NP-C.[1]

Genetic testing, as a rule, is mandatory in any suspected NP-C case and employs an arsenal of methods. Complete sequencing of all exons and adjacent intronic regions of the *NPC1* gene (chromosome 18, q11-q12, 25 exons) and/or *NPC2* gene (chromosome 14;q24.3, 5 exons) may be required. In complex cases, combined studies of genomic DNA/RNA and sequencing of promoters and deeper intronic regions may be added. Furthermore, exonic deletions may be identified by multiplex ligation-dependent probe amplification (MLPA).[1,3] In some patients, mutations could be identified in only 1 allele, and in a few patients no mutation was found at all.[1] Inconclusive genetic findings do not exclude a possible diagnosis of NP-C.[3] On the other hand, genetically full-tested NP-C heterozygotes can show some clinical and/or hematologic manifestations.[10]

Macrophagic involvement in NP-C (with similarity to other Niemann-Pick types or storage diseases) may become evident by the demonstration of storage macrophages in different tissues, including bone marrow. Storage cells seen in bone marrow smears can usually be classified according to cytologic criteria, for example as "Gaucher cells," "Niemann-Pick cells," or other storage cells, but the differentiation is not always easy. Typically, Niemann-Pick cells show a light, foamy, vacuolated cytoplasm, and these "foam cells" may have one or more, often shrunken, nuclei. During prolonged disease courses, the cytoplasm may not only display vacuoles but also "sea-blue" or dark granules (on the usual stains), and late stages of these storage cells appear as "sea-blue histiocytes," which have lost the vacuoles but are filled with blue or dark sometimes coarse granules containing ceroid pigment. In unclear NP-C cases, the demonstration of storage cells in bone marrow smears can be an essential diagnostic criterion.[3,11,12] Bone marrow infiltration with foam cells may reflect the disease burden, but cytologic findings may be not always distinct in patients with early[3,12] or attenuated systemic disease.

The use of so-called biomarkers in approaching a NP-C diagnosis has some importance: Plasma chitotriosidase activity is known as a marker of storage macrophages. It is extremely high in most patients with Gaucher disease and moderately enhanced in many patients with NP-C. Usually, the activity elevations are more distinct in patients with NP-C with an early clinical onset than in patients with late onset.[13-16] There is the caveat that about 6% of the general population is genetically chitotriosidase negative.[17] Plasma oxysterols, for example, cholestane-3β,5α and 6β-triol, have been reported to be distinctly elevated in most but not all patients with NP-C, and elevations can also be found in other disease conditions.[18] Recently, another biomarker named lysosphingomyelin-509 with a high sensitivity and specificity for NP-C1 disease when estimated in blood plasma has been described.[19]

Clinical Report

A wheelchair dependent, 20-year-old female patient was first referred to our neurology department in 2010. Neurological examination revealed impaired eye movements, ataxia, and dystonia involving her face and limbs, as well as brisk reflexes and a general severe slowing of movements. Further analysis of the disturbed eye motility strongly suggested the presence of VSGP: There was an almost complete blockade of vertical eye movements, and when trying to look upward, she compensated with lifting and dorsal flexing the head. However, the blockade was characteristically overcome by the vestibulo-ocular reflex. Her horizontal eye movements were massively slowed and, to the left side, reduced in amplitude (see video in Supplementary material S1). Repeated loss of head control, with an intermittent anterocollis-like appearance, was observed. She was able to follow simple commands and spoke single words, but the speech was distinctly dysarthric. Feeding and severe swallowing problems were reported. To the earlier history, an unremarkable perinatal period and normal early childhood development were reported. When she was first hospitalized at age 11, for a 1-year history of clumsiness and gait problems, she was suspected to have mild cerebral palsy. When again hospitalized at age 14 because of learning difficulties and repeated falls, she presented with cerebellar ataxia, orofacial dyspraxia, and severe dysarthria and dysphagia. Trunk muscles had reduced tone, reflexes in the lower limbs were brisk, and plantars were flexor. Cerebral magnetic resonance imaging (MRI) was normal. At age 15, rapid cognitive decline and progressive gait problems were obvious, and enuresis occurred. Slow eye movements, ataxia, severe dysarthria, and psychomotor slowing were observed. Cerebral MRI now showed a discrete atrophy of the cerebellar vermis. Cerebrospinal fluid (CSF) was normal (<4 white blood cell [WBC]/μL, total protein 31.1 mg/dL, lactate 1.17 mmol/L, normal immunoglobulin G index and synthesis rate, negative for oligoclonal bands). There was no evidence for polyneuropathy. Liver and spleen had normal size on abdominal sonography. Six months later, the present dementia, VSGP, severe orofacial dyspraxia, dysarthria, ataxia, increased reflexes, and dystonia were highly suggestive of an unknown neurodegenerative disease. Cerebrospinal fluid was again normal. The patient developed epileptic seizures at age 16, which partly responded to lamotrigine. She was still able to walk at age 17. At age 18, she was permanently incontinent and wheelchair dependent and had almost stopped speaking. Cognitive decline was severe. She was repeatedly hospitalized because of seizures and respiratory
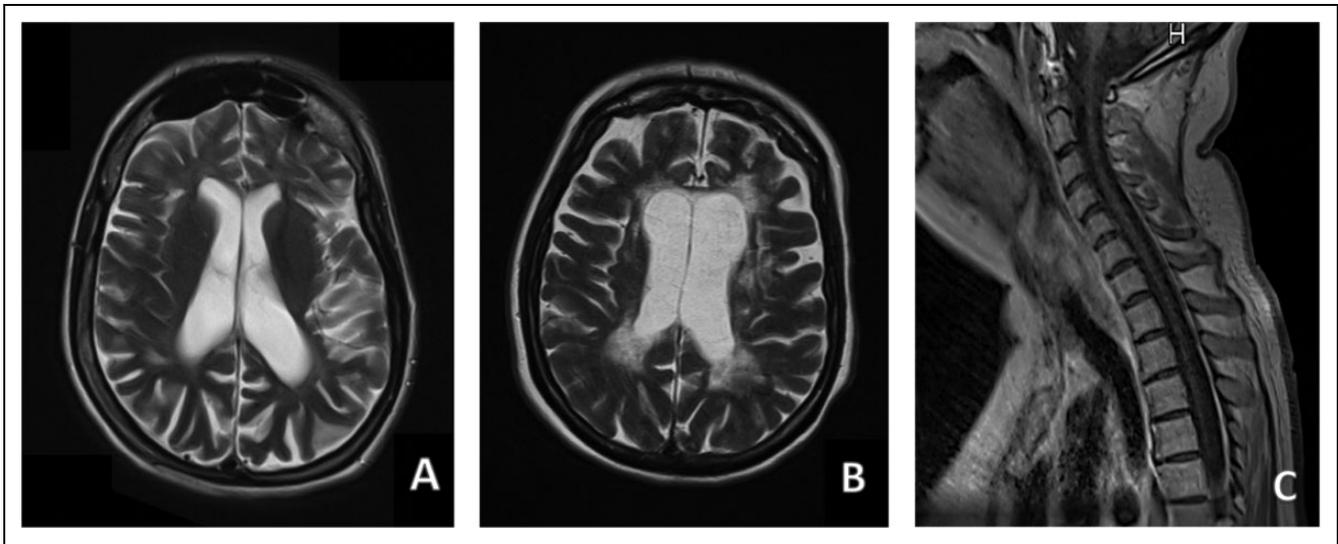
Figure 1. Cranial magnetic resonance tomography (MRT) from present patient (A, T2 weighted, 1.5 Tesla), and her mother (B, T2 weighted); note global atrophy and multiple white matter/subcortical lesions. Cervical spinal MRT from the mother (C, T1 weighted).

infections. At age 20, she had lost speech, and a gastrostomy was required because of dysphagia. Cerebral MRI now revealed global atrophy, which was frontally accentuated and affected also the corpus callosum, and there were diffuse white matter lesions (Figure 1A). The clinical features were now viewed as being highly suggestive of NP-C. However, the subsequent, complex laboratory including genetic work-up (see Results and Discussion section) did not absolutely confirm a NP-C diagnosis, though it still provided different parts of evidence, including at least 1 mutated *NPC1* allele, and a marked elevation of the lysosphingomyelin-509 NP-C biomarker for this diagnosis. The patient was started on treatment with miglustat, which was administered for 15 months without convincing benefit.

The patient's mother was diagnosed with multiple sclerosis (MS; CSF: WBC 126/µL; oligoclonal bands, positive) at the age of 30 years. After a short relapsing-remitting relapsing course, the disease became secondarily progressive leading to rapid cognitive decline and inability to walk without support by age 35. When she was first seen at our department at age 50, she was bedridden. Here cognitive decline and psychomotor slowing were striking. The severe dysphagia had required gastrostomy 3 months before. She did not speak spontaneously but could partly answer elementary questions with a marked tendency to perseveration. Neurological examination revealed VSGP, saccadic eye movements, bilateral gaze evoked nystagmus, ataxia, and spastic tetraparesis. Cerebral MRI showed massive white matter lesions and global atrophy (Figure 1B), particularly pronounced in the corpus callosum, cerebellum, and brain stem, whereas the cervical spinal cord was not visibly affected (Figure 1C). Ophthalmologically, at age 53 her narrow pupils did not react to light and her eye fundi showed extremely pale optic disks so that the optic nerves presumably were atrophic. She was reported to have been under psychiatric treatment at age 25 for anxiety and obsessive-compulsive disorder (onset of psychiatric symptoms in her teens) and to have had

episodes of optic hallucinations (seeing people in her home, calling the police) since age 38. She has the same genotype with 1 mutated *NPC1* allele (see Results and Discussion section) as her daughter, that is, the present patient. The lysosphingomyelin-509 NP-C biomarker tested in the mother's blood plasma was not enhanced, and its level was in the lower range of normal controls. The further family history was essentially unremarkable, in particular any neurological or psychiatric problems or diseases were denied when the relatives of our patient were interviewed 3 years ago; the pedigree in Figure 2 was outlined according to their reports.

Methods

Filipin testing was done according to Sokol et al.[20] Plasma chitotriosidase activity was estimated with a standard method (Hollak et al).[21] Acid sphingomyelinase in fibroblasts was assayed as described by van Diggelen et al.[22] Molecular analysis of the *NPC1* and *NPC2* gene was done after isolating genomic DNA from peripheral blood leukocytes using the Maxwell-DNA isolation kit by Promega (Mannheim, Germany). Molecular genetic analysis of the 25 coding exons of *NPC1* and the flanking intronic regions was performed by Sanger sequencing including macrodeletion screening by MLPA. Polymerase chain reaction conditions and primer sequences are available on request. Multiplex ligation-dependent probe amplification was done corresponding to the instructions of MRC-Holland (Amsterdam, The Netherlands) for using their NPC-MLPA-Kit 193.

Results and Discussion

In the present patient, skin fibroblasts cultured in low-density lipoprotein (LDL) enriched media revealed numerous strongly fluorescent perinuclear vesicles after filipin staining (Figure 3), but this cholesterol storage pattern was not seen in LDL-deprived
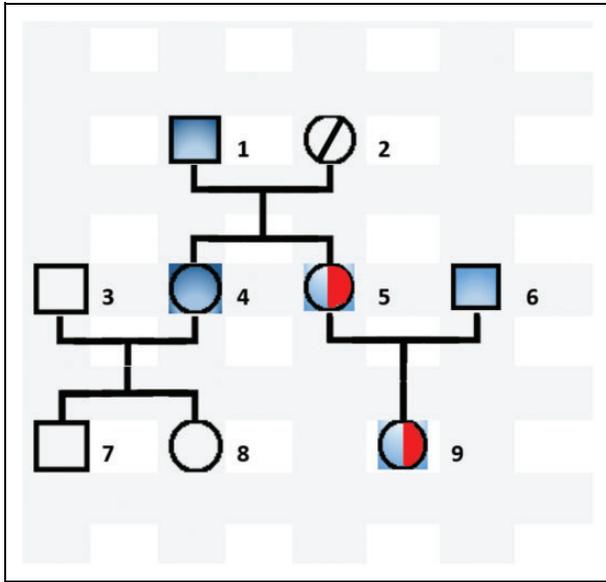
Figure 2. Pedigree of the present patient's family. Light blue symbols, *NPC1* genotype determined as wild-type; half-red symbols with blue background, heterozygous for the *NPC* variant p.N916del; white symbols, healthy except for family members 1 (urinary bladder carcinoma) and 2 (she had "rheumatic hands" and an operation on the cervical spine; she has deceased from cardiac failure at age 68 years). The present patient is member 9, her mother 5, and her father 6 (who was healthy).

media. The overall cholesterol storage pattern was that of the variant biochemical phenotype. Since such patterns have also been reported for heterozygous carriers of *NPC1* mutations,[1,8,9] the test result did not essentially support the hypothetical NP-C diagnosis in this patient. However, the cytological findings were, in our opinion, diagnostically relevant: The high numbers of foamy storage cells and some sea-blue histiocytes (Figure 4) seen in the bone marrow plates (May-Grünwald/Giemsa stain) represented a "Niemann-Pick disease pattern." The types A and B of the disease were excluded by the normal acid sphingomyelinase activity shown in cultured fibroblasts, so that NP-C remained as a likely diagnosis. There is only 1 important differential diagnosis for our cytological findings: Storage cell patterns resembling those in NP-C can be seen in late onset cholesteryl ester storage disease. However, the clinical phenotype of this disease is extremely different from the phenotypes usually observed in NP-C and from the phenotype in the present patient. The following negative findings in the patient are mentioned for completeness, although they do not speak against the presence of a type of Niemann-Pick disease: On abdominal sonography, the spleen and liver were not found to be increased in size. A pelvic bone marrow biopsy was reported to be histologically normal, which, however, is not uncommon even in cases with positive cytological findings in bone marrow smears. Gaucher disease, though not suspected clinically and cytologically, was excluded by a
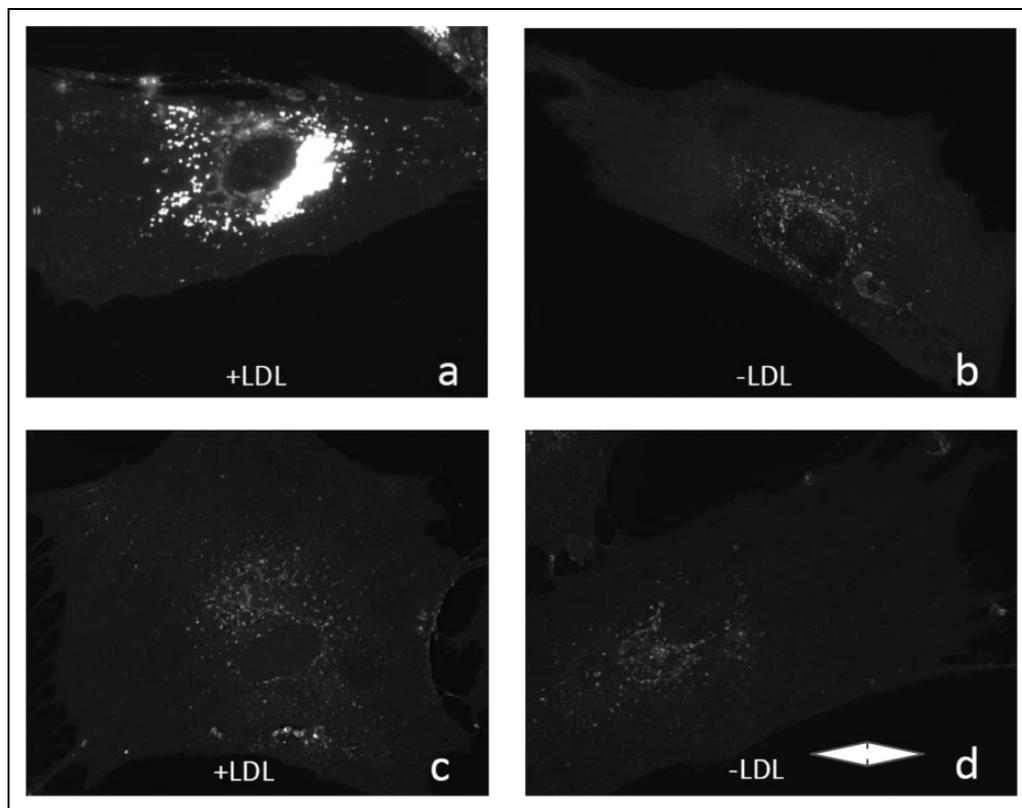

Figure 3. Filipin reactivity in single cells of fibroblast cultures. Symbols: a and b, present patient; c and d, mother of present patient. Brightly fluorescent perinuclear vesicles in (a) show cholesterol positivity in the presence of low-density lipoprotein (+LDL); weakly fluorescent vesicles in (b) in the absence of LDL, −LDL. Weakly fluorescent vesicles in (c) and (d), similar to findings in normal controls. Length of diamond in d, 10 μm.
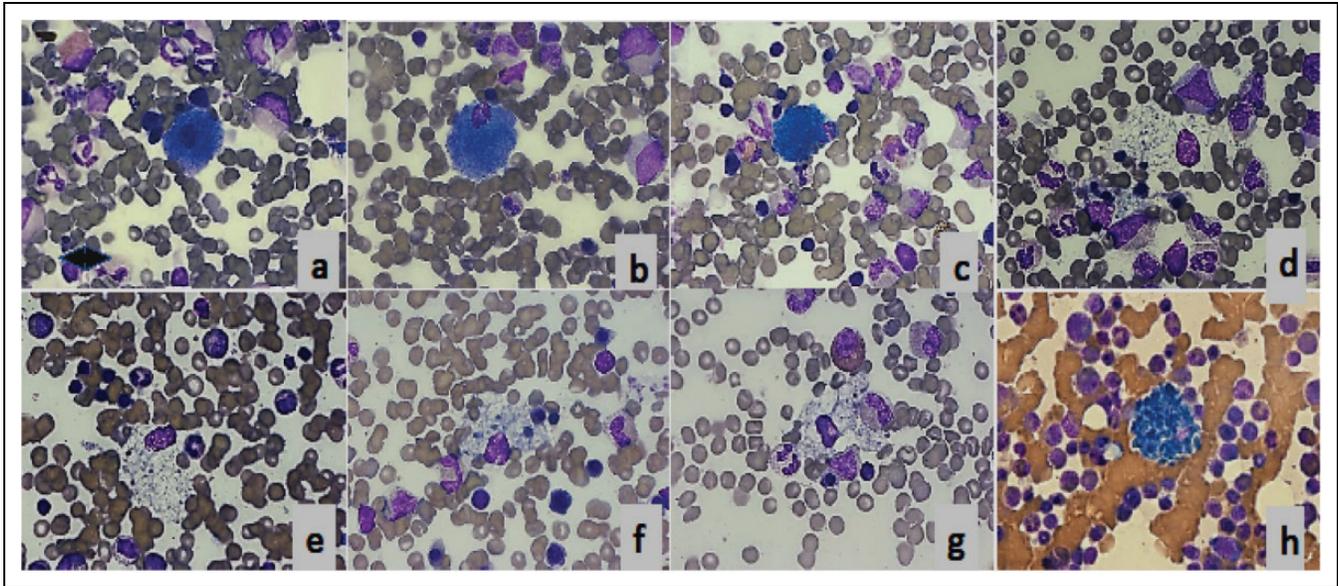
Figure 4. Storage macrophages in bone marrow smears (May-Grünwald/Giemsa stain). Identification: a to c, sea-blue histiocytes with dense cytoplasm and shrunken cell nuclei, in present patient; d to g, in the approximate mid of each photograph, a foamy storage cell with light cytoplasm containing small or less small granules and vacuoles and slightly shrunken, distinctly stained nuclei, in present patient; h, sea-blue histiocyte with coarsely granular cytoplasm and very small nucleus, in mother of present patient. Length of diamond in (a), 20 μm.

normal glucocerebrosidase activity found in cultured fibroblasts in the routine laboratory of one of us (K.H.). Very long chain fatty acids were found to be normal in blood plasma, excluding different types of adrenoleukodystrophy. Plasma chitotriosidase displayed normal activity, which illustrated once more that this "macrophagic marker" is not always positive even in a high load with storage macrophages such as is present in this patient. As to more specific markers, estimations of plasma oxysterols (cholestan-triol, 7-ketocholesterol, 24-hydroxycholesterol, and 27-hydroxycholesterol)[18] performed in 2 different laboratories revealed no abnormalities, but it is known that these compounds are chemically labile, and they may have been lost during sample preparation and transport. In contrast, a new biomarker for NP-C1, lysosphingomyelin-509,[19] revealed a distinctly positive result in our patient's blood plasma (marker enhanced up to the 2-fold mass of the used internal standard, while controls had less than 0.7-fold this mass; personal communication with members of the group of Giese[19]). According to the recent report,[19] the upper biomarker levels in NPC-1 heterozygotes slightly overlap with the lower levels in diagnosed patients with NP-C1. Might the high level heterozygotes still have subclinical, not as yet recognized NP-C disease?

Genetic testing in this patient included, as routine steps, analyses of the genes responsible for Friedreich ataxia; spinocerebellar ataxia types 2, 3, 6, 7, 8, 10, 12, 17; Huntington disease-like disorder 2; and dentatorubropallidoluysian atrophy, all with negative results. When then focusing on NP-C genetics, sequencing of the *NPC1* gene revealed a heterozygous in-frame deletion of 3 bp in exon 18, c.2746_2748delAAT; p.N916del, and no mutations in the *NPC2* gene. On repeated sequencing of *NPC1* and when using MLPA to exclude exonic deletions, no further coding *NPC1* changes were found, suggesting the presence of 1

wild-type allele. When our genetic studies were started, the 3 bp deletion (p.N916del) had not been known. However, in 2011, a patient with juvenile onset NP-C was described to have a classical biochemical phenotype on filipin staining of fibroblasts, on the background of a compound heterozygous genotype of *NPC1* with the known pathogenic p.A1151T and the novel p.N916del variant.[23] Notably, the p.N916del is known to cause a loss of asparagine in the cysteine-rich loop within a very conservative residue of the NPC1 protein, so that the p. N916del was likely to be pathogenic according to the POLYPHEN and PANTHER prediction programs (personal communication with members of the group of Macial-Vidal et al[23]). In fact, this 3 bp deletion has now been indicated in Human Gene Mutation Database (CD109545) to be pathogenic. Additionally, on studies at the transcript level, we observed that in quantitative terms messenger RNA (mRNA) transcribed from the p.N916del coding allele was overexpressed as compared to mRNA of the wild-type allele. The detailed description of this peculiar constellation, and the expected confirmation in additional patients and their families, is the subject of a just started study (by SB-W and PB).

In comparison with this patient, her mother who was diagnosed with MS showed the following characteristics: She had a negative filipin test (Figure 3), a small number of Niemann-Pick cells, including sea-blue histiocytes, in bone marrow smears (Figure 4). Genetic testing revealed a *NPC1* p.N916del/wild-type genotype. An allele overexpression as in her daughter was absent. Her early psychiatric problems and pronounced optic hallucinations were rather unusual for MS, while they can be observed in adult-onset NP-C; but this was not sufficient for explaining essential parts of her clinical picture with her heterozygous *NPC1* genotype, which, on the other hand, can explain the cytological findings.[10] A speculative

possibility is that the heterozygosity might have modified, but not caused primarily, the mother's disease course.

As a simple explanation of the present patient's disease, she could have also the second *NPC1* allele mutated (for example, in the noncoding regions), despite the negative findings. The present genetic test sensitivity still may be slightly lower than 100%. Thus, the patient can be viewed as having distinctly manifested NP-C, in which one of the blood plasma markers for NP-C[19] was positive, but the genetic characterization was incomplete. However, this view includes also the possibility that the reported overexpression of the mutated *NPC1* allele is involved in still unknown pathogenic mechanisms causing disease also without the second allele affected. However, also in the absence of the overexpression, as in the patient's mother, a "dominant" trait of a certain mutated *NPC1* allele would not be absolutely unrealistic when one regards the interesting findings in the spastic paraplegias, which strongly suggest that the nature and localization within the protein of a given mutation can change the mode of inheritance (and the expressed phenotype) of the mutated allele.[24] In such a model, also the present patient's mother should have NP-C (in addition to MS?), the less severe manifestation compared to her daughter being due to secondary variation. When additionally comparing the disease phenotypes in the mother and daughter with some known, dominantly inherited phenotypes including Perry syndrome,[25] which share the important symptom of VSGP with NP-C, these disorders seem to be very distinct from the phenotypes in our 2 females. In addition, a storage disorder was well characterized in the present patient by the distinct cytological abnormality, which would never be consistent with one of those disorders. Nevertheless, small last doubts may remain in the present NP-C diagnosis which is hoped to be corroborated by future studies including whole exome sequencing.

Conclusion

Neurologic and laboratory scientists are encouraged not to dismiss a possible NP-C diagnosis in patients with a clinical, cytological, and biochemical phenotype highly suggestive of NP-C but with only 1 *NPC1* allele found to be mutated. In a number of such patients, hitherto unclear genetic or other mechanisms (such as the here reported allele overexpression?) might complicate the simple NP-C heterozygous situation and promote a "full" manifestation of NP-C, while simple heterozygosity may be responsible for mild NP-C manifestations in some cases. Thus, "heterozygous" but full-manifesting NP-C patients, like the here described one, should be completely documented and be studied using the recent genetic techniques.

Authors' Note

Julia Jecel, Klaus Harzer, Eduard Paschke, Stefanie Beck-Wödl, Peter Bauer, Milos Hejtman, and Regina Katzenschlager declare that they have no conflict of interest.

Author contributions

JJ, KH, EP, SB-W, PB, and RK jointly wrote the first draft of the manuscript; MH critically revised the manuscript for important intellectual content; JJ was responsible for the clinical care of the patients. EP was responsible for filipin testing. SB-W and PB performed the genetic testing. KH and MH reviewed the bone marrow smears. EP and MH contributed the images of the manuscript. All authors read and approved the final manuscript. Guarantor: JJ.

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 the guardians of the patients for being included in the study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Supplemental Material

The online [appendices/data supplements/etc] are available at http://iem.sagepub.com/supplemental

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