A Riddle Wrapped in a Mystery: Understanding Niemann-Pick Disease, Type C

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Background: Niemann-Pick disease, type C (NPC), is a lipid storage disease that may present at any age from fetal life to the seventh decade. Its protean manifestations include hepatic and pulmonary failure, as well as a range of progressive neuropsychiatric phenotypes. Late onset disease has been increasingly recognized as the biochemical diagnosis of NPC has been more widely applied.

Review Summary: The phenotypes, biochemical, and molecular bases of NPC are reviewed. Indistinguishable phenotypes are produced by mutations in two distinct genes, designated NPC 1 and NPC 2, that play key roles in the intracellular trafficking of lipids. The diagnosis of NPC is challenging as the characteristic vertical supranuclear gaze palsy is difficult to recognize, organomegaly is often absent, and neuroimaging and standard biochemical screening studies are usually normal. Definitive diagnosis requires demonstration of the trafficking defect in cultured fibroblasts, supplemented in selected cases by genotyping. Animal studies have shown that inhibition of glycosphingolipid synthesis may delay the onset of disease and prolong survival; a human trial of this approach is underway.

Conclusions: NPC is a model for inborn errors of metabolism whose gene product mediates molecular trafficking rather than catabolizing macromolecules, as in classic lipid storage diseases. NPC should be considered in the differential diagnosis of progressive neurodegenerative disorders at any age. The astute clinician can provide great comfort to families afflicted by NPC by making an accurate diagnosis, notwithstanding the absence of definitive treatment.

Key Words: lipid storage, dementia, psychosis, vertical gaze palsy

"It is a riddle wrapped in a mystery inside an enigma."\(^1\)

Winston Churchill’s comment on the perplexing actions of Soviet Russia in 1939 serves as an equally apt characterization of Niemann-Pick disease, type C (NPC), an underrecognized lipid storage disease that presents throughout the life span in a variety of guises. Its protean clinical nature is matched by its biochemical complexity. Most lipidoses are characterized by the accumulation of lipids secondary to deficient lysosomal hydrolase activity, but in NPC lysosomal hydrolases are normal and lipid accumulation results from impaired intracellular trafficking of macromolecules.\(^2\) Mutations in either of 2 genes, NPC1 and NPC2, produce the Niemann-Pick disease, type C phenotype.

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NPC can present at any age from intrauterine life to the sixth decade with liver failure, incidental organomegaly, or a wide variety of neurologic and psychiatric symptoms and signs. Its incidence has been calculated as 1:150,000 for the Western European population, based on laboratory diagnoses over a 15-year period.\(^2\) The disease is more common in genetic isolates such as the Acadian population of Yarmouth County, Nova Scotia, where an incidence of 1:100, with carrier frequencies from 10 to 26% (95% CI), was reported.\(^3\)

HISTORY

The first recognizable description of NPC is found in the review of Niemann-Pick disease published in 1958 by Crocker and Farber.\(^4\) In this paper, the authors expanded the definition beyond the clinical phenotype, as described in

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1914, of an infant with massive hepatosplenomegaly who died at 18 months after rapid neurologic deterioration.\(^5\) Niemann recognized that this disorder differed from Gaucher disease, but it was Pick who delineated the distinctive pathology.\(^6\) Key findings included hepatosplenomegaly and the presence of foam cells in the viscera. In the following decade, Klenk described the storage of sphingomyelin as a hallmark of the disease.\(^7\) Crocker and Farber expanded the compass of Niemann-Pick disease based on anatomic and chemical findings, rather than conformity to the classic clinical course. In this manner, patients with more protracted neurologic illnesses and some who lacked neurologic manifestations altogether were included under the aegis of Niemann-Pick disease.

In 1961, Crocker classified Niemann-Pick disease into four groups.\(^8\) Group A corresponded to the aggressive infantile form first described by Niemann, group B included patients with only visceral manifestations, and groups C and D had later onset neurologic disease. Patients in group D were separated from those in group C because the former shared a common Acadian Nova Scotian ancestry, subsequently traced to a founder who had settled in Nova Scotia in 1673.\(^5\) Niemann-Pick disease, type D, is now known to be allelic with NPC\(^9\) and the separation of this group from NPC is no longer justified.

In the early and late 1960s, reports of what is now recognized as NPC accumulated in the literature under a bewildering variety of guises. These included juvenile Niemann-Pick disease,\(^10\) dystonic juvenile idiocy without amaurosis,\(^11\) juvenile dystonic lipidosis,\(^12\) lactosylceramidosis,\(^13-15\) neurovisceral storage disease with vertical supranuclear ophthalmoplegia,\(^16\) and DAF (down gaze paralysis, ataxia, foam cells) syndrome,\(^17\) among other terms.

Brady’s description of acid sphingomyelinase (ASM) as the defective enzyme in Niemann-Pick disease, type A,\(^18\) marked a crucial turning point in the understanding of the Niemann-Pick diseases. ASM was subsequently found to be deficient in Niemann-Pick disease, type B,\(^19\) but its relationship to Niemann-Pick disease, type C, was unclear. ASM activity in the tissues of patients with NPC, although generally low, was not depressed to the same extent as in primary sphingomyelinase deficiencies. This observation led to a proposed classification of Niemann-Pick disease into two groups\(^20\): group I (primary sphingomyelinase deficiency) (that included Crocker groups A and B) and group II (secondary sphingomyelinase deficiency) (groups C and D).

The next advance came from animal studies. In the late 1970s, Arliss Boothe, a veterinary pathologist working in Arkansas, recognized a progressive neurodegenerative disorder in an inbred Balb/c mouse colony. Pentchev subsequently found that the tissues of these animals contained excessive amounts of lipids including unesterified cholesterol and glycosphingolipids, including glucosylceramide, lactosylceramide, and GM1 and GM2 gangliosides. Subsequently, this group demonstrated a dual deficiency of glucocerebrosidase and acid sphingomyelinase in these Balb/c mice.\(^21\) Studies focusing on unesterified cholesterol, the major stored metabolite in peripheral tissues, led to recognition of the lipid trafficking defect in the mutant mice\(^22\) and subsequently in human NPC.\(^23,24\) This murine phenotype is now designated npc\(^{ala}\) to distinguish it from another murine model, C57BLKS/J spm.\(^25\)

The defect was demonstrated by culturing fibroblasts in lipoprotein-deficient serum and then exposing these cells to a pulse of LDL-derived cholesterol. Control cells showed rapid activation of the homeostatic responses to cholesterol loading, including down-regulation of low-density lipoprotein (LDL) receptors and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and up-regulation of acyl-CoA: cholesterol acyltransferase (ACAT). Murine and human NPC cells showed markedly delayed and diminished responses to this stimulus.\(^26\) The greatest difference between controls and mutant cells was observed in cholesterol re-esterification (catalyzed by ACAT), leading to selection of this activity as a key biochemical assay for the diagnosis of NPC. The consequence of these delayed and diminished responses was the accumulation of unesterified cholesterol in perinuclear membrane bound vesicles.\(^27\) Staining with filipin,\(^28\) an antifungal polyene macrolide biosynthesized by Streptomyces filipinensis that is widely used for the detection and quantitation of cholesterol in biomembranes, readily demonstrated this accumulation.

In 1993 linkage of NPC to chromosome 18 was established\(^29\) and the following year, the existence of a second complementation group was demonstrated\(^30\) and subsequently confirmed.\(^31\) Positional cloning demonstrated in 1997 that the defect in the major complementation group was in a previously undescribed gene, designated NPC1.\(^32\) In 2000 the NPC 2 gene was found to be the entity previously described as HE1.\(^33\) Mutations at this locus accounted for the handful of patients in the minor complementation group.

The most recent developments have focused on the mechanisms of disturbed intracellular trafficking, revealing that the NPC1 protein has a central role in trafficking lipids from late endosomes to the plasma membrane and endoplasmic reticulum.\(^34,35\)

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**PHENOTYPE**

Niemann-Pick disease, type C, can present at any time from intrauterine life to old age. Several cases have been
reported in which fetal ascites has been detected by ultrasound.\textsuperscript{36,37} All of these children had severe hepatic dysfunction at birth and succumbed to their illness in infancy. Fifty percent of neonates with NPC have jaundice; most recover uneventfully. Ten percent of jaundiced children with NPC have progressive and ultimately fatal hepatic disease without manifesting neurologic signs.\textsuperscript{38–44} The diagnosis has likely been missed frequently in the past. Light microscopic examination of hepatic biopsies shows the nonspecific features of neonatal hepatitis.\textsuperscript{44} Electron microscopy of liver or bone marrow may detect polymorphous cytoplasmic bodies, but biochemical confirmation of the diagnosis is essential.\textsuperscript{45}

These infants have cholestatic jaundice that has been misattributed to atresia of the bile ducts. The liver and spleen are markedly enlarged. The organomegaly gradually regresses over time in those who survive infancy. Several patients with hepatic disease had foam cell infiltrates of the lungs that can cause clinically significant impairment of gas exchange.\textsuperscript{43,46,47} Pulmonary infiltrates may be characteristic of NPC 2.\textsuperscript{47} These children do not survive beyond infancy.

A less severe phenotype with infantile onset of hypotonia and developmental delay has been reported in children in North Africa and Europe.\textsuperscript{41,48,49} Subsequent observation confirms the progressive course of the illness as additional signs, including tremor and spasticity, appear. Vertical supranuclear gaze palsy has not been reported in these cases. These children have a rapidly degenerative course and succumb to the illness by 5 years of age. Such cases have rarely been recognized in the United States. Other unusual manifestations of NPC have included peripheral neuropathy\textsuperscript{50} and a rigid-akinetic syndrome.\textsuperscript{51}

The best-recognized presentation of NPC is in early to middle childhood,\textsuperscript{2} frequently with incidental splenomegaly. In such cases the diagnosis is often missed, and children may be free of neurologic symptoms for a period of years thereafter. Other children present with behavioral or academic difficulties that progress in such an insidious fashion that diagnoses of learning disability or attention deficit hyperactivity disorder may be entertained. With time, the progression of cognitive impairment becomes apparent together with more overt signs of motor dysfunction. There is often a history of frequent stumbling and falling, although children may not have overt ataxia when such symptoms present. Eventually gait ataxia becomes apparent and as this progresses, dysarthria and dysphagia appear.

In some children, action dystonia is a prominent early feature, typically presenting with posturing of one hand or foot while walking. Dystonia subsequently spreads to involve the other limbs and axial muscles and can be a major contributor to disability later in the course of the illness.

The first neurologic sign detected in children who have been followed prospectively after presenting with incidental splenomegaly is vertical supranuclear gaze palsy.\textsuperscript{2,17} This begins with an increase in vertical saccadic latency, subsequently progressing to saccadic slowing and eventual paralysis. There may be more profound weakness in up or down gaze initially, but ultimately the entire range of movement is lost. It is less well appreciated that horizontal gaze is also impaired, albeit later than vertical gaze, and that in the terminal stages of the illness there is complete vertical and horizontal supranuclear gaze palsy. Separate mechanisms appear to be operative in feedback control of vertical and horizontal saccades, perhaps explaining the differential involvement in NPC.\textsuperscript{52}

Later onset presentations are more fragmentary. In adolescence and adulthood, psychiatric or cognitive dysfunction may dominate the picture. In a series of 16 adults, dysarthria, dementia, and ataxia were the most frequent presenting complaints.\textsuperscript{53} Almost one-half of these patients had neither visceromegaly nor vertical supranuclear gaze palsy. Only 6% experienced seizures in the course of their illness. Psychosis may precede the expression of overt neurologic signs by years.\textsuperscript{54}

Gelastic cataplexy is characteristic of NPC, although it occurs in only about 20 per cent of affected individuals.\textsuperscript{55–58} Episodes of cataplexy may be subtle, manifesting as simple head nodding attacks or a feeling of “rubberiness” at the knees, or may present as fully developed attacks of atonia, generally provoked by a stimulus perceived as humorous by the patient. Such episodes may occur hundreds of times a day and be quite disabling. Cataplexy generally responds well to treatment with protriptyline or clomipramine. The anticholinergic actions of these agents are incidentally helpful in controlling drooling. Sleep inversion may develop later in the course of NPC and may pose major management problems.

Seizures, either partial, generalized, or a mixture of both, occur in between one-third and one-half of the patients with NPC. These patients have no special requirements for the management of seizures. In some cases seizures may be refractory to medical therapy, although as the disease progresses and neuronal fallout increases, the seizures typically come under better control.

Some adults have been described with visceromegaly and the biochemical phenotype of NPC without neurologic manifestations, raising the possibility of a nonneuronopathic form of NPC.\textsuperscript{59–61} The later development of neurologic signs in these patients cannot be excluded.

**PATHOLOGY**

Gross pathology in NPC includes enlargement of the liver and, to a greater extent, the spleen. Organomegaly is most prominent in childhood and may regress later in the course of the illness. Organomegaly does not occur in many NPC patients, particularly those with later onset disease.\textsuperscript{62} These organs, together with the bone marrow and lymph nodes, contain large numbers of foam cells. Foam cells are
macrophages that stain variably with Luxol fast blue (LFB), periodic acid Schiff (PAS) and Sudan black, and are acid phosphatase positive. Sea blue histiocytes may also be recognized, particularly in cases of longer standing. Sea blue histiocytes occur in a number of disorders, most commonly myeloid diseases. Nonetheless, these findings are highly suggestive of the diagnosis of NPC in the appropriate circumstances. In some cases, relatively few such cells may be present within the tissues. Thus, a negative bone marrow or tissue biopsy does not rule out the diagnosis of NPC.

Giant cell transformation of hepatocytes (‘neonatal’ or ‘giant cell’ hepatitis) has been reported in neonates and younger children with NPC, usually in association with cholestasis.

Children with severe, early onset hepatic disease may have interstitial infiltrates of foam cells in the lungs (see previous section on phenotype) that may cause tachypnea and exercise intolerance. One case report described a response to corticosteroid therapy when the infiltrates were mistakenly treated as viral pneumonitis. The author has personal experience of one such case where there was clear evidence of clinical improvement in gas exchange after the use of oral corticosteroids.

Neuronal pathology occurs throughout the brain, but its earliest manifestations are localized to the brainstem and cerebellum. Gross changes are not apparent until the disease has progressed to a late stage. By this phase of the illness, cerebellar atrophy, particularly in the superior vermis, is apparent and is often associated with atrophy of the brainstem. Cortical atrophy and ventricular dilatation with myelin pallor in the periventricular regions may be prominent late in the illness.

Balloononed neurons are found throughout the nervous system. Cortical neurons, particularly large pyramidal cells in deep cortical layers, have distended cytoplasm with fine vacuolation. Fine cytoplasmic granular inclusions in these neurons stain variably with PAS, LFB, and minimally with cholesterol stains. Golgi preparations show meganeurite formation and ectopic dendritogenesis in these neurons. Ectopic dendritogenesis is characteristic of NPC and other sphingolipidoses in which ganglioside GM2 is stored in excess. GM2 is normally expressed only during fetal life when normal dendrite formation is occurring. Subsequently, expression is very low in both animal models and humans. In NPC and a number of related disorders, the accumulation of ganglioside GM2 correlates with the formation of ectopic dendrites directly from neuronal soma and from the axon hillock. The presence of such additional dendrites may form an anatomic substrate for the development of aberrant electrical circuits that contribute to cognitive impairment and seizures. The recognition of the pathogenic role of glycosphingolipids in NPC has provided the impetus for studies of substrate balance therapy (see Management section).

Small pyramidal neurons in more superficial layers of the cortex usually store little, if any, excess lipid.

Neuroaxonal dystrophy occurs throughout the central nervous system (CNS), with concentrations of axonal sphingolipids in the thalamus, dentate nucleus, and midbrain. Cerebellar involvement is variable; in severe cases, there may be almost complete loss of granular and Purkinje cells, with residual gliosis. Surviving Purkinje and Golgi cells, anterior horn cells, and neurons of the myenteric plexus all show evidence of excess lipid storage. Studies in the NPC mouse have demonstrated preferential loss of cerebellar neurons in a striped pattern that correlates with the presence of heat shock proteins. Virtually identical findings have been reported in the murine acid sphingomyelinase knockout (ASMKO). In both cases, the fate of morphologically indistinguishable neurons is determined by their molecular phenotype.

Neurofibrillary tangles (NFTs) have been observed in longstanding cases of NPC. The NFTs consist of paired helical filaments (PHF) with PHF tau resembling that seen in Alzheimer disease. The distribution of PHFs in NPC differs from that seen in Alzheimer diseases, as PHFs are generally found in association with ballooned neurons, both in perikarya and meganeurites.

“The hallmark of this disease is the presence of polymorphous cytoplasmic bodies.”

The macroscopic and light microscopic findings are characteristic but not pathognomonic of NPC. In contrast, ultrastructural findings may be diagnostic. The hallmark of this disease is the presence of polymorphous cytoplasmatic bodies (PCBs). These consist of structures resembling membranous cytoplasmatic bodies; “zebra bodies”; lipofuscin-like bodies; or compound structures surrounded by a single membrane, sometimes containing Golgi profiles. The combination of multiple inclusions appears to be unique to NPC. Such changes may be detected in biopsies of the CNS, rectum, skin, and conjunctiva. False negatives may occur because of sampling error, as is true for light microscopic studies.

Other characteristic histopathological findings include meganeurites and axonal spheroids. Electron microscopy of axonal spheroids shows a large number of characteristic profiles reflecting impaired microtubular trafficking within the axons and dendrites.
**BIOCHEMISTRY AND CELL BIOLOGY**

The biochemical hallmark of NPC is the intracellular accumulation of multiple lipids. In peripheral tissues, unesterified cholesterol predominates, but in the CNS, glycosphingolipids (GSLs) are proportionately more significant and likely play key roles in the pathogenesis of NPC. Glucosylceramide, lactosylceramide, sphingosine, & monoacyl glycerophosphate (bi-MAG), GM1, & GM2 gangliosides are present in excess, ranging from five times control values for glucosylceramide to twice control values for GM2 ganglioside. GM2 ganglioside expression is down-regulated after early development where it plays a key role in normal dendritogenesis. The accumulation of GM2 ganglioside in NPC and several other sphingolipidoses correlates with ectopic dendritogenesis and megaleneurite formation. These observations have formed the basis for studies of substrate inhibition therapy in NPC and related disorders (see Management section).

The accumulation of GSLs and unesterified cholesterol appears to be coupled.

The gene product of NPC1, the NPC 1 protein, is localized to Rab7 and GTPase; LAMP-2, and VAMP-7 positive, mannose-5-phosphate receptor negative vesicles consistent with late endosomes. Fluorescence videomicroscopy studies indicate that NPC1 protein plays a central role in the trafficking of vesicular lipid cargos between the late endosome, endoplasmic reticulum and plasma membrane. The movement of these vesicles is sterol-sensitive—high levels markedly inhibit the process. Cultured fibroblasts transfected with dominant negative Rab 7 and 9 constructs have reproduced the NPC phenotype, and overexpression of Rab 7 and 9 in cultured NPC fibroblasts has lead to clearance of stored lipids, as demonstrated by reduced perinuclear filipin positive staining and increased neutral red staining (presumed to reflect reduction in free cholesterol and increase in cholesterol esters). In addition to casting light on the pathogenesis of NPC, these studies point to a novel approach to therapy. Before such an approach can be pursued, several issues must be resolved—to answer these questions.

**MOLECULAR BIOLOGY**

Most patients with NPC have mutations in the NPC 1 gene; mutations in NPC 2 have been recognized in fewer than a dozen reported and unpublished cases to date.

NPC 1 is a 4673 bp gene mapping to chromosome 18q11. The gene product is a 1278 amino acid protein that is heavily glycosylated and contains 13 transmembrane domains, a unique N-terminal NPC domain, and regions with homology to the sterol-sensing domain of the Drosophila morphogen patched, 3-hydroxy 3-methyl glutaryl CoA reductase (HMG CoA reductase) and SCAP [S = (sterol regulatory element binding protein); CAP = (cleavage activating protein)].

The NPC 1 domain contains a dileucine motif, presumed to play a role in lysosomal targeting. Deletion of this domain in the CT 60 cell system is associated with accumulation of the NPC1 protein in the endoplasmic reticulum. The NPC 1 protein is expressed in all tissues, with notably high levels in murine cerebellum. The protein is synthesized and undergoes initial cotranslational glycosylation in the ER, followed by further processing in the Golgi apparatus, whence it is trafficked in vesicles to a late endosomal compartment. Studies in cell culture systems have shown that these NPC1-bearing organelles normally move at high speed between perinuclear regions and the periphery of the cell. Trafficking in this pathway is accelerated by overexpression of NPC1, but is markedly impaired when the sterol-sensing domain of NPC 1 is knocked out.

**“The NPC1 compartment functions as a dynamic, sterol-modulated sorting organelle . . . .”**

The NPC 1 compartment is enriched with glycolipids, including GM2 and lactosylceramide. The glycolipid content of the NPC1 compartment is modulated by LDL uptake and the accumulation of lysosomal cholesterol. The NPC1 compartment thus functions as a dynamic, sterol-modulated sorting organelle that traffics plasma membrane-derived glycolipids as well as plasma membrane and endocytosed LDL cholesterol in control cells. In contrast, mutant cells accumulate NPC 1 protein in perlysosomal vesicles containing immobilized unesterified cholesterol. GM2 is excluded from...
these vesicles in null mutants but reappears when full-length NPC 1 is introduced to the system.98

The precise mechanism by which NPC 1 mediates intracellular trafficking remains unknown. One unconfirmed study has suggested that NPC 1 is the first recognized eukaryotic member of the resistance-nodulation-division family of bacterial proteases that function as molecular pumps.85 These investigators expressed NPC1 in Escherichia coli and found that it facilitated the transport of acriflavine and oleic acid but not cholesterol or cholesterol-oleate across the plasma membrane. It has been speculated that NPC 1 might act by enhancing the exchange of lipids between late endosomes and the endoplasmic reticulum.99

Much less information is available concerning NPC 2. The gene maps to 14q24.3, and has a single 0.9 kb transcript coding for a 151 amino acid glycoprotein containing the mannos-6-phosphate lysosomal targeting signal.33 The gene product had previously been described as HE1 (human epidermal protein 1), a ubiquitously expressed soluble lysosomal product had previously been described as HE1 (human epidermal protein 1), a ubiquitously expressed soluble lysosomal product that is most abundant in epidermal fluid.33 HE 1 binds cholesterol100 and may thus play a role in the efflux of cholesterol from sperm plasma membranes during the process of capacitation,101 as well as more globally. The reduction in plasma membrane cholesterol alters membrane fluidity and hence the organization of microdomains, including lipid rafts. Accumulation of lipid rafts is a feature of lipid storage diseases, including NPC,102 and may represent one potential disease mechanism in NPC 2.

The published cases of NPC 2 were associated with null mutations and an aggressive infantile phenotype. One unpublished case associated with the presence of functional HE1 has a milder, juvenile onset phenotype.103

Culture of NPC 2 mutant cells in HE1 conditioned medium resulted in reduced cholesterol accumulation. NPC 1 mutant cells did not show this response, suggesting that the NPC 2 defect is specific.33

"Any individual with unexplained dementia or psychiatric illness and cognitive impairment, particularly when accompanied by ataxia, dystonia, or vertical supranuclear palsy, should be investigated for NPC.”

**DIAGNOSIS**

The diagnosis of NPC requires a high index of suspicion, particularly in late onset and atypical cases. Any individual with unexplained dementia or psychiatric illness and cognitive impairment, particularly when accompanied by ataxia, dystonia, or vertical supranuclear gaze palsy, should be investigated for NPC. The diagnosis should also be considered in neonates or infants with unexplained cholestatic jaundice and older children with progressive liver disease.

The absence of organomegaly, normal computed tomography (CT) or magnetic resonance imaging (MRI) of the head, and normal bone marrow biopsy do not rule out the diagnosis. Late in the course of the disease, neuroimaging typically shows cerebellar atrophy with lesser degrees of cerebrat atrophy, periatrial myelin loss, and thinning of the corpus callosum.104 The diagnosis is strongly supported by the finding of polymorphous cytoplasmic bodies on EM examination of skin, rectal, or conjunctival biopsies. The definitive test in new cases remains skin biopsy for fibroblast culture and cholesterol esterification studies with filipin staining (vide supra).

The ‘classic’ biochemical phenotype is of negligible or absent cholesterol esterification at 6 hours when compared with controls, with an intense pattern of perinuclear fluorescence following filipin staining. About 20% of otherwise typical cases of NPC have lesser degrees of impairment of cholesterol esterification.105 In such cases reliance must be placed on filipin staining patterns, as cholesterol esterification values may be difficult to distinguish from those of obligate heterozygotes.106

Genotyping may be helpful in confirming the diagnosis if the biochemical studies are equivocal, although the size of the NPC1 gene and frequency of polymorphisms and “private” mutations makes detection and interpretation of changes in the DNA sequence difficult in many cases. When two (or more) mutations are detected in trans, the diagnosis is established with certainty. Genotyping is now the preferred method for antenatal diagnosis. This represents an advance on biochemical diagnosis in pregnancy, which required several weeks to accomplish and was not useful in biochemical variant cases, where the results can overlap with heterozygotes.

**MANAGEMENT**

There is no definitive therapy for NPC. A variety of approaches have been employed over several decades. The earliest studies of dietary restriction and the use of cytotoxic agents were performed in the 1950s by Alan Crocker and colleagues.107 These small, uncontrolled studies showed no evidence of benefit. The first randomized study of therapeutic intervention in Niemann-Pick disease, type C, was based on the hypothesis that unesterified cholesterol was the major offending metabolite in NPC.108

Patients with NPC were treated with a low-cholesterol diet and several combinations of cholesterol-lowering agents. Patients were block randomized to five treatment groups, five
patients per group. This short-term study demonstrated that a combination of low-cholesterol diet and three cholesterol-lowering agents substantially reduced the concentration of unesterified cholesterol in the liver biopsies after 4 months of treatment. Owing to lack of funding, no formal follow-up clinical study was carried out. The majority of families involved in the study elected to continue on treatment. Patients continued to progress on this regimen and ultimately succumbed to their illness. These uncontrolled observations suggest that the benefit of a cholesterol-lowering strategy, if any, for the management of NPC would be small. Subsequent studies in mice with other and more powerful cholesterol-lowering agents have failed to demonstrate improvement in survival or pathology.\textsuperscript{109,110}

Alternative approaches have included bone marrow transplantation in humans\textsuperscript{111} and mouse models and hepatic transplantation in humans.\textsuperscript{112} There is no evidence of amelioration of neurologic disease with either of these approaches.

One report from Europe suggested that fetal hepatic cells could halt the progression of neurologic disease in the NPC mouse.\textsuperscript{113,114} This study has not been reproduced, and it is possible that the original animals studied were in fact heterozygotes, rather than homozygotes, as the study took place before the cloning of the NPC I gene. Two humans treated in this fashion have not shown clinical benefit.\textsuperscript{115}

An alternative strategy for the treatment of glycosphingolipid storage diseases has been advanced in the last decade. Given that there are no techniques yet identified to transduce the defective gene product to the brain or to repair or replace the mutated gene in situ in these disorders, investigators have studied inhibition of synthesis of the accumulating substrates. Imino sugars and their analogues, including N-butyldexylojirimycin (NB-DNJ)\textsuperscript{116} and N-butyldexoxygalactonojirimycin (NBD-GJ),\textsuperscript{117} competitively inhibit glucosylceramide synthase, leading to a partial block in the synthesis of glucosylceramide and hence higher-order glycosphingolipids in that pathway.

Treatment with N-butyldexylojirimycin produced diminution of ganglioside storage in the murine Tay-Sachs model\textsuperscript{118} and increased longevity in the Sandhoff mouse.\textsuperscript{119}

Subsequent studies of N-butyldexylojirimycin in the murine and feline models of NPC showed delayed onset of symptoms and increased survival in treated animals compared with controls.\textsuperscript{120} Reduction of GM2 storage was found in immortalized murine Schwann cells exposed to the same compound, although filipin staining was unaltered in this system.\textsuperscript{121} A clinical trial NBD-NJ in human NPC is currently underway. NBD-NJ inhibits a number of enzymes in addition to glucosylceramide synthase. This lack of specificity likely explains a number of adverse effects, principally diarrhea and weight loss, that are avoided by the use of the more specific glucosylceramide synthase inhibitor, NBD-GJ.\textsuperscript{122}

While awaiting definitive therapy for NPC, a number of symptomatic therapies are available to the clinician treating NPC. Cataplexy responds well to propranolol or clonipramine.\textsuperscript{55,56} The anticholinergic actions of these agents may also be beneficial in treating drooling in patients with impaired swallowing.

Standard antiepileptic drugs should be used to treat seizures. There are no data to suggest the superiority or inferiority of any specific agent in NPC. Dystonia may respond at least transiently to trihexyphenidyl; less experience is available with other agents.

Physical therapy is essential to maintain fitness of affected individuals and to keep them mobile as long as possible. In addition, swallowing must be regularly assessed and managed.\textsuperscript{123} By the time dysarthria has become sufficiently severe to impair intelligibility of speech, patients are at high risk of aspiration. Anticholinergics can be a helpful temporizing measure. Ultimately, maintenance of nutrition and prevention of aspiration require consideration of gastrostomy feeding with or without gastric fundoplication. This issue must be addressed well in advance of the necessity to fashion enteral access, since doing so constitutes an important psychologic step for affected individuals and their caregivers.

An important aspect of care in NPC, as for any neurodegenerative disease, is multidisciplinary support for the affected individual and family. The diagnosis of this disease is devastating for families that must not only face the inexorable progress of the illness but also a lack of comprehension from family, friends and many caregivers who are unfamiliar with the disease. To this end, it is important to ensure that an appropriate social network is in place and that the family has ready access to community support and funding agencies. In addition to government agencies, voluntary foundations have been established to provide a wide range of support services to families. In the United States, the best established of these is the National Niemann-Pick Disease Foundation (www.nnpdf.org) that also funds research. Similar groups have been established in the United Kingdom, Australia, and European countries. Other foundations that have been established exclusively to support research funding for this disease include the Ara Parseghian Medical Research Foundation (www.parseghian.org) and the Jim Lambright Niemann-Pick Foundation (www.lambrightfoundation.com/).

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