

PART 16: LYSOSOMAL DISORDERS

Chapter 145: Niemann-Pick Disease Type C: A Lipid Trafficking Disorder

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Abstract

1. Niemann-Pick disease type C (NP-C) is an autosomal recessive lipidosis with protean clinical manifestations, distinguished biochemically by a unique error in cellular trafficking of exogenous cholesterol that is associated with lysosomal accumulation of unesterified cholesterol. A majority of patients with this phenotype are linked genetically to chromosome 18, the locus of Niemann-Pick disease type 1 gene (NPC1). *NPC1* is a novel gene whose predicted protein product contains between 13 and 16 transmembrane domains, and a sterol-sensing domain with homologies to Patched, HMG-CoA reductase, and sterol regulatory element binding protein [SREBP] cleavage-activating protein (SCAP). A region designated the NPC domain, conserved in yeast, nematode, and mouse, contains a leucine zipper. NP-C is distinct at clinical, biochemical, and molecular levels from the primary sphingomyelin lipidoses (Niemann-Pick disease types A [NP-A] and Niemann-Pick disease types B [NP-B], respectively), with which it has traditionally been grouped. Niemann-Pick disease type D (NP-D) is allelic with NP-C, and should be regarded as a variant phenotype associated with a genetic isolate, rather than a distinct entity. A small group of patients belong to a second genetic complementation group that does not link to chromosome 18. These individuals are believed to have mutations in a gene provisionally designated Niemann-Pick disease type 2 gene (NPC2).
2. The clinical manifestations of NP-C are heterogeneous. Most patients with NP-C have progressive neurologic disease, although hepatic damage is prominent in certain cases, and may be lethal in some. Variable hepatosplenomegaly, vertical supranuclear ophthalmoplegia, progressive ataxia, dystonia, and dementia characterize the "classic" phenotype. These children present in childhood, and die in the second or third decade. Other phenotypes include presentations with fetal ascites, fatal neonatal liver disease, early infantile onset with hypotonia and delayed motor development, and adult variants in which psychiatric illness and dementia predominate.
3. NP-C is panethnic. Genetic isolates have been described in Nova Scotia (formerly Niemann-Pick disease type D) and southern Colorado. Complementation studies have demonstrated two distinct groups. About 95 percent of patients link to chromosome 18q11, and thus to *NPC1*; the remainder are believed to have mutations in a second gene, provisionally designated *NPC2*.
4. NP-C has an estimated prevalence of approximately 1:150,000, making it a more common phenotype than NP-A and NP-B combined. It is likely that the true prevalence of the disease has been underestimated because of confusing terminology, the lack of a definitive diagnostic test prior to the discovery of the abnormalities of cellular cholesterol processing, and failure to recognize the clinical phenotypes.
5. Foam cells or sea-blue histiocytes are found in many tissues. Such cells are not specific for NP-C and may be absent, particularly in cases lacking visceromegaly. Characteristic inclusions (polymorphous cytoplasmic bodies) may be identified in skin and conjunctival biopsies. Neuronal storage with cytoplasmic ballooning and a variety of inclusions is present throughout the nervous system. Neurofibrillary tangles, meganeurites, and axonal spheroids are also seen.

6. In most cases of **NP-C**, the primary molecular defect lies in **NPC1**. Unesterified cholesterol, sphingomyelin, phospholipids, and glycolipids are stored in excess in the liver and spleen. Glycolipids are elevated in the brain, the principal target of this disease. There is no overt increase in cholesterol in the brain in human NP-C or its animal models. Partial sphingomyelinase deficiency, observed only in cultured cells (and never in leukocytes or solid tissues), represents a variable, secondary consequence of lysosomal cholesterol sequestration. Cultured fibroblasts show a unique disorder of cellular cholesterol processing, in which delayed homeostatic responses to exogenous cholesterol loading are impaired in association with cholesterol accumulation in lysosomes.
7. The diagnosis of **NP-C** requires both documentation of a characteristic pattern of filipin-cholesterol staining and measurement of cellular cholesterol esterification in cultured fibroblasts during LDL uptake. Candidates for such testing are identified chiefly by clinical presentation with or without supportive findings from neurophysiological tests and tissue biopsies. There is considerable variability in the degree of impairment of cholesterol trafficking in NP-C. Consequently, antenatal diagnosis has been restricted to families in which the biochemical abnormalities are pronounced. Molecular diagnosis will offer a desirable alternative in families where mutations in **NPC1** have been identified in the index case.
8. Symptomatic treatment of seizures, dystonia, and cataplexy is effective in many patients with **NP-C**. Combination drug regimens have been shown to lower hepatic and plasma cholesterol levels in human NP-C. There is no evidence that such therapy alters the progression of the disease in humans or murine models.
9. Animal models with clinical, pathologic, and biochemical features resembling **NP-C** have been described in two species of mice, as well as in the cat and boxer dog. The murine ortholog of **NPC1** is mutated in the C57BLKS/J and BALB/c models.

Major advances in our understanding of Niemann-Pick disease type C (**NP-C**) have occurred since the last edition of this text. Complementation studies have demonstrated that the NP-C phenotype results from mutations in two distinct genes. The gene associated with the smaller complementation group, accounting for perhaps 5 percent of NP-C patients, has not yet been identified. This gene has been provisionally designated **NPC2**.

The **NPC1** gene was cloned in 1997, conclusively demonstrating that **NP-C** and the sphingomyelin lipidoses (Niemann-Pick disease types A and B [**NP-A** and **NP-B**, respectively]) are distinct entities at a molecular level, confirming their earlier separation on clinical and biochemical grounds. Furthermore, it was found that NP-C is allelic with Niemann-Pick disease type D (**NP-D**), justifying the elimination of NP-D as an entity separate from NP-C.

As well as providing a new tool for the investigation of cellular homeostasis, the identification of **NPC1** will also provide a new means of investigating the poorly understood pathogenesis of neurodegeneration in **NP-C**. The latter goal is a necessary condition for the development of effective therapy for this devastating neurologic disorder.

This chapter (a) traces the historic evolution of **NP-C**; (b) characterizes its distinct and diverse clinical features; (c) defines its unique cellular lesions; (d) describes and evaluates various diagnostic procedures; (e) discusses potential therapeutic strategies; and (f) speculates on the role of **NPC1** in cellular lipid trafficking.

HISTORY

The first recognizable description of NP-C appeared in Crocker and Farber's review of Niemann-Pick disease in 1958.¹ Until then, Niemann-Pick disease was restricted to infants conforming to Niemann's original description² of infantile neurodegenerative disease with hepatosplenomegaly. Crocker and Farber based their diagnosis of Niemann-Pick disease on the presence of foam cells and increased tissue sphingomyelin, thus including children with indolent or absent neurologic disease in this category. All of the classic neurologic features of NP-C were present in their patient 15, namely: vertical supranuclear gaze palsy; ataxia; dystonia; dementia; cataplexy; dysarthria; spasticity; and seizures. They also described several other presentations of NP-C, including prolonged neonatal jaundice with death in infancy (patient 2); hypotonia and delayed motor milestones (patient 9); isolated organomegaly (patient 10); seizures (patient 13); and learning and behavioral problems at school (patient 14).

Crocker later classified Niemann-Pick disease into four groups based on biochemical and clinical criteria.³ Group A (NP-A) included the classic patients with neurodegenerative disease leading to death in infancy; group B (NP-B) patients had organomegaly without nervous system disease; group C (NP-C) patients had slowly progressive neurologic illness; and group D (NP-D) closely resembled group C, except for its restriction to a genetic isolate in Nova Scotia. Nonneural tissues in the latter two groups had relatively less sphingomyelin, and more cholesterol storage than tissue from group A and B patients.

Numerous reports in the 1960s and 1970s described a disorder now clearly recognizable as NP-C. The diagnoses included atypical cerebral lipidosis;⁴ juvenile Niemann-Pick disease;⁵ dystonic juvenile idiocy without amaurosis;⁶ juvenile dystonic lipidosis;^{7–9} giant cell hepatitis;¹⁰ lactosylceramidosis;¹¹ neurovisceral storage disease with vertical supranuclear ophthalmoplegia;¹² maladie de Neville;¹³ DAF (down gaze paresis, ataxia, foam cell) syndrome;¹⁴ adult dystonic lipidosis;¹⁵ and adult neurovisceral lipidosis.¹⁶ The confused terminology notwithstanding, the salient clinical features of vertical supranuclear gaze palsy (VSGP),^{17, 18} ataxia, dystonia, and dementia were well described and clearly established NP-C as a distinct disorder.

In 1966 Brady and coworkers¹⁹ demonstrated severe generalized sphingomyelinase deficiency in NP-A, a finding that was soon extended to type B, but not to types C and D,²⁰ indicating that the two latter types constituted separate entities. Hypotheses invoking the deficiency of a specific sphingomyelinase isoenzyme²¹ or of a sphingomyelinase activator protein²² were disproved^{23–25} and the concept of secondary sphingomyelin lipidosis was strengthened by observations of multiple lipid storage in NP-C.^{23, 26} Somatic cell hybridization studies²⁷ supported the concept of NP-C as a separate entity. In 1982, a consensus was reached in Prague to separate the sphingomyelinase-deficient forms (NP-A and NP-B) from the other forms of Niemann-Pick disease (NP-C and NP-D).²⁸

In 1984, the seminal observation by Pentchev and coworkers²⁹ of defective cellular esterification of exogenously derived cholesterol in the BALB/c murine model of the disease^{30, 31} led to the discovery of an identical lesion in NP-C and further demonstration of unique abnormalities of intracellular transport of LDL-derived cholesterol with sequestration of unesterified cholesterol in lysosomes in the disease^{32–35} (see "Pathophysiology" below). From that time on, the concept of NP-C evolved from a primary sphingomyelin storage disorder to a primary cholesterol lipidosis. Strategies based on cholesterol trafficking abnormalities facilitated the early diagnosis of patients.^{36, 37} Mapping of the murine *spm* gene to chromosome 18³⁸ and subsequent correction of the metabolic lesion in mutant murine fibroblasts by human chromosome 18³⁹ facilitated linkage studies in the human disease. Genetic heterogeneity, with two complementation groups, was demonstrated in NP-C using combined cell hybridization and linkage studies.^{40, 41} The gene involved in more than 95 percent of the patients (*NPC1*) has been mapped to

18q11.^{42, 43} Definitive progress has been achieved with the isolation of the *NPC1* gene⁴³ and its murine ortholog.⁴⁴

CLINICAL MANIFESTATIONS

NP-C is a disorder with protean manifestations that can present at any time from intrauterine life to adulthood (see Table 145-1). Manifestations may be primarily hepatic, neurologic, or psychiatric. Patients with NP-C may thus present to perinatologists, pediatricians, family practitioners, hematologists, gastroenterologists, neurologists, internists, or psychiatrists, all of whom should be familiar with this disease.

Table 145-1: Clinical Presentations of NP-C

Age of onset	Presentation
Perinatal period	Fetal ascites Neonatal jaundice Benign, self-limiting Rapidly fatal Hepatosplenomegaly VSGP usually absent
Early infantile	Hypotonia Delay of motor milestones Hepatosplenomegaly VSGP usually absent
Late infantile	"Clumsy" frequent falls (ataxia) Isolated organomegaly
VSGP may be present	
Juvenile	School failure (intellectual impairment and impaired fine movements) Behavioral problems Ataxia, dysarthria, dystonia Seizures Cataplexy VSGP present
Adolescent and adult	Progressive neurologic deterioration Dementia Psychosis VSGP may be present

Classic Niemann-Pick Disease Type C

The “classic” NP-C patient is the product of a normal pregnancy; about half of these children have transient neonatal jaundice. Although development in early childhood is usually unremarkable, the child may be labeled as ill-behaved on entering kindergarten or school. Many years may pass before it becomes apparent that the child is slowly dementing. Meanwhile, the child is labeled as clumsy and suffers frequent falls before overt ataxia is recognized. Eye blinking or head thrusting on attempted vertical gaze may be noted. Gelastic cataplexy may appear at this time, with manifestations as subtle as head nodding or as dramatic as atonic collapse with injury. Dysarthria, dysphagia, and drooling contribute to educational problems by impairing communication and exposing the child to ridicule. Dystonia first manifests as posturing of a hand or foot when walking or running, and gradually becomes generalized. Partial, generalized, or mixed seizures may begin in childhood or later. Enlargement of the liver or spleen is often first detected in early childhood and usually regresses over time. In at least 10 percent of patients, hepatosplenomegaly is never detected.

The child suffers increasing physical and intellectual disability through late childhood and adolescence, eventually becoming chairbound and incapable of continuing at school. Psychiatric disturbances including psychosis may coincide with the onset of puberty. Severe dysphagia now imperils nutrition, and the upper airway is poorly protected. In many cases, spasticity or rigidity (or both) add to the burden of nursing care. Death from pulmonary complications occurs in the teenage years or early adulthood.

Variant Phenotypes

The “classic” clinical profile accounts for 50 to 60 percent of cases of NP-C in large series. Many alternative presentations have been described.

Four children with fetal ascites^{45, 46} have been described; only one survived the first year. Two died from hepatic failure and the other from respiratory failure.⁴⁷ Foam cells were found in the pulmonary interstitium in this patient,⁴⁷ and in another patient with progressive neonatal liver failure.⁴⁸ One child presented at 4 months with respiratory symptoms.⁴⁹ Further cases presenting with pulmonary involvement have been described.⁵⁰ A link between this aggressive pulmonary phenotype and complementation group two has been suggested.⁵¹

As many as half the patients with NP-C have neonatal cholestatic jaundice that is usually self-limiting;⁵² in as many as 10 percent of NP-C cases, terminal hepatic failure without neurologic symptoms occurs.^{10, 48, 49, 52–55} NP-C has been reported as the second most common genetic cause of liver disease in infancy in the United Kingdom, after α_1 -antitrypsin deficiency.⁵⁶ Neonatal jaundice without overt liver disease may herald a more aggressive clinical course, with neurologic abnormalities appearing in the first 4 years of life.⁵⁷ In exceptional cases, neurologic dysfunction is not apparent until adolescence.

Some children present with hypotonia and delayed motor development before 2 years of age.^{52, 58, 59} These children invariably have hepatosplenomegaly, usually do not learn to walk, develop intention tremor and generalized spasticity, and die between 3 and 5 years of age. VSGP is not seen. This phenotype is more frequently recognized in patients from southern Europe, the Middle East, and North Africa than in those from the U.S.⁶⁰ One child was described with symptomatic peripheral neuropathy.⁶¹ Other childhood presentations include visceromegaly discovered in the course of intercurrent illness, cataplexy with⁶² or without narcolepsy,⁶³ or a rigid-akinetic syndrome.⁶⁴ There is considerable overlap among these early onset groups.

In contrast to these aggressive presentations, insidious onset and slow progression characterize late onset cases. Cognitive and psychiatric disturbances are prominent, and may overshadow other findings. Shulman and colleagues⁶⁵ reviewed 16 cases of NP-C with adult onset, including a new case of their own. Age of onset ranged from 18 to 59 years (mean 32 years). Signs at presentation included dysarthria (44 percent), dementia (31 percent), psychosis (25 percent), limb ataxia (25 percent), and gait ataxia (25 percent). During the course of the illness, the signs observed included dementia (81 percent), gait ataxia (75 percent), limb ataxia (69 percent), dysarthria (56 percent), VSGP (56 percent), splenomegaly (50 percent), psychosis (38 percent), extrapyramidal signs (38 percent), pyramidal signs (31 percent), dysphagia (25 percent), hepatomegaly (13 percent), and seizures (6 percent).^{15, 16, 65–70} One patient was described with clinical and imaging findings mimicking multiple sclerosis.⁷¹

Two recent, biochemically well-documented observations suggest the existence of a nonneuronopathic (or, alternatively, very delayed neurologic onset) variant of NP-C. The first patient was diagnosed at the age of 46 because of an enlarged spleen that was ruptured in a traffic accident.⁷² Another patient with splenomegaly and thrombocytopenia was diagnosed at age 52, following splenectomy (Massenkeil, Harzer, and Vanier, unpublished). Other similar, albeit less well documented, patients are known.^{73, 74} This raises the question of a possible relationship with the ill-defined patients reported as Niemann-Pick type E.⁷⁵

Specific Symptoms and Signs

Certain manifestations of NP-C deserve separate comment. VSGP is the neurologic hallmark of this disease, and it has been present in all juvenile and adult cases examined by one author (M.C.P.). Early and late onset patients in whom this sign was not found have been reported.^{65, 74} Increase in saccadic latency, followed by subtle slowing of vertical saccades (upward or downward) begins in childhood, and may be accompanied by blinking or head thrusting. Older patients complain of difficulty negotiating stairs, or that their eyes become stuck at extremes of vertical gaze. Voluntary vertical gaze is completely paralyzed in the late stages of the illness, and horizontal eye movements may also be affected. Oculocephalic reflexes are preserved. The sign is easily missed if not specifically sought. Saccades should always be examined in addition to pursuit movements. For cooperative patients, an optokinetic stimulus may be helpful. Recent studies suggest that VSGP in NP-C most likely reflects selective dysfunction of vertical burst neurons in the brain stem^{76, 77} (see “Pathology” below).

Cataplexy usually occurs late in the neurologic illness,^{78, 79} but may be the presenting feature.^{62, 63} Typically, the loss of postural tone is evoked by a humorous stimulus (gelastic cataplexy), and the resulting falls may lead to repeated injury.⁷⁸ This symptom also correlates with brain stem disease.^{63, 78–83}

EPIDEMIOLOGY AND CLINICAL GENETICS

NP-C shows autosomal recessive inheritance. Clinical heterogeneity within families is limited,⁸⁴ except when one sib has a rapidly fatal cholestatic form and another sib survives to show neurologic signs.^{84, 85} Few data are available on the epidemiology of NP-C, but the disease is clearly panethnic. Two genetic isolates have been described, French Acadians in Nova Scotia (formerly NP-D)³ and Spanish-Americans in southern Colorado.⁸⁶ Their phenotypes, although variable, are indistinguishable from other patients with NP-C. A study in Yarmouth County, Nova Scotia⁸⁷ found a 1 percent incidence of NP-C and an estimated a carrier frequency between 10 and 26 percent (95 percent confidence limits). The disease is apparently much less common in the general population. A prevalence of about 1/150,000 live births has been calculated for France, West Germany, and the U.K. from the number of cases diagnosed over a 15-year period (M.T.V.). In those countries, NP-C thus appears more frequent than NP-B and NP-A

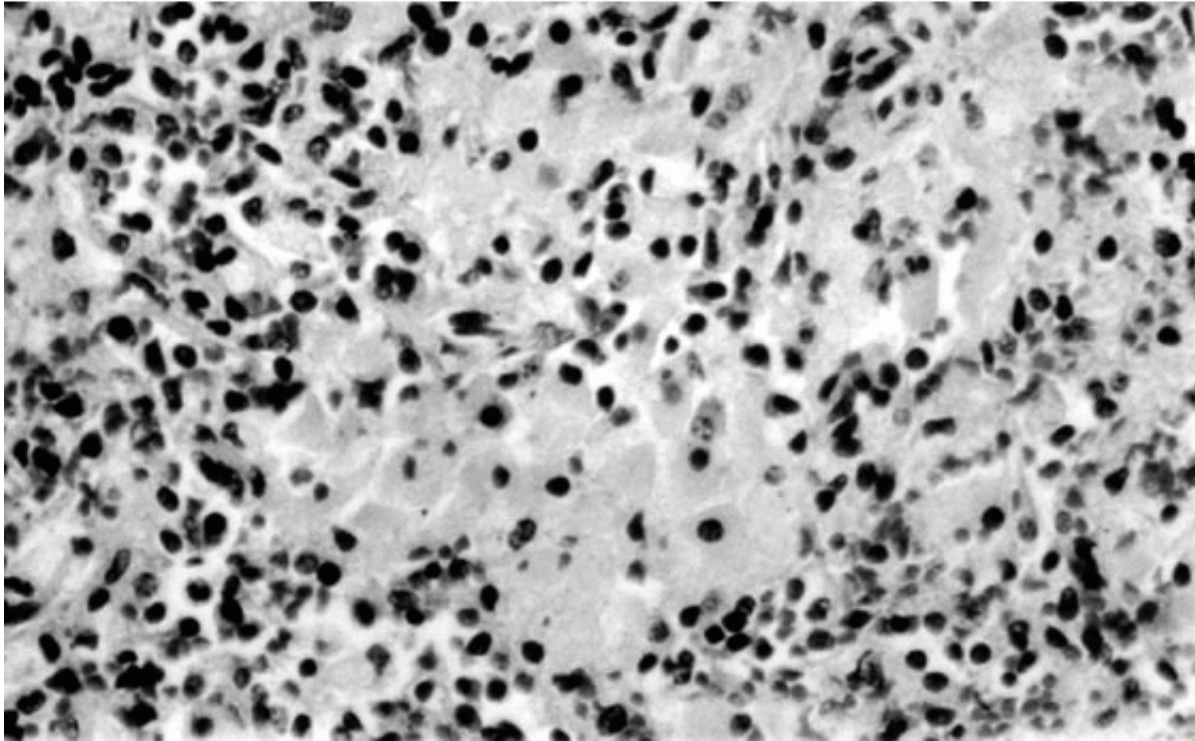
combined. The prevalence of NP-C has undoubtedly been underestimated in the past, due to confusing terminology, the previous lack of specific biochemical tests, and the many variant forms of the disease.

PATHOLOGY

Human Disease

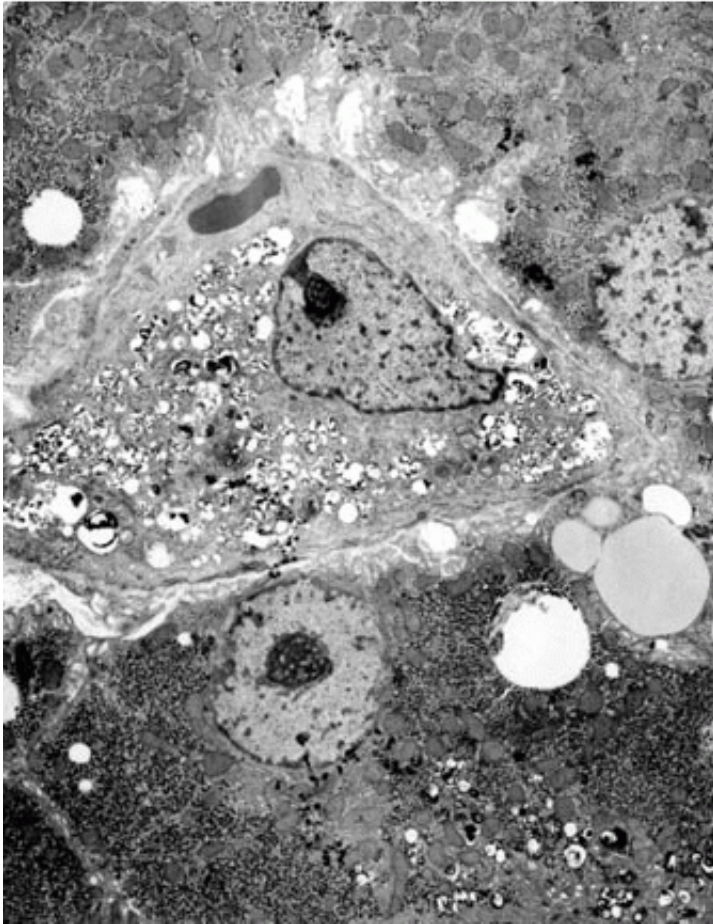
In all clinical forms of **NP-C**, the cardinal pathologic features are those of neurovisceral storage disease with foamy storage cells in the visceral organs and an accumulation of storage materials in neurons and glial cells in the nervous system. The severity of the pathologic manifestations varies considerably, reflecting the clinical heterogeneity of the disease.⁸⁸

The most notable gross pathologic feature is splenomegaly with or without associated hepatomegaly. Hepatic involvement may be prominent in early life. In most juvenile or adult patients, hepatomegaly or lymphadenopathy is not seen.⁶⁰ Histopathologically, two distinct types of cells, foamy cells (macrophages) and sea-blue histiocytes are seen in bone marrow preparations.^{12, 15, 86} Intermediate forms also occur. These cells stain variably with Luxol fast blue (LFB), periodic-acid Schiff (PAS), and Sudan black stains, and are strongly positive with the Schultz reaction for cholesterol and for acid phosphatase.^{9, 12, 89–92} Foamy cells and sea-blue histiocytes are conspicuous in spleen (Fig. 145-1), tonsils, lymph nodes, liver, and lung. These cells tend to be clustered in the red pulp in spleen and within the sinusoids in the liver. Microvacuolation may be found in the hepatocytes.^{90, 91} Hepatic pathology is usually more conspicuous in the early onset cases, in which giant cell transformation of hepatocytes (“giant cell hepatitis”), cholestasis, or both have been reported.^{10, 48, 53, 55, 93} In some infantile cases, severe pulmonary involvement causing death by respiratory failure has been reported.^{47, 50, 51} Three of these early lethal pulmonary involvement cases were found to belong to the rare genetic complementation group 2 (see “Molecular Genetics” below). In adult cases, only a few foamy cells may be found in the hepatic sinusoid without significant storage in hepatocytes.¹⁶

Fig. 145-1:

Clusters of foamy macrophages in the spleen (H&E, x520).

Dumontel and coworkers reported the pathology of a 20-week fetus including detailed ultrastructural analysis of storage cells in the liver and spleen. They found many pleomorphic lysosomes and variously shaped crystalline structures resembling cholesterol crystals.⁹⁴ Similar crystalline structures have not been reported in postnatal NP-C cases in humans, but are seen in macrophages in the brain and liver of the murine model of NP-C⁹⁵ (see “Animal Models” below). Pathologic involvement of skin, skeletal muscle, and eye may be subtle on routine histopathologic preparation, but abnormal inclusions can be found in histiocytes at the ultrastructural level.^{96–99} The cytoplasmic inclusions are polymorphic structures of various electron densities, consisting of stacked, closely packed osmiophilic membranes of varying size and thickness admixed with multiple electron-lucent vacuoles^{100, 101} (Figs. 145-2 and 145-3). Similar pleomorphic membranous inclusions can be also found in conjunctival epithelial cells; endothelial cells and pericytes; keratinocytes; lens epithelium; retinal ganglion cells; retinal pigment epithelium; pericytes; Schwann cells; smooth muscle cells; and fibroblasts.^{96–98} Sea-blue histiocytes contain concentrically arranged, tightly packed electron-dense inclusions and lipofuscin granules.^{9, 89}

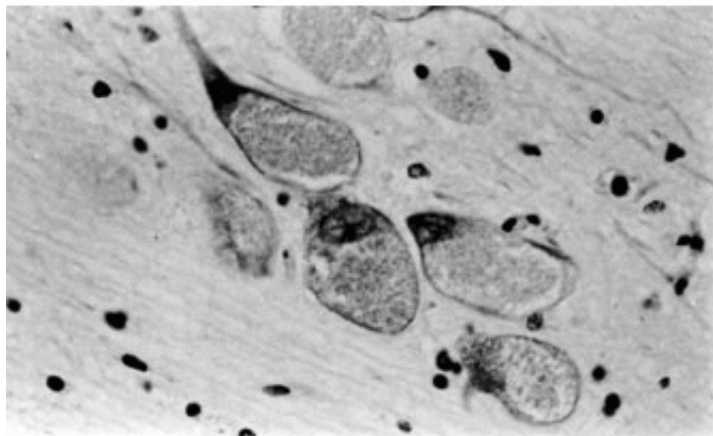
Fig. 145-2:

Electron micrograph of liver showing numerous inclusions in a macrophage (Kupffer cell) in the center and surrounding hepatocytes (×8600).

The brain is often atrophic, severely so in patients with a slowly progressive clinical course. Neuronal storage is the most conspicuous cerebral pathology (Fig. 145-4). Cortical neurons, in particular large pyramidal neurons in the deep cortical layers, show distended cytoplasm. In hematoxylin and eosin (H&E) preparations, pale gray-blue granular inclusions or fine vacuolation are noted in the perikarya, and axonal hillocks appear swollen (meganeurites). These granular inclusions are variably stained with PAS and/or **LFB** but are negative¹⁰² or only weakly positive⁸⁹ for the histochemical stain for cholesterol. In the basal ganglia and thalamus, larger neurons tend to be more affected. The degree of neuronal cytoplasmic ballooning and distribution of such storage neurons may vary considerably in individual cases.^{5, 100} Golgi preparations of the cerebral cortex reveal meganeurites, ectopic neurites, and irregular focal swellings of dendrites of affected pyramidal neurons in the deep cortical layers.^{103, 104} These features are very similar to those of storage neurons in the gangliosidoses (see Chaps. 151 and 153). Small pyramidal neurons in the layers II, III, and IV are often devoid of storage materials.^{103, 104} The cerebral white matter is usually normal, although demyelination with perivascular collections of macrophages containing sudanophilic granules has been reported.¹⁰² Neuroaxonal dystrophy in the form of axonal spheroids is found throughout the neuraxis, in particular in the thalamus (Fig. 145-5), dentate nucleus, and midbrain,

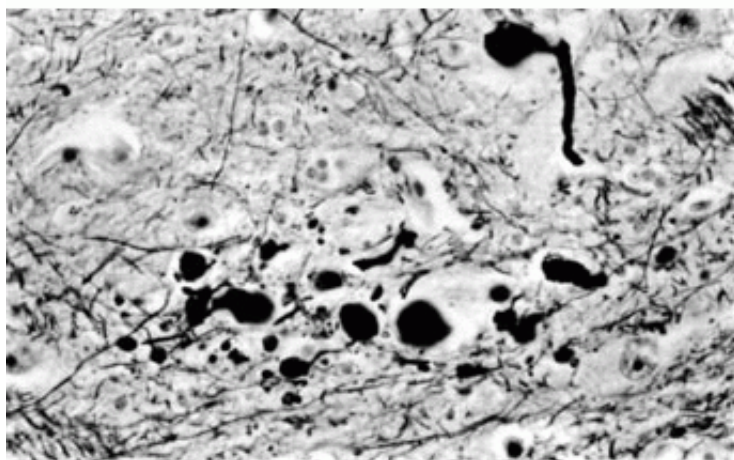
including substantia nigra.^{59, 102} The cerebellum is variably affected by this process. In severe cases, both Purkinje and granular cells are lost and replaced with dense fibrillary gliosis.^{5, 59, 100} Most of the surviving Purkinje cells and Golgi cells show the perikarya distended with storage materials. Antler-like swelling of Purkinje cell dendrites may be seen.⁵ The perikarya of both anterior and posterior horn neurons in the spinal cord are distended with storage materials. Neuronal storage is also prominent in the myenteric plexus.⁵⁹

Fig. 145-4:



Storage neurons with ballooned perikarya in the substantia nigra (Nissl stain, x520).

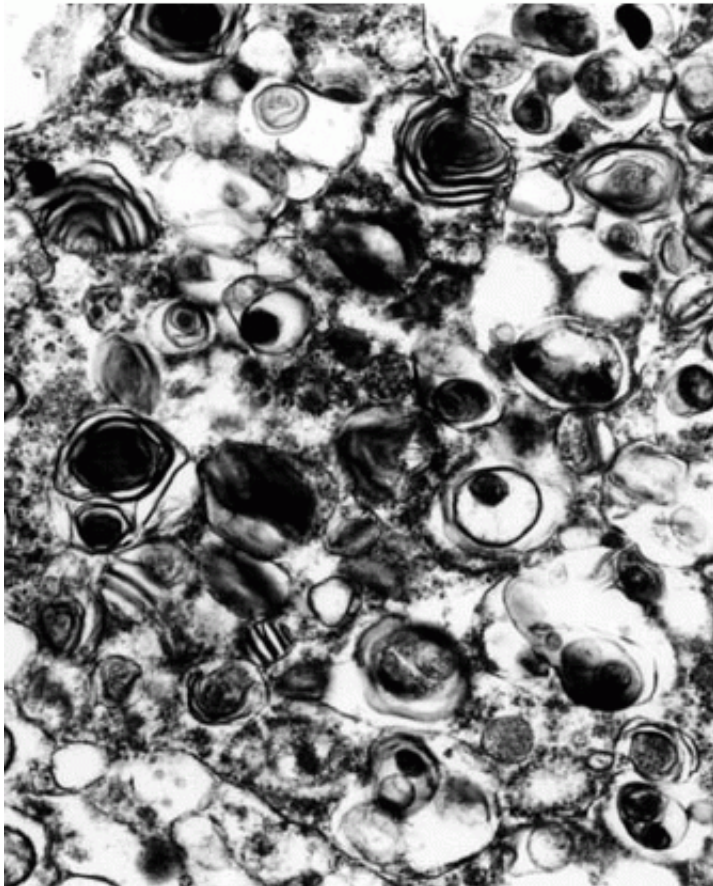
Fig. 145-5:



Clustered axonal spheroids in the thalamus (Bielschowsky stain, x520).

Ultrastructurally, neuronal inclusions consist of heterogeneous lamellar structures, called polymorphous cytoplasmic bodies (PCBs) (Fig. 145-6). Some neurons contain cytosomes resembling the membranous cytoplasmic bodies (MCBs) of gangliosidoses, “zebra bodies,” or both. Lipofuscin-like bodies or compound PCBs surrounded by a single membrane may be found in some neuronal perikarya. These complex PCBs are often associated with dilated smooth membrane profiles of Golgi apparatus. Microglia may be enlarged and often contain PCBs.^{4, 6, 9, 89, 100, 101} Similar inclusions are also found in hepatocytes (Fig. 145-4).

Fig. 145-6:



Electron micrograph of polymorphous inclusions (PCBs) in neuronal perikarya (×66,600).

Recent studies have shown that neurofibrillary tangles (NFTs) associated with neuropil threads are consistent findings in the brains of NP-C patients with a prolonged clinical course. These NFTs consist of paired helical filaments (PHF).^{67, 68, 105–108} Paired helical filament tau (PHFtau) in NP-C is similar to that of Alzheimer disease (AD).¹⁰⁹ Unlike AD, NFTs in NP-C predominantly involve the deeper layers of the cerebral cortex, thalamus, basal ganglia, hypothalamus, brain stem, and spinal cord. Entorhinal cortex, orbital gyrus, and cingulate gyrus are commonly affected. The distribution of NFTs generally parallels that of the swollen storage neurons. Paired helical filaments (PHFs) are found in the swollen perikarya as well as in the meganeurites. However, neurons without swollen perikarya may contain NFTs. NFTs can be

found in a number of non-Alzheimer cases,¹⁰⁸ and thus the presence of NFTs in NP-C cases may simply represent a nonspecific neuronal response to metabolic perturbation. However, the frequent association of a prolonged clinical course and the presence of NFTs, even in patients as young as 10 years,¹⁰⁸ may suggest that the association is more than coincidental and reflects unique neuropathology in NP-C.

Morphologic studies of the peripheral nerves are scarce. Axonal degeneration and membrane-bound lysosomal inclusions have been reported in Schwann cells, endoneurial fibroblasts, macrophages, pericytes, and endothelial cells.^{61, 110}

Animal Models of NP-C

A feline model,¹¹¹ canine model,¹¹² and two murine models (BALB/c npc^{nih} and C57BLKS/J *spm*),^{95, 113–115} are known. The C57BLKS/J mouse, also known as the “sphingomyelinosis” mouse, was reclassified as a model of NP-C rather than of NP-A.¹¹⁶ Light and electron microscopic features of these models closely resemble human NP-C. In both canine and feline models, infiltration of foamy macrophages is extensive in the lung, liver, spleen, and lymph nodes. Hepatosplenomegaly is absent in the canine model.

Neuronal storage is prominent throughout the cerebrum. Cortical pyramidal neurons in laminae II, III, and V in the feline model exhibit meganeurite formation with or without ectopic dendritogenesis.¹⁰⁴ Axonal swelling or axonal spheroids is very frequent in the feline model¹¹¹ but is relatively mild in the canine model.¹¹² These axonal spheroids demonstrate immunoreactivity for glutamic acid decarboxylase (GAD).¹⁰⁴ A marked loss of Purkinje and granular cells is documented in the cerebellum of the canine model while Purkinje cells are relatively well preserved in the feline model. Segmental myelin loss is noted in the spinal roots in feline NP-C.

Two murine models have almost identical histopathology. Hepatomegaly is not apparent. Foamy macrophages can be detected in young asymptomatic mice and their numbers increase significantly with age. These foamy cells stain positively with various lectins, indicating the heterogeneous nature of the storage material.¹¹⁷ Alveolar macrophages in younger mice contain osmiophilic dense granules and annulolamellar structures, but larger multilamellar concentric structures are found more frequently in the older mice.¹¹⁸ The brain of the affected mice is smaller than controls, with atrophy of the cerebellum and midbrain region including colliculi.^{95, 114} In both murine models, neuronal storage and axonal spheroids are very conspicuous. In BALB/c npc^{nih} mice, hypomyelination and myelin degeneration have been reported,^{95, 115} whereas myelination is well preserved in the C57BLKS/J mice.¹¹⁴ In the older mice, loss of Purkinje cells and abnormal dendritic arborization of the surviving Purkinje cells are observed.^{114, 119, 120} Ultrastructural features of these storage neurons are closely similar to those described in feline and human NP-C and numerous concentric lamellar inclusions are found in the perikarya. Inclusions are also seen in astrocytes, oligodendrocytes, microglia, and vascular endothelial cells. Myelination of the spinal roots and peripheral nerves appears normal. However, teased fiber preparations of the sciatic nerve show accumulation of myelin ovoids in practically all paranodal regions of the Schwann cells in BALB/c npc^{nih} mice,¹²¹ but myelin sheaths are reported to be preserved in C57BLKS/J mice.

Pathology in Heterozygotes

In lysosomal storage diseases, heterozygotes are usually normal. However, foamy bone marrow cells and inclusions in skin fibroblasts have been reported in the humans heterozygous for NP-C.^{122, 123} Furthermore, in feline obligate heterozygotes, occasional polymorphic membranous cytoplasmic inclusions have been reported in hepatocytes, biliary epithelial cells, and Kupffer cells.¹²⁴

PATHOPHYSIOLOGY

Tissue Lipids

Liver and Spleen.

A complex lipid storage pattern, with no compound predominating, is observed in liver and spleen in NP-C.^{23, 26} Apart from a moderate (twofold to fivefold) increase in sphingomyelin and unesterified cholesterol,^{1, 16, 26, 54, 55, 59, 75, 85, 125–127} bis(monoacylglycero)phosphate,^{8, 23, 26, 58, 126, 127} glucosylceramide,^{26, 58, 59, 89, 128, 129} and, to a lesser extent, other phospholipids and glycolipids accumulate. More lipid accumulates in spleen than in liver,²⁶ where alterations may be subtle.^{9, 130} Abnormal lipid storage is already present in the fetus and in fetal liver glucosylceramide accumulates to a greater extent in NP-C than in Gaucher disease.^{37, 59, 94, 130, 131} Very similar lipid abnormalities have been reported for the murine and feline models.^{30, 111, 132, 133}

Brain.

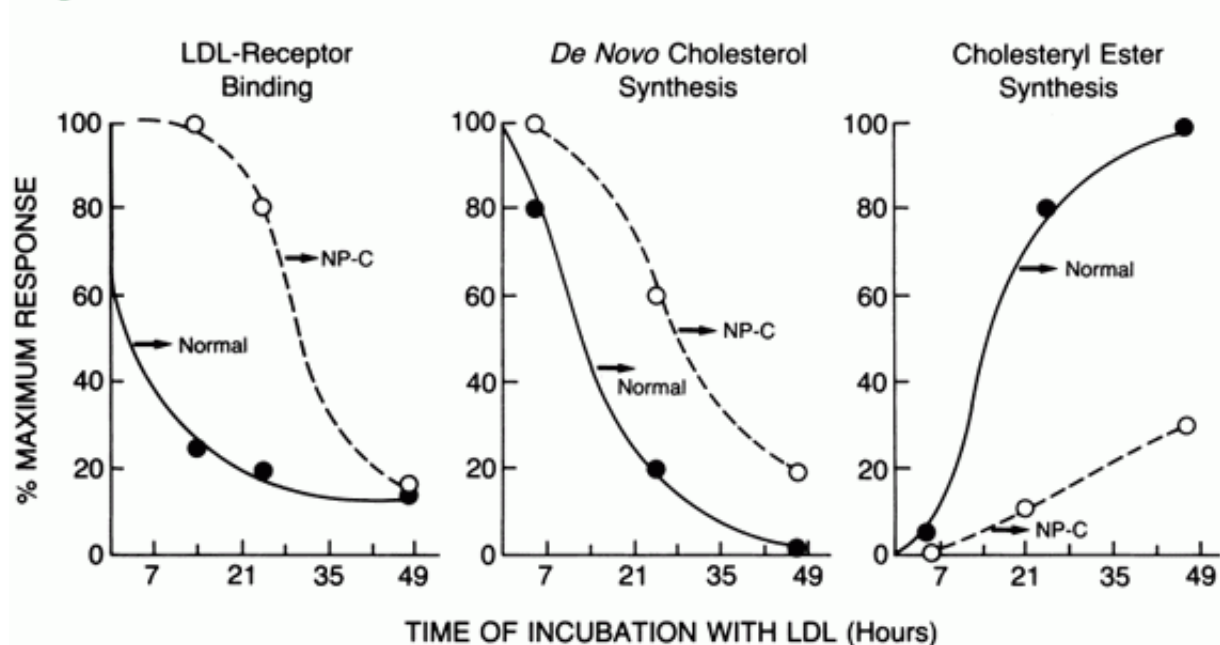
Brain lipids have been analyzed in a number of patients with NP-C, without evidence of a pathologic increase in cholesterol or sphingomyelin.^{3, 5, 53, 54, 75, 90, 91, 126, 127, 134–139} In cerebral cortex, the sole reported abnormalities pertain to glycolipids.^{9, 59, 90, 91, 102, 127, 129, 130, 137, 139–142} The most striking alterations are a many-fold increase of glucosylceramide (up to levels observed in type II Gaucher disease) and of lactosylceramide to the point that one case was initially described as lactosylceramidosis.^{11, 142} G_{M3} and G_{M2} gangliosides, as well as asialo-G_{M2} ganglioside, are significantly elevated. These alterations are not present in fetal brain and are discrete in the first months of postnatal life.^{130, 139} In white matter, these abnormalities are less marked but a varying decrease in myelin lipids has been observed. In cases with infantile onset and rapid progression of the neurologic disease, severely decreased galactosylceramide values indicate profound demyelination.^{59, 130, 136, 139} Milder changes in galactosylceramide are observed in the juvenile forms.^{9, 127, 130} Phospholipid and cholesterol concentrations are decreased in proportion to the degree of demyelination. The biochemical findings in human NP-C brains parallel those reported for the mutant BALB/c mouse.¹¹⁵

Cellular Cholesterol Lipidosis

Elucidation of the cellular basis of NP-C occurred serendipitously in the course of the study of mutant BALB/c mice that, in retrospect, had clear biochemical and pathologic similarities to human NP-C (see “Tissue Lipids” and “Animal Models” above).^{30, 133} A lesion in the intracellular processing of exogenous cholesterol was demonstrated in this murine model.²⁹ This led to the seminal discovery that cultured NP-C fibroblasts were also deficient in their ability to synthesize cholesteryl esters during endocytic uptake of LDL^{31, 32} and stored abnormal amounts of unesterified cholesterol in an intravesicular compartment when cultured in a cholesterol-enriched medium.^{33, 143, 144} Later studies from several laboratories led to more detailed understanding of the disruption in intracellular cholesterol metabolism. The subject has been reviewed by several authors.^{145–150}

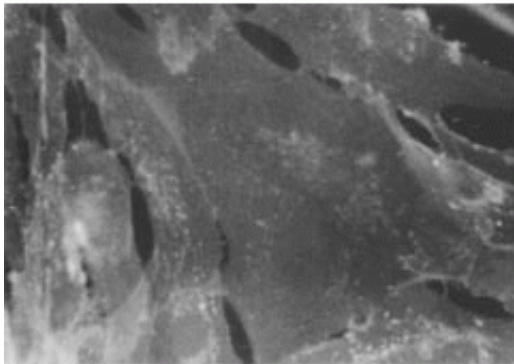
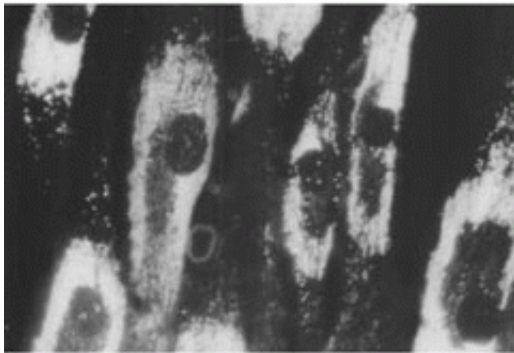
Internalization of LDL, its subsequent transport to lysosomes, and lysosomal hydrolysis of cholesteryl ester are not impaired in **NP-C**.^{33, 151} However, endocytosed cholesterol is sequestered in lysosomes and transport to the plasma membrane and the endoplasmic reticulum (ER) is retarded.^{33–35, 143, 145, 147, 152–154} There are three principal homeostatic responses following cellular uptake of LDL cholesterol¹⁵⁵ (see Chap. 120): (a) attenuation of *de novo* cholesterol synthesis; (b) depression of receptor-mediated LDL uptake; and (c) activation of cellular cholesterol esterification. All of these are compromised in NP-C fibroblasts³³ (Fig. 145-7), but are normal when the same cells are treated with 25-hydroxycholesterol.¹⁵¹ This indicates that the defect specifically affects the processing of endocytosed cholesterol and that the ability of the cell to respond is intact. Newly synthesized cholesterol does not appear to pass directly through the lysosome and is not affected by the sterol trafficking error.³⁵

Fig. 145-7:



Induction of cholesterol homeostatic responses in fibroblasts cultured with LDL. Normal and NP-C fibroblasts were cultured in lipoprotein-deficient serum to activate the LDL-receptor pathway. Cells were subsequently challenged with LDL to measure their ability to respond to the endocytic uptake and accumulation of the lipoprotein-derived cholesterol. (Adapted from Pentchev et al. 33 Used by permission.)

The intracellular distribution of cholesterol has been monitored with filipin, a fluorescent probe that forms specific complexes with unesterified cholesterol.¹⁵⁶ In cholesterol-depleted medium, little filipin-fluorescent staining is seen in either normal or **NP-C** fibroblasts. In normal cells, adding LDL to the medium results in enhanced filipin-cholesterol staining of the plasma membrane and some fluorescent staining of intracellular structures (Fig. 145-8A). In contrast, LDL uptake by NP-C cells is marked by striking filipin-cholesterol staining of perinuclear vacuoles (Fig. 145-8B), identified immunocytochemically as lysosomes.³⁴ This lysosomal sequestration of exogenous cholesterol is associated with impaired relocation to the plasma membrane.^{34, 35}

Fig. 145-8:**A****B**

Filipin-stained localization of unesterified cholesterol in fibroblasts cultured with LDL. Cholesterol-depleted normal (A) and NP-C (B) fibroblasts were cultured with LDL (50 mg/ml) for 24 h. Cells were subsequently fixed and stained with filipin to fluorescently label the intracellular distribution of LDL-derived cholesterol. A, In normal cells, cholesterol accumulation was associated with some enhanced filipin staining of the perinu...

In control fibroblasts, cholesterol egress from lysosomes may be compromised by hydrophobic amines (U-18666A, imipramine, natural sphingoid bases),^{156–158} by progesterone,¹⁵⁹ or by inhibitors of H^+ -ATPase such as bafilomycin.¹⁶⁰ The two first pharmacologic models have been widely used to study cellular trafficking of LDL-cholesterol.

The cellular consequences of the NP-C lesion may be traced in this order: the primary mutation leads to lysosomal sequestration of endocytosed cholesterol, to delayed induction of homeostatic regulation, and to cellular cholesterol lipidosis. The cellular distribution of cholesterol follows the descending concentration gradient: plasma membrane to endosomes to *trans*-Golgi to *cis*-Golgi to smooth endoplasmic reticulum to rough endoplasmic reticulum. The existence of this gradient implies the presence of specific trafficking mechanisms for its creation and maintenance. Exogenous cholesterol in the form of LDL enters the cell by receptor-mediated uptake,¹⁵⁵ and the endocytosed lipoproteins are targeted to lysosomes for hydrolysis of proteins and cholesteryl esters.¹⁵⁵ The mechanism of cholesterol efflux from lysosomes and transport to other sites in the cell is not well defined. Transfer of cholesterol to the plasma membrane from lysosomes appears rapid and constitutive,^{161, 162} but the route of trafficking is still under investigation. There is evidence for a plasma membrane-independent pathway from lysosomes to endoplasmic

reticulum.^{163, 164} Bidirectional circulation between lysosome and plasma membrane has been suggested.¹⁶⁵ The NP-C mutation has provided compelling evidence that the Golgi complex plays an active role in the transport of lysosomal cholesterol to other cellular membranes.^{148, 150, 163, 166} Filipin staining of NP-C cells following LDL uptake has revealed unusually early staining of cholesterol in the Golgi apparatus in addition to lysosomal cholesterol accumulation.¹⁵³ High-resolution electron microscopy of such cells subjected to freeze-fracture cytochemistry has shown accumulation of cholesterol in the *trans*-Golgi cisternae and deficient cholesterol relocation to *cis*-medial cisternae and adjacent condensing vacuoles.¹⁶⁶

It is not known if cholesterol first accumulates in the lysosome or the Golgi complex. Although the bulk of endocytosed unesterified cholesterol accumulates in lysosomes, the primary trafficking block in **NP-C** could affect cholesterol transport simultaneously at several organelles. Alternatively, block at an obligatory transport step at the Golgi complex could account for an upstream accumulation of cholesterol in the lysosomes. Indeed, disruption of this organelle by brefeldin-A redirects the flow of lysosomal cholesterol from the plasma membrane to the endoplasmic reticulum.¹⁶³ The abnormal distribution of cholesterol in the Golgi complex may therefore be linked to the finding that lysosomal cholesterol movement to the plasma membrane is retarded in NP-C cells.^{35, 163}

Notwithstanding these documented errors in cellular LDL cholesterol metabolism in cultured cells, the plasma lipoprotein profile of **NP-C** patients is normal. A recent study has, however, reported that intracellular trafficking of LDL-derived cholesterol is also defective in vivo in such patients.¹⁶⁷ Males with NP-C and same-sex controls were given [¹⁴C]mevalonate intravenously, as a tracer of *de novo* synthesized cholesterol, in addition to [³H]cholesteryl linoleate in LDL to monitor lipoprotein-derived sterol. The release of unesterified [¹⁴C]cholesterol into the plasma and bile was normal in controls. In marked contrast, the appearance of LDL-derived [³H]cholesterol in the plasma and bile was retarded in the affected individuals. The relative extent to which cholesterol transfer was delayed in the three NP-C patients correlated with the degree to which LDL cholesterol metabolism was affected in cultured fibroblasts derived from these same individuals. The kinetic data obtained in this fashion can be analyzed by compartmentalized modeling to provide a measure of both the size and turnover of the NP-C-induced cholesterol pools in vivo. This may provide a measurable endpoint for therapeutic interventions, if indeed extraneural cholesterol storage is relevant to disease progression (see “Treatment” below).

Sphingolipid Metabolism

Early studies attempted to link the **NP-C** mutation to a deficiency of sphingomyelin catabolism (see “History” above). Levels of sphingomyelinase activity are normal or elevated in tissues and leukocytes of NP-C patients.^{20, 75, 126, 127, 131, 168} In cultured fibroblasts, partial deficiency of sphingomyelinase activity^{86, 168, 169} with decreased degradation of exogenous sphingomyelin^{170–172} is found, albeit inconsistently.^{52, 171} It has been shown convincingly that depression of sphingomyelinase activity in NP-C-cultured fibroblasts is a secondary and reversible consequence of excessive cholesterol sequestration.^{36, 173} Glucosylceramidase activity is not decreased in solid tissues but often shows partial deficiency in cultured cells.¹⁷⁴ In contrast to human patients, solid tissues from the murine models show partial deficiencies of both sphingomyelinase and glucosylceramidase.^{30, 132}

G_{M2} ganglioside accumulates in lysosomes of cultured cells of patients, in spite of normal in vitro activities of hexosaminidase A.¹⁷⁵ There was evidence of an increased rate of biosynthesis, and accumulation occurred independently of the LDL-cholesterol uptake. Impaired cellular transport of G_{M2} ganglioside in **NP-C** has been suggested as a mechanism for this observation.^{175–177} Nevertheless, the explanation for the storage of several glycolipids, all with a short oligosaccharide chain, in human tissues (especially

brain) remains elusive, and is an area of continuing investigation.

Another intriguing observation is the many-fold elevation of free sphingoid long-chain bases, shown both in the BALB/c mouse model¹⁷⁸ and in human patients' tissues and cultured cells, that is apparently not modulated by LDL.¹⁷⁹

Other Biochemical Abnormalities

An as yet totally unexplained observation is the deficient ferritin immunoreactivity demonstrated in liver and spleen of NP-C patients.^{180, 181} Some abnormal features described in the BALB/c mouse model, such as increased dolichol phosphate¹⁸² or partial decrease of some peroxisomal β -oxidation enzymes and catalase activity in brain and liver,¹⁸³ have not been investigated in humans. One NP-C patient from a highly consanguineous family was described with additional defective peroxisomal β -oxidation of branched-chain substrates,¹⁸⁴ but the most likely explanation was that the patient was expressing mutations in two separate genes. Very long chain fatty acids in plasma studied in 6 NP-C patients showed normal results (Vanier, unpublished data).

Some other reported abnormalities most likely pertain to the alterations in trafficking of membrane lipids. Cystine has been shown to accumulate in lysosomes of tissues and cultured cells from both the murine BALB/c model and human NP-C, possibly as a consequence of cholesterol storage.¹⁸⁵ Other observations made by individual groups include high levels of caveolin-1 expression observed in liver of heterozygote mutant BALB/c mice¹⁸⁶ or fibroblasts of NP-C heterozygotes,¹⁸⁷ defective processing of apolipoprotein D found in astrocytes of the BALB/c mutant mouse,¹⁸⁸ and the decrease in membrane fluidity observed in cultured cells from the C57BLKS/J mouse model and from human patients.¹⁸⁹

Two observations suggest some impairment of cell signaling in NP-C: the attenuated elevation of cytoplasmic calcium concentration following LDL uptake¹⁹⁰ and the correlation between free sphingosine levels and inhibition of phorbol dibutyrate binding in NP-C cells.¹⁹¹ Alterations in neurotransmitters (serotonin, glycine, glutamate, and GABA) have also been described in the cerebellum and cortex of the BALB/c mouse model.¹⁹²

MOLECULAR GENETICS

Genetic Heterogeneity in NP-C

The 11 patients included in the initial cell hybridization study were found to belong to the same complementation group,⁶⁹ but subsequent work⁴⁰ disclosed the existence of a minor second complementation group. A larger investigation including genetic linkage analysis definitively established that there were two separate genetic loci responsible for NP-C disease.⁴¹ The gene defective in the large majority of NP-C families (approximately 95 percent) has been localized to chromosome 18, cloned, and designated *NPC1* (see "Identification of the *NPC1* Gene" below). The *NPC2* gene, defective in, at most, 5 percent of NP-C families (only six separate families known to date), has not yet been mapped or identified. Crossbreeding¹⁹³ and complementation¹⁹⁴ experiments in the C57BLKS/J and the BALB/c murine models have shown that both have allelic mutations and belong to the same complementation group as human *NPC1*.

The phenotypes of patients belonging to the two complementation groups have been thoroughly studied. Half of the patients in the *NPC2* group had suffered from the rare, rapidly fatal form with prominent lung involvement.⁴¹ Apart from this fact, variability in the clinical and cellular phenotypes occurred in both

groups, and no biochemical marker could be found that was specific to one of the groups.^{41, 175} This strongly suggests that the two respective gene products may function in tandem or sequentially.^{41, 43}

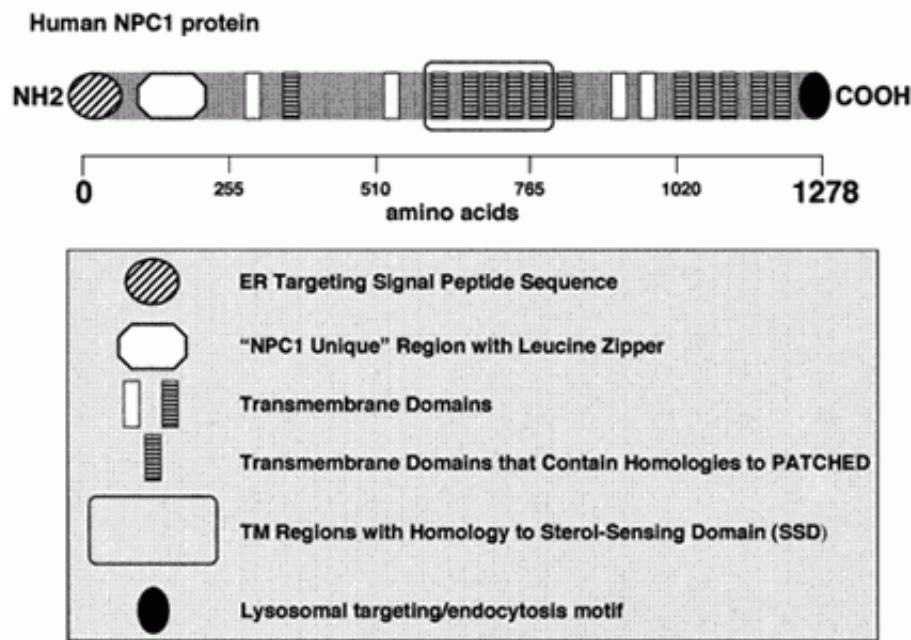
Identification of the *NPC1* Gene

The *NPC1* gene was identified through human positional cloning with the aid of the NP-C murine models. The studies of human NP-C have consistently paralleled characterization of the murine disease. In 1991, investigators linked the murine NP-C mutation in the *spm* mouse model to chromosome 18.³⁸ Human genomic regions that were syntenic to mouse chromosome 18 included portions of human chromosomes 5 and 18. Thirty-one stringently diagnosed NP-C families participated in a linkage study that mapped the NP-C gene to the pericentromeric region of chromosome 18.^{42, 43} Microcell transfer of human chromosome 18 into NP-C mouse derived 3T3 cells resulted in the restoration of cholesterol transport.³⁹ Linkage studies in Nova Scotia Niemann-Pick disease type D localized to 18q11, confirming that type C and type D were allelic.¹⁹⁵

The NP-C critical interval on chromosome 18 was refined through genetic examination of an extended pedigree of Bedouin Arab descent, which exhibited extensive consanguinity, suggesting a homoallelic mutation. Within this family a genetic recombination narrowed the *NPC1* interval to a 1-cM region defined by markers D18S44 to D18S1388.⁴³ The NP-C interval was further characterized with a yeast artificial chromosome (YAC) physical map consisting of three overlapping genomic clones. These genomic clones were introduced into NP-C-like mutant CHO cells via spheroplast fusion.¹⁹⁶ One of the three YACs complemented the NP-C phenotype. Transcripts were identified within the YAC-defined interval through sequence, exon trapping, and database analyses. The *NPC1* gene was identified based on mutation analysis of affected individuals as well as the ability to rescue the normal phenotype with the introduction of the NP-C cDNA into cultured human NP-C cells.⁴³ Identification of a major deletion in the murine ortholog confirmed the character of the *npc^{nih}* murine model.⁴⁴ The cloned human NP-C transcript was 4673 bp in length.⁴³

Translation of the open-reading frame of the *NPC1* gene predicts a protein of 1278 amino acids with a molecular weight of 142 kDa. Based on amino acid sequence analysis, the NPC1 protein is predicted to be an integral membrane protein with 13 to 16 putative transmembrane domains (Fig. 145-9). The N-terminus contains a peptide sequence associated with targeting to the endoplasmic reticulum. At the C-terminus, there is a lysosomal/endocytic-targeting signal (LLNF). Amino acids 73 to 94 comprise a leucine zipper motif. This lies within a region from amino acids 55 to 165 that has high homology with other *NPC1* orthologs. A potential tyrosine phosphorylation site is located at amino acid 506. The human and mouse NPC1 proteins have 14 potential glycosylation sites. The NPC1 human protein has high homology (percent identity/percent similarity) to other NP-C orthologs: mouse (85/93), yeast *Saccharomyces cerevisiae* (34/57), and the nematode *Caenorhabditis elegans* (30/55).⁴³

Fig. 145-9:



Structure of the human NPC1 protein.

NPC1 has extensive homology to Patched. This homology lies within 12 of the 16 predicted transmembrane domains of NPC1.¹⁹⁷ Patched is the defective protein in basal cell nevus syndrome; it is also known to be involved in the Sonic hedgehog signaling pathway. The significance of this homology has yet to be defined.

The **NPC1** protein also has homology to other proteins that are involved in cholesterol homeostasis. Within a five-transmembrane region of amino acids 615 to 797, there is significant homology to the sterol-sensing domains of HMG-Co A reductase, involved in cholesterol synthesis, and to the sterol regulatory element-binding protein (SREBP) cleavage-activating protein, **SCAP**, an activator of a transcription factor in cholesterol biosynthesis.^{198, 199} The sterol-sensing domains of these proteins are thought to be involved in their regulation based on the levels of sterols within the cell. It is not known whether cholesterol binds to this domain.

NP-C is a panethnic disorder with only two described founder populations, French Acadians in Nova Scotia^{1, 3, 200} and Spanish Americans in Colorado.⁸⁶ Outside of these populations, ongoing studies in several laboratories found that a majority of patients have private mutations and are compound heterozygotes, which will complicate genotype/phenotype correlations. Eight mutations in nine unrelated individuals were defined for the identification of the **NPC1** gene, including two deletions, one insertion, and five missense mutations.⁴³ Mutations in the Japanese populations from 11 unrelated NP-C families have been identified, of which 2 are splicing errors.²⁰¹ The mutation in the French Acadian population of Nova Scotia has been recognized as a missense mutation.²⁰⁰

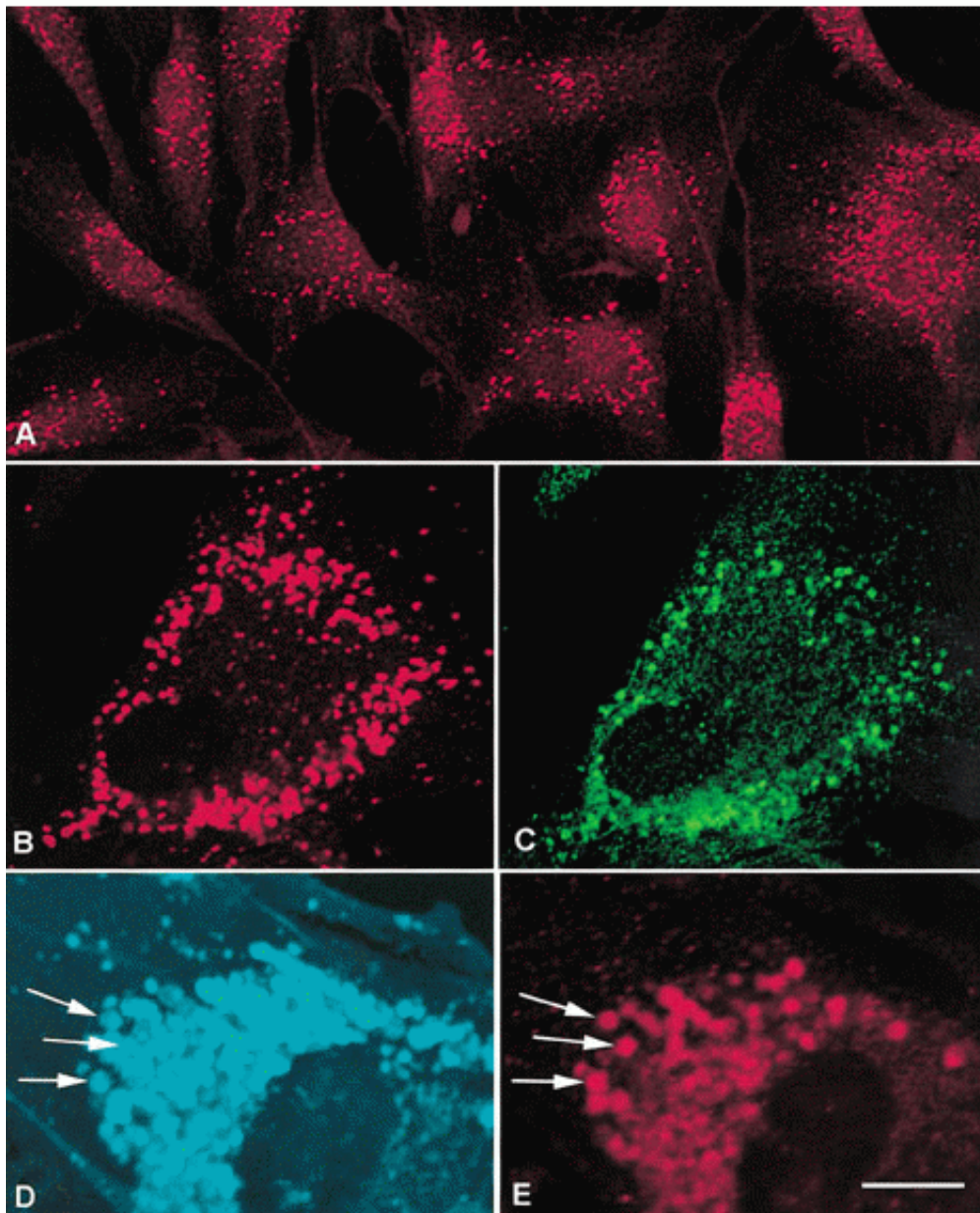
Murine Mutations

The npc^{nih} murine mutation is an insertion of an 824-bp retrotransposon-like sequence along with a 703-bp deletion of the genomic sequence consisting of 44 bp of exon sequence and 659 bp of intron sequence.⁴⁴ The resulting **NPC1** transcript has a frameshift mutation resulting in a truncated protein that is one-third the size of the **NP-C** protein, and that excludes 11 of the 13 transmembrane domains and the putative sterol-sensing domain. The availability of genotyping enhances the value of the npc^{nih} mouse for the study of NP-C.

CURRENT RESEARCH

The identification of the **NPC1** gene (see “Molecular Genetics” above) and its protein product promises to provide further insights into the mechanisms governing lysosomal cholesterol mobilization, and other aspects of intracellular lipid trafficking. In one study, polyclonal antiserum to a C-terminal region (amino acid residues 1256 to 1274) of the NPC1 protein has been used in immunocytochemical studies to determine its intracellular localization in cultured human fibroblasts.²⁰² In control cells, as well as **NP-C** cells whose mutations do not affect the expression of this particular epitopic region, NPC1 colocalizes with lysosome-associated membrane protein (lamp)-positive vesicles in the perinuclear region of the cell (Fig. 145-10A, B, and C). These vesicles are distinct and separate from the lamp-positive vesicles in which cholesterol accumulates after LDL uptake (Fig. 145-10B, and C). This segregated distribution of NPC1 and endocytosed cholesterol into separate lamp-positive vesicles is abolished when drugs are added that block lysosomal cholesterol egress.²⁰² The addition of the hydrophobic amine U-18666A¹⁵⁶ to cultured fibroblasts during the endocytic uptake of LDL causes both cholesterol and NPC1 to accumulate in the same lamp-positive vesicles (Fig. 145-10D and E). This altered relocation of NPC1 into cholesterol-engorged lysosomes suggests that NPC1 vesicles normally interact transiently with the cholesterol-enriched lysosomes to affect a relocation of this sterol pool. Further characterization of these NPC1 vesicles remains a focal point of current research.

Fig. 145-10:



A, Control human fibroblasts incubated with LDL for 24 h, immunostained with anti-peptide antibodies to the C-terminus of NPC1 protein, and viewed with confocal fluorescence microscopy. NPC1 immunofluorescence is present in small granules that are distributed throughout the cytoplasm of cells. B and C, Control fibroblast incubated with LDL for 24 h and immunostained with both (B) antibodies to a lysosomal membrane glycoprotein...

Recent studies in NP-C cells show that the cellular defect encompasses a more global transport error than targeted disruption of cholesterol trafficking alone. Because a majority of lysosomal cholesterol relocates to the plasma membrane,²⁰³ intracellular routing of endocytosed cholesterol can be viewed, at least in part, as tracing a retroendocytic vesicular pathway in which lysosomal components are transported to the plasma membrane.²⁰⁴ This consideration encouraged a reevaluation of the trafficking defect in NP-C. Measurement of the cellular egress of an endocytosed fluid phase marker ([¹⁴C]sucrose) was carried out in cultured control and NP-C fibroblasts to study trafficking through this retroendocytic vesicular pathway.²⁰² A discernible delay in the loss of [¹⁴C]sucrose from the monolayers of NP-C fibroblasts was noted. Compartmentalized kinetic modeling of the data suggests that the difference in [¹⁴C]sucrose clearance from normal and NP-C cells can be accounted for by delayed movement from a vacuolar compartment separate from, but in direct communication with, the mature lysosomes. The extent of delayed movement of sucrose through this NP-C targeted compartment (50 percent of normal)²⁰² closely approximates the measured delays in cholesterol relocation from lysosomes to the plasma membrane in NP-C cells.^{35, 163}

A model for the intracellular distribution of lysosomal cholesterol can be proposed based on these and other data. Mobilization of a majority of the lysosomal cholesterol to other membranes is likely to occur through a vesicular-mediated pathway because both specific-drug perturbations¹⁶⁴ and critical temperature transitions²⁰² known to affect vesicular trafficking markedly suppress sterol movement. Several inferences can be drawn from the [¹⁴C]sucrose egress studies. The data strongly reinforce the notion of vesicular-mediated sterol trafficking because the vesicular-dependent clearance of sucrose is defective to the same extent as cholesterol in NP-C cells. That NPC1 mutations affect the intracellular trafficking of two substances as disparate as sucrose and cholesterol suggests that the primary functional defect may target a vesicular trafficking pathway shared by these two substances. This, in turn, suggests that metabolites other than cholesterol might assume the role of offending metabolites in cells where their trafficking between the lysosomes and plasma membranes is of major physiologic significance. Solid tissues and cultured cells in NP-C contain a heterogeneous profile of accumulating material that includes several lipids in addition to cholesterol (see “Tissue Lipids” above). The possibility of alternate metabolites may explain the absence of cholesterol accumulation in the brain in humans or animals with NP-C (see “Tissue Lipids” above). Cholesterol for synthesis of myelin appears to be made locally, not imported into brain.²⁰⁵ The brain in vivo has little capacity or opportunity to metabolize lipoproteins rich in cholesterol through receptor-mediated endocytosis targeted to lysosomal processing.²⁰⁶ On the other hand, gray matter from NP-C brains shows extensive accumulation of glycolipids (see “Tissue Lipids” above) that are normal components of the plasma membrane^{207, 208} and are enriched in this organ. It might be hypothesized that the NP-C lesion leads to impaired vesicular trafficking that in turn results in cholesterol accumulation in peripheral tissues and glycolipid accumulation in the brain, where G_{M2} in particular may play an important role in pathogenesis.^{209, 210}

DIAGNOSIS

Accurate diagnosis of NP-C requires recognition of the many clinical phenotypes, narrowing of the differential diagnosis by the use of ancillary testing, and final confirmation by biochemical testing. The NPC1 gene has been cloned, but to date, the majority of affected individuals are compound heterozygotes with private mutations. Thus, molecular diagnosis is not a suitable tool for primary diagnosis, although it holds great promise for improvements in antenatal diagnosis and identification of heterozygotes in probands' families.

The known clinical presentations of **NP-C** are protean (see “Clinical Manifestations” above). The differential diagnosis is broad (see Table 145-2). A frequent clinical conundrum is posed by the classification of individuals with hepatosplenomegaly and foam cells in the bone marrow. Such individuals could have **NP-B**, but also NP-C prior to the onset of neurologic symptoms. Sphingomyelinase always shows a normal activity in leukocytes of NP-C patients, so this assay can be used to exclude sphingomyelinase-deficient types of NPD. Depression of fibroblast sphingomyelinase in NP-C is variable, but not as pronounced as in NP-B. The blood count, biochemical profile, plasma lipids, urinalysis, and cerebrospinal fluid (cells, glucose, and protein) are normal in NP-C, except in patients with hypersplenism or cholestatic jaundice.

Table 145-2: Differential Diagnosis of NP-C [Separate Window](#)

Clinical manifestations	Alternative diagnosis
Severe neonatal jaundice	Biliary atresia, congenital infections, α_1 -antitrypsin deficiency, tyrosinemia
Isolated splenomegaly	Leukemia, lymphoma, histiocytosis, storage diseases (e.g., Gaucher, NP-A, NP-B), infections (e.g., malaria)
VSGP	G _{M2} gangliosidosis, mitochondrial diseases, glycine encephalopathy, maple syrup urine disease, dorsal midbrain syndrome
School difficulty	Attention-deficit disorder, learning disabilities, absence seizures, other dementing illnesses
Dystonia	Idiopathic torsion dystonia, doparesponsive dystonia, Wilson disease, amino and organic acidopathies (e.g., glutaric aciduria type 1), G _{M2} gangliosidosis
Dementia	Pseudodementia (depressive disorder), neuronal ceroid lipofuscinosis, subacute sclerosing panencephalitis, HIV encephalopathy
Cataplexy	Other sleep disorders, seizures, syncope, periodic paralysis

Modified with permission from Schiffmann.²⁴⁷

Imaging and neurophysiologic studies are nonspecific. MRI and CT scans may be normal or show atrophy, in particular cerebellar atrophy. Changes in the periventricular white matter have been seen in some cases.⁹⁹ Proton magnetic resonance spectroscopic imaging (MRSI) in 10 patients has shown evidence of diffuse brain involvement in **NP-C**, beyond that apparent on routine imaging studies.²¹¹ Patients with NP-C had significantly decreased N-acetylaspartate (NA)/creatine plus phosphocreatine (Cre) ratios in the frontal and parietal cortices, centra semiovale, and caudate, and significantly increased choline/Cre in the frontal cortex and centra semiovale, when compared to controls. The authors also noted some correlation between proton MRSI findings and clinical stage of disease as previously defined.⁵⁷ One earlier study had included a single patient who showed loss of an abnormal lipid peak on proton MRS

at 13 and 19 months of cholesterol-lowering therapy.²¹² Owing to differences in technique, this case cannot be readily compared to the more recent series.

The electroencephalogram may show diffuse slowing or a variety of epileptiform discharges. The latter do not always correlate with the occurrence of seizures. Central conduction times may be slowed in somatosensory and visual evoked potentials.²¹³ The audiologic profile, in particular the acoustic reflex, may provide evidence of brain stem dysfunction early in the course of the illness.²¹⁴ Changes in the brain stem auditory-evoked response begin with inversion of the wave V:I amplitude ratio, followed by increase in the wave I to V interpeak latency, progressing, finally, to loss of late wave forms.

Several characteristic pathologic findings have been described (see “Pathology” above). Foam cells and sea-blue histiocytes have been detected in many tissues in NP-C, but they are nonspecific.²¹⁵ Failure to demonstrate these cells in biopsies does not rule out the diagnosis of NP-C. Cases have been reported in which bone marrow biopsy was initially unrevealing,²¹⁶ only to show storage cells when the test was repeated years later.²¹⁷ Ultrastructural studies of tissue biopsies are more specific than light microscopy, and may strongly support the diagnosis of NP-C.^{99, 218} In early onset cases in which a liver biopsy is performed, the diagnosis of NP-C can rapidly be confirmed using as little as 5 mg of frozen tissue analyzed by thin-layer chromatography of the lipids.

Biochemical Diagnosis

The diagnosis of NP-C should no longer rely on histopathologic or tissue lipid findings alone. The discovery of abnormal LDL-cholesterol processing has established the rationale for specific biochemical testing.^{32, 144} The tests are usually performed on cultured fibroblasts,^{32, 36, 88, 110, 219, 220} although lymphocytes have been used.²²¹ To date, the diagnosis is best achieved by the concomitant demonstration of (a) intralysosomal accumulation of unesterified cholesterol as shown by a characteristic pattern of intense perinuclear fluorescence after challenge with LDL-enriched medium and staining with filipin (Fig. 145-8B), and (b) abnormal intracellular cholesterol homeostasis as defined by impaired LDL-induced cholesterol esterification.^{36, 60, 220} A majority of NP-C cell lines (approximately 80 percent) express profound alterations of cholesterol homeostasis (classical biochemical phenotype),^{32, 36, 52, 88, 110, 223} but some have milder changes, especially in their esterification ability (intermediate and variant phenotypes).^{36, 52, 130, 154, 219, 220, 224, 225} The filipin test is more sensitive than cholesterol esterification assays in detecting patients with the variant phenotype.^{36, 60, 154, 220, 226} Filipin staining also increases specificity because impairment of cholesterol esterification may be found in other disorders such as acid lipase deficiency, familial hypercholesterolemia, and I-cell disease. The filipin pattern in I-cell disease is abnormal, but can be differentiated from NP-C in expert hands.^{36, 227} In the experience of one author (M.T.V.), slight lysosomal cholesterol storage may be seen in cells from patients with sphingomyelinase-deficient types of Niemann-Pick disease.

In comprehensive investigations, no strict correlation has been observed between the severity of alteration in intracellular cholesterol homeostasis and the clinical phenotype, especially in the late infantile and juvenile onset forms.^{69, 74, 220} A trend for adult onset patients to show a “variant” biochemical phenotype has been reported.⁷⁴ On the other hand, a consistent biochemical phenotype has been the rule within a sibship.^{36, 84}

More than half the obligate heterozygotes tested show a level of LDL-induced lysosomal cholesterol accumulation and possibly of cholesterol esterification intermediate between normal individuals and affected homozygotes.^{36, 52, 74, 143, 220} Abnormalities in a few cases may be as pronounced as in some “variant” patients.²²⁶ The remaining heterozygotes overlap with controls. Thus, generalized NP-C carrier

screening using this strategy is not possible. Typical storage cells in bone marrow or inclusions in skin biopsies have been reported in a few parents of children with NP-C.^{122, 123}

Provided marked abnormalities (classical phenotype) have been demonstrated in the index case, similar tests can be used for prenatal detection of affected fetuses using cultured chorionic villus cells or amniotic fluid cells.^{37, 228} Because some heterozygotes may show significant alterations, prior study of both parents is advisable. By the summer of 1998, at least 150 at-risk pregnancies had been monitored in our laboratories and in those of Drs. D. Wenger, M. Ziegler, A. Fensom, and O. Van Diggelen.

Analysis of the lipids in a frozen liver biopsy (see “Tissue Lipids” above) is generally diagnostic, but occasionally may be inconclusive. Partial deficiency of sphingomyelinase activity in cultured skin fibroblasts is nonspecific and inconstant. Chitotriosidase has been found to be modestly elevated in plasma in several lysosomal diseases, although marked elevations of diagnostic value are seen only in Gaucher disease.²²⁹ The usefulness of this enzyme as a disease marker is diminished by its relatively frequent deficiency in the general population.²³⁰

Molecular Diagnosis

In NP-C patients belonging to complementation group 1, at least four laboratories have initiated mutational analysis of the *NPC1* gene. In the *NPC1* cloning paper,⁴³ eight different mutations were reported. Most of the patients were compound heterozygotes. Subsequently, the mutation in the Nova Scotia isolate of NP-C was identified.²⁰⁰ Preliminary results from the authors' laboratories and the laboratory of Dr. K. Ohno in Japan have shown that a transcript is made in most patients and that mutations occur at various locations of the gene. Most of the mutations appear to be private. A number of polymorphisms have already been observed. Consequently, with current technology, direct molecular diagnosis is not practical and may be misleading, except in specific populations such as the French Acadians in Nova Scotia and the Hispanics from southern Colorado. Nevertheless, identification of the mutation(s) in a demonstrated NP-C case is important for subsequent genetic counseling in the proband's family. Finding the mutations will make prenatal diagnosis possible for the so-called “variant” families, in which biochemical tests were not reliable. In other families, this would permit prenatal diagnosis using uncultured chorionic villi, and thus provide a result a month earlier than traditional biochemical methods. Mutational analysis would also allow accurate heterozygote testing in the proband's family.

TREATMENT

There is no specific treatment for NP-C. The identification of *NPC1* and its predicted protein product will eventually lead to new therapeutic strategies. The *NPC1* gene product appears to be an integral membrane protein, which is not likely to be readily replaced from exogenous sources including tissue transplantation. Indeed, attempts at nonspecific replacement therapy by tissue transplantation have been disappointing. In the C57BLKS/J murine model of NP-C, bone marrow transplantation^{231–233} and combined liver and bone marrow transplantation²³³ partially reversed tissue storage of cholesterol and sphingomyelin, but did not influence the continued neurologic deterioration.

Liver transplantation in a 7-year-old girl with NP-C and cirrhosis was similarly successful in restoring hepatic function, but failed to slow neurologic progression.²³⁴ There has been one report of transplantation of fetal liver cells into BALB/c mutant mice, with apparent correction of the phenotype in many cells.^{235, 236} This study, which has not been replicated, must be interpreted cautiously, as it was performed prior to description of the murine ortholog of *NPC1*, so that genotyping of the mice prior to transplantation was not possible. Transplantation of fetal liver cells has been used in two humans.²³⁷ The

one patient for whom follow-up was available to us was engrafted twice at the ages of 8.5 and 9 years, without significant improvement of the natural history of the disease. To date, there have been no reports of bone marrow transplantation in human NP-C.

Treatment strategies to reduce intracellular cholesterol accumulation were formulated based on the hypothesis that cholesterol is an offending metabolite in NP-C. Several lines of evidence supported this assumption. Cholesterol is stored in excess in the nonneural tissues in NP-C, and the defect in cholesterol trafficking is closely associated with the primary mutation. Combinations of cholesterol-lowering agents were found to reduce hepatic stores of unesterified cholesterol in NP-C patients,²³⁸ but there is no evidence that their outcome was improved, and several patients who elected to continue the diet long-term have subsequently succumbed to NP-C. There is a case report of a child treated with a cholesterol-lowering regimen in whom serial magnetic resonance spectroscopy studies were interpreted as showing diminution of stored cerebral lipids.²¹² Follow-up data have not been published. Cholesterol-lowering drugs may be ineffective in influencing neurologic disease because cholesterol is not an offending metabolite in the brain, or, alternatively, because the agents employed do not penetrate the blood-brain barrier. Dimethyl sulfoxide, which corrects partial sphingomyelinase deficiency²³⁹ and substantially reverses the cholesterol-trafficking abnormalities²⁴⁰ in NP-C fibroblasts, has been used as a single agent in two patients. Stabilization of neurologic disease was reported in an 8-year-old girl,²⁴¹ but a 3-year-old boy showed no apparent benefit.²⁴²

Regardless of the availability of specific treatments for NP-C, a great deal can be offered to patients and their families in the form of symptomatic management. We found that dystonia and tremor respond to anticholinergic agents in some patients, and seizures can be controlled or diminished in frequency with antiepileptic drugs, at least early in the course of the illness. No controlled studies of the effectiveness of such treatments are available. Protriptyline,^{78, 79} clomipramine, and modafinil are effective in controlling cataplexy and managing accompanying sleep disturbances.

Therapy to delay or prevent puberty has been proposed as a means of ameliorating the progress of NP-C. The rationale for this approach is based on the observations (a) that progesterone can induce an NP-C like phenotype in control fibroblasts,¹⁵⁹ (b) that progesterone exacerbates the biochemical abnormalities in NP-C fibroblasts,²⁴³ and (c) that symptoms of NP-C often worsen at the time of menarche. Gonadotrophin-releasing hormone analogs have been administered to some girls in an uncontrolled fashion. No data on clinical efficacy are available.

Two siblings with late onset disease, presenting with psychiatric symptoms, were treated with prednisolone with apparent improvement in symptoms, and recurrence of symptoms after steroid withdrawal.²⁴⁴

Physical therapists, speech therapists, and occupational therapists should all be involved in the care of the patient with NP-C, in order to address problems in the areas of impaired mobility, contractures, dysarthria, dysphagia, and occupation. Medical therapy notwithstanding, support of the patient and family in the course of NP-C, following long-established principles,²⁴⁵ is of paramount importance. Counseling services should be available for patients and family members. Support for families with NP-C is available through umbrella organizations for children with inherited or metabolic diseases in many countries. Support groups devoted to Niemann-Pick disease have been established in the U.S. (<http://www.nnpdf.org>), the U.K. (<http://www.nnpdf.org/npdg-uk>) France (<http://www.aafnp.org>), and Germany (<http://www.niemann-pick.de/wirveberuns.html>).

Animal models will be critical for future studies of potential therapies. The genotyping of the BALB/c murine model of **NP-C** will facilitate its use in such experiments. Prior studies focused on reduction or removal of the accumulated offending metabolite. A new approach that has shown promise in the murine model of Tay-Sachs disease is to inhibit synthesis of a known offending metabolite, in this case G_{M2} ganglioside, prior to its accumulation.²⁴⁶ Given the evidence for the role of G_{M2} ganglioside in NP-C, a trial of this agent (*N*-butyldeoxynojirimycin) in the *npc^{nih}* mouse may be worthwhile.

ADDENDUM

Since the manuscript for this chapter was submitted, substantial advances have occurred in our knowledge of **NP-C**. These are summarized in the following paragraphs.

PATHOLOGY

Falk and co-workers²⁴⁸ investigated developmental expression of the **NPC1** (*Npc1*) mRNA with a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). The transcript was expressed in the cerebellum in vivo and in vitro during early postnatal life, as well as in the adult cerebellum. In in vitro studies, Purkinje cells and some glial cells were immunoreactive to the antibody to anti-*Npc1* antibody. They concluded that since *Npc1* is expressed at similar levels throughout the development in both neurons and glia, the vulnerability of Purkinje cells in **NP-C** is likely to involve disruption of an interaction with other developmentally regulated proteins. In mouse brain, Prasad and co-workers²⁴⁹ detected *Npc1* mRNA in neurons as early as embryonic day 15. In this study, the **NPC1** gene was widely expressed in neurons, the highest levels occurring in the cerebellum and pons. The high expression in the cerebellum may account for the vulnerability of the Purkinje cells in NP-C.

Henderson et al.²⁵⁰ demonstrated that embryonic striatal neurons in primary cultures from *npc^{nih}* mice can take up LDL-derived cholesterol but with significantly lower hydrolysis and re-esterification than in wild type neurons. Cholesterol is thought to be critical for membrane localization and function of many signaling proteins in membrane domains or "lipid rafts." These cultured **NP-C** neurons failed to increase neurite outgrowth in response to brain-derived neurotrophic factor (BDNF), in contrast to the response of wild type neurons. Western blotting revealed that Trk receptor expression was similar in striatal neurons from wild type and mutant mice, yet BDNF-induced tyrosine phosphorylation of Trk was essentially eliminated in neurons from *npc^{nih}* mice. Trk activation was also abolished in cholesterol-depleted wild type neurons. Thus, abnormal cholesterol metabolism occurs in neurons of *npc^{nih}* mice, even at embryonic stages, and is associated with notable lack of BDNF responsiveness. Given the importance of BDNF in neuronal survival, growth, and differentiation, the lack of BDNF responsiveness might contribute to the loss of neural function in NPC.

In contrast to the diffuse expression reported in rodents, an immunoelectron microscopic investigation of the brains of macaque monkeys found that **NPC1** was expressed predominantly in astrocytic process located in the presynaptic region, rather than in neurons.²⁵¹ These investigators also reported that the terminal fields of axons and dendrites were the earliest sites of degeneration in *npc^{nih}* mice, based on studies with amino-cupric-silver staining. This work remains unconfirmed to date.

Wu and co-workers²⁵² have found that increasing numbers of neurons were lost by apoptosis in **NP-C** brains with progression of the disease process in both humans and mice. Apoptosis was demonstrated in Purkinje cells in the cerebellar vermis, in cerebral cortical neurons, and in some cells in the white matter as early as 30 days of age in NP-C mice, prior to detectable neurological symptoms. The numbers of apoptotic cells increased with age.

In **NP-C** brains, gangliosides G_{M2} and G_{M3} accumulate. The possible role of these accumulated gangliosides in the dysfunction and eventual death of neurons in NP-C was tested by generating double knockout mice. *npc^{nih}* and *GalNAcT -/-* mice (that lack the activity of the β -1,4-GalNAc transferase responsible for the synthesis of complex gangliosides) were interbred, and the clinical, pathologic, and biochemical characteristics of their double knockout offspring were studied.²⁵³

Positive stains for filipin and PAS best demonstrate neuronal storage in *npc^{nih}* mice. Neither filipin nor PAS stains were positive in the neurons of the double knockout mice, indicating absence of neuronal storage. Electron microscopic study found no neuronal inclusions typical of **NP-C**, and thin layer chromatography confirmed the absence of G_{M2} and complex gangliosides in the double mutant brain. The double knockout mice showed no improvement of the clinical course or neuronal degeneration compared to *npc^{nih}* controls. Macrophages and microglia containing cholesterol were still numerous in the brain, and visceral pathology was unchanged. Apoptotic neuronal loss appeared more pronounced and loss of Purkinje cells was clearly observed in the double knockouts by comparison with *npc^{nih}* controls. The absence of G_{M2} ganglioside storage from conception does not significantly influence the clinical phenotype of the NP-C in this model system.

Animal Models of NP-C

The feline model of **NP-C** was demonstrated to belong to the **NPC1** complementation group²⁵⁴ and the generation of mutations of the homologue of *NPC1* in the nematode *C. elegans* has provided a new model system allowing studies of NP-C disease in a thoroughly characterized animal that reproduces rapidly.²⁵⁵

PATHOPHYSIOLOGY

Cell Biology

The exact function of the **NPC1** gene product is not yet elucidated and this area is under active investigation by several groups. A clear consensus has yet to emerge, but major findings may be summarized as follows.

Neufeld et al. found that the **NPC1** protein was localized to late endosomes immunocytochemically and that the clearance of endocytosed ^{14}C -sucrose as well as cholesterol was defective in **NP-C** cells studied biochemically.²⁰² Both general retroendocytic trafficking and mobilization of multiple lysosomal cargo appeared defective at a late endosomal trafficking step. This mechanism would account for the accumulation of multiple lipids in NP-C cells and tissues. Site-directed mutagenesis of the NPC1 protein targeting domains of the protein responsible for its cholesterol transport function caused loss of function.²⁵⁶ Confocal microscopy of living cells, using an NPC1-green fluorescent chimeric protein, documented an unusual mode of tubular membrane trafficking for the NPC1 endocytic compartment that appears linked to the NPC1 protein and cellular cholesterol content.²⁵⁷ Using cytochemical techniques, it was found that cellular cholesterol levels modulate the glycolipid profile of the *NPC1* compartment. The results indicate that the *NPC1* compartment serves as a sorting station in the endocytic trafficking of both cholesterol and glycolipids. Enriching the cholesterol content of lysosomes recruits the NPC1 protein into endocytic vesicles containing glycolipids. In the presence of elevated cholesterol levels, certain glycolipids are restricted from entering the lysosomal compartment for degradation and are efficiently recycled in NPC1 sorting vesicles to the plasma membrane. Glycolipids that accumulate in NP-C cells and tissues, such as G_{M2} , are those sorted through the NPC1 compartment, while non-accumulating glycolipids such as CTH and G_{D3} are shown to traffic on to the lysosomes for probable degradation.²⁵⁷ Other workers have drawn similar conclusions.^{258–260}

Studies in CT60 and CT43 CHO mutants²⁶¹ disclosed that the initial movements of LDL-derived cholesterol to the plasma membrane did not require participation of the **NPC1** protein. The authors concluded that the NPC1 protein cycles cholesterol from an intracellular compartment to the plasma membrane or to the endoplasmic reticulum after (and not prior to) newly hydrolyzed LDL-derived cholesterol appears in the plasma.²⁶¹ A further report concluding that cholesterol moves freely from the lysosomes to the plasma membrane in **NP-C** cells has appeared.²⁶² The latter authors considered that cholesterol accumulation in NP-C lysosomes results from an imbalance in the brisk flow of cholesterol among membrane compartments. While most authors consider that in NP-C cells, cholesterol essentially accumulates in lysosomes,^{202, 257, 262} two reports conclude that accumulation takes place in late endosomes.^{261, 263}

Cholesterol balance and metabolism were quite extensively studied in mutant *npc^{nih}* mice. The cholesterol pool was found to expand continuously from birth in homozygous affected animals.²⁶⁴ While cholesterol entering tissues through the coated-pit pathway became sequestered in the lysosomal compartment and was metabolically inactive, cholesterol that was newly synthesized or that entered cells through the SR-BI pathway was metabolized and excreted normally.^{265, 266}

Several reviews have addressed the subject of the role of the **NPC1** protein in the broad context of intracellular cholesterol trafficking.^{267–269} In general, current opinion favors a key role for the NPC1 protein in modulating vesicular trafficking of both glycolipids and cholesterol.

MOLECULAR GENETICS

NPC1 Gene Organization, **NPC1** Protein Structure and Topology, Functional Domains, and **NPC1** Mutations

The genomic organization of **NPC1** is now known. The gene spans greater than 47 kb and contains 25 exons.²⁷⁰ Key promoter regions have been defined.^{270, 271} The NPC1 protein appears to be N-glycosylated, and shows a size of 170 and 190 kDa by western blotting, in CT-60 CHO cells transfected with wild-type NPC1²⁵⁶ or as native protein in human skin fibroblasts.²⁷² There is further evidence that the NPC1 protein resides in late endosomes and interacts transiently with lysosomes and the *trans*-Golgi network.²⁵⁸ Topological analysis of NPC1 has revealed that this glycoprotein contains 13 transmembrane domains, 3 large and 4 small luminal loops, 6 small cytoplasmic loops, and a cytoplasmic tail.²⁷³ The putative sterol-sensing domain has the same orientation as those in HMG-CoA reductase and **SCAP**.²⁷³ Information regarding the functional role of different domains of the protein has been deduced from clinical studies, and by transfection of CT-60 cells with mutant NPC1. The N-terminal domain and the lysosome-targeting motif appear essential for cholesterol mobilization.²⁷⁴ Mutations in the leucine-zipper motif and sterol-sensing domain inactivate the protein.²⁵⁶ Homozygote missense mutations in the sterol-sensing domain correlate with a severe infantile neurological onset disease and absence of detectable NPC1 protein.^{275, 276} Studies in patients revealed that a cluster of mutations was located on the third large luminal, cysteine-rich loop, which is highly conserved in NPC1 orthologues, and has a sequence showing resemblance to the RING finger motif of protein kinase C.²⁷⁷ This domain was shown to bind zinc²⁷¹ and also appears of particular functional interest.

Taking into account recent studies^{201, 272, 275, 277, 278} and data from seven groups presented in the International Workshop “The Niemann-Pick C lesion and the role of intracellular lipid sorting in human disease” (National Institutes of Health, Bethesda, MD, October 1999), the number of **NPC1** mutations known to date exceeds 100. Mutations have been found widely distributed on the gene (leucine zipper domain excepted), with, however, about 1/3 of them located in the cysteine-rich luminal loop. With few exceptions, most mutations appear to be private. In Caucasians, only one common mutant allele, I1061T,

has been identified.²⁷⁸ This mutation, which in the homozygous state correlates with a juvenile neurological onset form of the disease, constitutes about 15% of alleles in patients of Western European (especially British and French) descent. The same mutation is prevalent in the Hispanic-American isolate of Colorado and New Mexico, suggesting a founder effect.²⁷⁸ Mutational analysis of the *NPC1* gene is complicated by the large number of polymorphisms already recognized.^{201, 270}

NPC1 Gene Homologues

By EST screening, a new gene with homology to *NPC1*, named *NPC1L1*, has been isolated. It has been suggested that *NPC1* and *NPC1L1* form part of a new family of proteins that may have similar functions at different subcellular locations.²⁷⁹

TREATMENT

Bone marrow transplantation was performed on an NP-C patient aged 3 years and 5 months. Regression of hepatosplenomegaly and decreased infiltration of foamy macrophages in the bone marrow and lung were described 6 months after the transplant. Predictably, given the nature of the *NPC1* gene product, and the early onset of disease in this patient, neurological status continued to deteriorate, with progressive brain atrophy on serial magnetic resonance imaging (MRI). The authors concluded that bone marrow transplantation is unlikely to be an adequate treatment for NPC.²⁸⁰

Erikson et al.²⁸¹ reported that modifications of somatic cholesterol do not significantly alter the neurological course in *npc^{nih}* mice, confirming earlier studies.

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