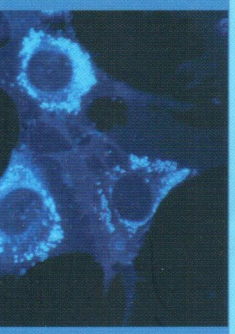
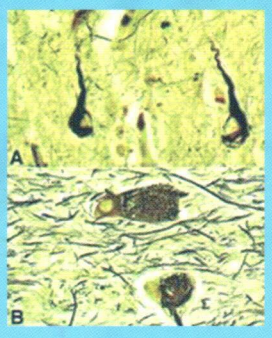
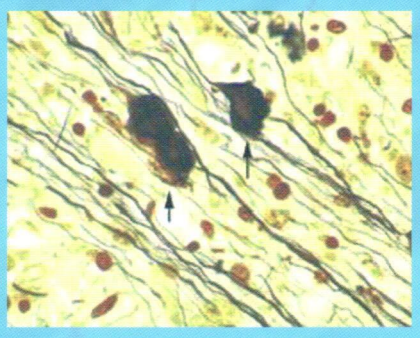
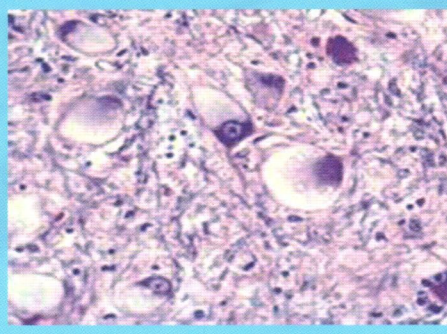
Understanding Niemann-Pick disease type C and its potential treatment









J. E. Wraith and J. Imrie

Understanding Niemann–Pick disease type C and its potential treatment

Blackwell Publishing

J. E. Wraith and J. Imrie

Blackwell Publishing, Inc., 350 Main Street, Malden, Massachusetts 02148-5020, USA
Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK
Blackwell Publishing Asia Pty Ltd, 550 Swanston Street, Carlton, Victoria 3053, Australia

ISBN: 978-1-4051-5702-5

Set in Franklin Gothic Medium Condensed by Sparks, Oxford -- www.sparks.co.uk

Printed and bound in the UK by rpm print and design, Chichester

Niemann-Pick
DISEASE GROUP (UK)
Registered Charity No. 1061881

Associazione
Italiana
Niemann Pick
ONLUS

Selbsthilfegruppe e.V.
Deutschland
Niemann-Pick

This publication has been made available through an educational grant from Actelion Pharmaceuticals Ltd

Contents

Glossary of Terms

Term	Definition
Amyloid precursor protein	APP; protein from which beta-amyloid (the main component of neuritic plaques in Alzheimer's disease) is derived by proteolysis
Ascites	Abnormal accumulation of serous fluid between tissues and organs
Ataxia	Inability to coordinate voluntary muscular movements
Atonia	Lack of physiological (muscular) tone
Autophagy	Digestion of cellular constituents by enzymes of the same cell
Cataplexy	Sudden loss of muscle control/tone while conscious, following emotional stimulus
Dysarthria	Difficulty in articulating words
Dysphagia	Difficulty in swallowing
Dystonia	Abnormal tonicity of muscle with involuntary sustained muscle contractions
Ectopic dendritogenesis	Formation/outgrowth of new, malformed (ectopic) dendrites from neurones
Endoplasmic reticulum	Membranous/vesicular network involved in intracellular transport of materials
Filipin	Antifungal agent used in fluorescent diagnostic staining of cultured NPC fibroblasts
Foam cell	A swollen reticuloendothelial cell filled with lipid-laden vacuoles
Gelastic [cataplexy]	Cataplexy associated with mirthless laughter due to strong emotional stimulus
Glycoprotein	Conjugated protein in which the non-protein group is a carbohydrate
Glycosphingolipid	Carbohydrate-attached lipids (glycolipids) containing sphingosine
Hypocretins (orexins)	Excitatory neuropeptide hormones involved in sleep regulation
Hypotonia	A state involving general or localised low muscle tone
Lipoprotein	Conjugated protein with lipid portion; transports ingested lipids from food
Lysosome	Sac-like intracellular organelle that contains various hydrolytic enzymes
Meganeurite	Swollen, distended portion of a neurone occurring in neuropathological conditions
Miglustat	*NB*-DNJ, N-butyldeoxynojirimycin, OGT918; a small iminosugar molecule that inhibits glycosphingolipid synthesis
Neurofibrillary tangles	Pathological protein aggregates found in neurones in neurological disease
NPC (or NPA or NPB)	Niemann–Pick disease type C (or A or B)
NPC1 and NPC2	Genes in which mutations give rise to NPC
Sphingomyelinases	Group of enzymes that catalyse the hydrolysis of sphingomyelin
Saccades (saccadic)	Small, rapid movements of the eye as it jumps from one fixation point to another

Niemann-Pick Disease Type C: A Scientist's Perspective

Niemann-Pick type C disease (NPC) is a devastating and very rare genetic lysosomal storage disorder characterised by a wide range of clinical manifestations. It is inherited in an autosomal recessive manner, with an estimated prevalence of approximately 1 : 150,000. However, this may be an underestimate as signs and symptoms as well as biochemical findings are not always disease specific. Cases have been identified post mortem following stillbirth due to foetal ascites and liver disease at one end, and in the sixth decade of life with relatively few, but severe symptoms at the other extreme. Cases can present at all levels and across all disciplines of medicine including hepatology, paediatrics, haematology, adult neurology and psychiatry.

So far there are over 250 mutations known to cause NPC, which leads to challenges in counselling the families of affected individuals. Education and increased awareness of this devastating disease is vital at all levels of healthcare, in order to diagnose cases quickly and to ensure proper counselling and provision of appropriate symptom control therapies. Ideally, those affected by NPC should be managed at a specialist centre for inborn errors of metabolism, where multidisciplinary care can be offered.

The social and healthcare impact of NPC cannot be overestimated, and patient associations and family support groups play a vital role. With continued efforts, it is to be hoped that the future will lead to a cure for NPC. Meanwhile international research is in progress toward the development of further therapies that could play a huge role in the fight against this disease.

Dr Ed Wraith, MB, ChB, FRCP, FRCPCH
Consultant Paediatrician
Ed.Wraith@CMMC.nhs.uk

Jackie Imrie, SRN, RSCN, MSc
Clinical Nurse Specialist Niemann-Pick Disease
Jackie.Imrie@CMMC.nhs.uk

Willink Biochemical Genetics Unit
Royal Manchester Children's Hospital
Pendlebury
Manchester
UK

A Call to Action: The Patient Association View

When a child or individual is diagnosed with NPC it adversely affects the whole family. Their hopes and dreams for the future are destroyed: they suddenly have to face the fact that their child or family member will die prematurely, but no one can tell them when or how. The path to this diagnosis is often a difficult one, due to the rarity of the condition and the fact that most people, including medical professionals, know little about it. This can lead to additional problems such as conflicting advice and a lack of clear information. Health and social care services can vary around the country, leaving families feeling bewildered and isolated.

For many families, a diagnosis of NPC does not happen instantly, but is the point from which they begin to contemplate their future. In order to understand the nature of the disease, families need accurate, clear information that they can relate to. During this period of adjustment, families face a neverending series of appointments with the long list of professionals who will now be involved in caring for their loved one. As the condition is so rare, they will invariably have to tell their story time and time again. This in itself can be extremely difficult; families often find they know more about the condition than the professional.

As the disease progresses, those affected by NPC develop complex needs that can change rapidly. Families struggle to cope with the range of emotional and financial implications, which can severely affect the quality of family life.

We aim to make a difference to those affected by NPC through the provision of care, support and accurate information. Our associations give practical help as well as emotional support, and have developed a strong family support network in efforts to reduce feelings of isolation and despair.

Working in collaboration with Patient Groups across the world, we also aim to raise awareness of the disease, its clinical manifestations and its impact. Niemann-Pick Patient Associations share information and resources to maintain up-to-date, useful data for professionals. We encourage local, national and supranational authorities to improve patients' quality of life, keeping attention alive on the impact of this very rare disease on affected families and on society.

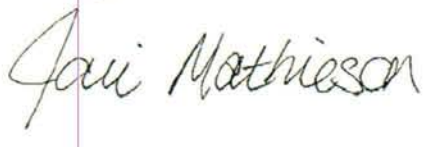
The promotion of research that will improve understanding of NPC and aid in the development of new therapies is crucial. Research is currently taking place, both into the causes of the disease and into potential treatments. We would like to ensure that in the future, families affected by NPC may be given the hope of a treatment – or even a cure – for this life-limiting condition.

Luigi Bonavita
Chairman
Associazione Italiana Niemann Pick e Malatti Affini (IT)

Gabriele Grillenberger
Chairman
Niemann-Pick Selbsthilfegruppe e.V. (Germany)

Edmund Fabianski
Treasurer
Niemann-Pick Selbsthilfegruppe e.V. (Germany)

Toni Mathieson
National Development Manager
The Niemann-Pick Disease Group (UK)



Niemann–Pick Disease in Summary

NPC is an extremely rare genetic disorder arising from neuronal accumulation of glycosphingolipids, due to abnormal lipid trafficking.[1] Cholesterol that enters cells from the circulation through the low-density lipoprotein (LDL) receptor is not processed in a timely, normal manner in parenchymal organs and the central nervous system (CNS). Lipids accumulate in toxic quantities as unesterified cholesterol, sphingomyelin, phospholipids and glycolipids, causing structural and functional damage in cells and tissues.[1,2]

Niemann–Pick type C disease (NPC) is pan-ethnic and arises sporadically across populations, regardless of race, although genetic isolates have been identified in Nova Scotia, Colorado, and New Mexico.[1] Based on published estimates,[3] it is believed that NPC arises in 1:150,000 live births. However, it is considered very likely that this is an underestimate due to a mixture of factors – chiefly, failure to recognise the clinical phenotypes and a lack of definitive diagnostic tests.

Fatal in most cases, NPC has an extremely heterogeneous clinical presentation, but is characterised by a range of progressive neurological problems that become severe and limiting at late stages of the disease.[1] It can present, either with or without associated hepatosplenomegaly in infants, children or adults,[1,4] and is characterised by eye movement abnormalities, dysphagia and dysarthria, ataxia and progressive cognitive dysfunction leading to dementia. The plight of patients with NPC is perhaps best represented by the patient journey in the 'classical' phenotype of NPC.

Classical NPC: the patient journey

The products of normal pregnancies, children affected with 'classical NPC' often show transient neonatal jaundice. Development in early childhood is usually unremarkable, although the child may be labelled as ill-behaved at kindergarten or school. It may be years before the child begins to show signs of progressive dementia, by which time they can often become known as clumsy, suffering frequent falls before overt ataxia is recognised. Eye blinking or head thrusting on attempted vertical gaze then become apparent, and gelastic cataplexy (limited or generalised atonia accompanied by mirthless laughter) may be seen, with manifestations ranging from head nodding to atonic collapse.

Dysarthria, dysphagia and drooling can contribute to educational and relational problems by impairing communication. Dystonia then appears, initially with posturing of a hand or foot during walking or running, and gradually becomes generalised. Seizures can also arise during childhood or later. Enlargement of the liver or spleen is often first detected in early childhood and progresses over time in some cases. However, hepatosplenomegaly remains undetected in at least 10% of cases.

The child then begins to suffer increasing physical and intellectual disability through late childhood and adolescence, eventually becoming chair-bound and incapable of continuing at normal school due to cognitive deterioration. Psychiatric disturbances including psychosis may occur around puberty.

Severe dysphagia now begins to endanger the child's nutritional status, and the upper airway becomes vulnerable to frequent aspiration. In many cases spasticity or rigidity (or both) add to the burden of nursing care. Eventually many patients die in the teenage years or early adulthood. Death is frequently due to pulmonary complications such as aspiration pneumonia.

Major advances in diagnostics, a greater awareness of common and variant clinical manifestations, and the possible arrival of the first specific therapies for NPC now offer new hope for the improved recognition and management of this tragic disease.[1,5]

History: Niemann–Pick Disease Types and Nomenclature

In 1914, German paediatrician Albert Niemann (1880–1921) described a young child with brain and nervous system impairment and hepatosplenomegaly.[6] Later, in the 1920s, Ludwig Pick (1868–1944) studied tissues after the death of such children and provided evidence of a new disorder, distinct from lipid storage disorders described previously.

In 1958, Crocker and Farber published a case series on patients with varied presentations of Niemann–Pick disease, according to diagnoses based on the presence of 'foam cells' (lipid-laden macrophages) and increased tissue sphingomyelin.[7] Because their patients included children with characteristic neurological symptoms as well as children showing little or no neurological symptoms, Crocker later classified Niemann–Pick disease into four separate categories based on biochemical and clinical criteria.[8]

Group A (Niemann–Pick disease type A [NPA]) included patients with classic neurodegenerative disease leading to death in early infancy, Group B (NPB) included those showing organomegaly without nervous system involvement, Group C (NPC) patients showed slowly progressive neurologic illness.[8] Group D (previously known as NPD) closely resembled Group C except that it was restricted to a genetic isolate from Nova Scotia.[8] Importantly, it was noted that non-neural tissues in NPC and NPD patients had relatively less sphingomyelin and more cholesterol storage compared with NPA and NPB. More recent research has shown that this was a highly relevant observation, as it reflects substantial differences in the underlying biochemical defect and pathophysiology of NPC compared with NPA and NPB (see *Pathophysiology, p. 12*).

In 1966, Kanfer *et al.* demonstrated that the primary biochemical defect in NPA and NPB (but not NPC) was severe generalised sphingomyelinase deficiency.[9] This early finding, coupled with the observed accumulation of multiple complex glycosphingolipids in NPC (i.e., not only sphingomyelin), indicated that NPC may be considered as a separate entity from NPA and NPB. In 1982 an expert consensus decision was taken in Prague to formally separate NPC from types A and B.[10]

Niemann–Pick diseases are still classified today according to Crocker,[8] although significant advances in knowledge on the genetic causes and underlying pathophysiological mechanisms have been achieved. In particular, characterisation of the genes responsible for each subtype have established that NPC is distinct at the molecular and biochemical levels from NPA and NPB, in line with differences seen clinically (Table 1; see *Pathophysiology, p. 12*).

Table 1. Niemann–Pick disease types[4,11]

Niemann–Pick disease type	Distinguishing feature	Primary pathophysiology	Genetic cause
Type A	Infant disease with very poor prognosis	Acid sphingomyelinase deficiency	*SMPD1* gene mutation
Type B	Juvenile form with lung involvement	Acid sphingomyelinase deficiency	*SMPD1* gene mutation
Type C	Pan-ethnic subtype with non age-related occurrence and brain complications	Defect in cellular cholesterol trafficking	*NPC1* or *NPC2* gene mutation
Type D*	Same as Type C but with Nova Scotian ancestry	Defect in cellular cholesterol trafficking	*NPC1* gene mutation

**Type D should no longer be regarded as a separate subtype; it is biochemically and clinically indistinguishable from NPC.*

Based on molecular genetic testing, NPC is now divided into two subtypes – NPC1 and NPC2 – as each is caused by a different gene mutation (see *Genetics of NPC, p. 9*). These names are currently preferred, as they relate back to the mutated genes responsible for the clinical phenotype. Niemann–Pick disease type D (NPD), which is still sometimes used to describe the genetic isolate from Nova Scotia, should no longer be considered as a separate

condition as it is biochemically and clinically indistinguishable from NPC, and is now known to result from mutations in the *NPC1* gene.[1,4,12]

In 1984, Pentchev *et al.* reported defective cellular esterification of exogenous cholesterol in a BALB/c mouse model of NPC.[13] This was a seminal study as an identical defect was later shown in human NPC, and it led to the elucidation of unique abnormalities in the intracellular trafficking of LDL-derived cholesterol, with sequestration of unesterified cholesterol in cell lysosomes in various tissues (see *Pathophysiology, p. 12*).[1,14,15] Diagnostic strategies for the identification of cholesterol trafficking deficits soon followed, allowing earlier identification of NPC patients.[16] Researchers were also able to map the gene now known to be responsible for the majority of cases of NPC.[17,18]

The following sections of this book focus on NPC, its genetic, biochemical and clinical profile, its impact on patients, caregivers and healthcare, and its clinical management. However, NPA and NPB are mentioned briefly, where relevant, for completeness and context.

Genetics of NPC

In 1997, researchers identified the location of the *NPC1* gene on chromosome 18 (Table 2).[18] Complementation studies, linkage analysis and mutation scanning studies have established that alterations or mutations in this gene cause the vast majority (90–95%) of cases of NPC.[17,19–21] Notably, genetic linkage studies by Greer *et al.*[20] established that the gene mutation responsible for so-called NPD was in fact an allelic variant of *NPC1*.

In a second, much smaller, group of patients with NPC, mutations in *NPC1* do not cause the disease. A second gene – *NPC2* – is responsible for the NPC phenotype in these patients. HE1, mapped to chromosome 14, was recently identified as the gene underlying the *NPC2* phenotype (Table 2).[22,23]

Table 2. Molecular genetics of NPC (adapted from Patterson[4])

Gene symbol	Chromosomal locus	Frequency/mutations	Protein name
NPC1	18q11–q12	90% of cases (sequence alterations)	Niemann–Pick C1 protein
NPC2	14q24.3	4% of cases (sequence alterations)	Epididymal secretory protein E1

Sources: Gene symbol from HUGO gene nomenclature committee; chromosomal locus, locus name, critical region and complementation group from Online Mendelian Inheritance in Man database (OMIM); protein name from Swiss-Prot protein knowledgebase.[4]

Although there is currently no direct evidence indicating other gene loci for NPC, some patients exhibiting the typical clinical and biochemical NPC phenotype have been shown not to possess mutations in either *NPC1* or *NPC2*, and this has led researchers to suggest that other NPC gene loci may exist.

Mutations

NPC1

The *NPC1* gene, mapped to chromosome 18 (18q11–q12) contains 25 coding regions (exons), varying in size from 74 to 788 bp and spread over 47 kb.[24] More than 50 exonic polymorphisms have been described that do not give rise to any pathologic effect – they are simply non-adverse genetic variants.[25] However, approximately 200 allelic variants have been identified for *NPC1* that are suspected to have pathological effects.[26,27]

Most affected individuals with *NPC1* mutations are compound heterozygotes (i.e., possess two different abnormal alleles, one on each of a chromosome pair) with single-base (point) mutations producing mis-sense mRNA transcripts that are unique to their family.[25] Nonsense mutations, base-pair deletions and splice-site mutations have also been reported. One study in 143 unrelated patients with NPC demonstrated an overall mutation detection rate of 88%.[21] Cases negative for *NPC1* mutations showed a high proportion of inconclusive results in genetic complementation studies, which could be due to: a) a third genetic subgroup for NPC or, b) non-specificity of NPC biochemical testing.

NPC1 mutations commonly seen within distinct patient groups include G992W, a point mutation almost uniformly seen in Acadian patients from Nova Scotia who have previously been described as having NPD,[28] and I1061T, a mutation that accounts for 15–20% of mutated alleles in Western Europe and the US.[25] I1061T is followed in prevalence by the mutation, P1007A.[25] In general, correlations between mutant *NPC1* genotypes and NPC clinical phenotypes are difficult to pinpoint because of their compound heterozygous nature, although some distinct phenotypes have been suggested. In particular, I1061T appears to be associated with non-infantile forms of NPC,[29] while A1054T, Q775P and C177Y mutant alleles were associated with early-onset disease with either biochemical or severe neurologic symptoms.[27,30]

NPC2

The *NPC2* gene, which has been mapped to chromosome 14 (14q24.3), has five exons and produces a single mRNA transcript of 0.9 kb in all tissues where it is expressed.[31] *NPC2* mutations are a little more varied than those in *NPC1*, with affected individuals expressing pathologic alleles in either homozygous or heterozygous fashion.[22] One study conducted in eight families with *NPC2* mutations found five mutations amongst sixteen mutant alleles, all except one of which were homozygous.[23] A more recent study has reported a current total of 13 disease-causing mutations in *NPC2*.[32]

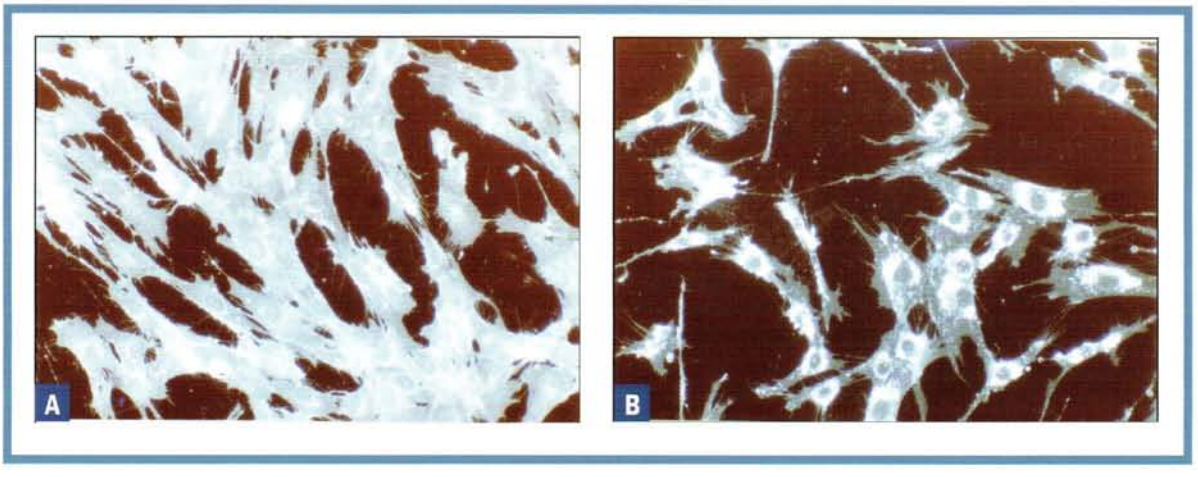
Genotype–phenotype correlations have also been identified for *NPC2* mutations. Of the five mutations identified by Millat *et al.*,[23] all but one were associated with a severe phenotype characterised by pulmonary infiltrates, respiratory failure, and death by age 4 years. Q45X, C47X and C99R mutations have also been associated with neonatal or infantile onset NPC, with death occurring in early childhood.[32] However, adult-onset disease or survival into middle adult life has been described in association with V39M and S67P mutations in *NPC2*.[32,33]

Pathophysiology

NPC defects in lipid trafficking

The central biochemical defect in NPC is severely impaired intracellular trafficking of lipids,[1] and is very distinct from acid lysosomal sphingomyelinase deficiencies that characterise NPA and NPB.[10,35] At the cellular level, the unique cholesterol trafficking defect in NPC occurs when endocytosed LDL-cholesterol becomes sequestered in lysosomes around the cell nucleus.[1] Transport from these perinuclear lysosomes to the cell membrane and endoplasmic reticulum is retarded in some, as yet, unknown way. As a result, unesterified cholesterol accumulates in great excess, which can be visualised by filipin staining of cultured NPC fibroblast cells (Figure 1).[1,36,37] It has been suggested that, in turn, this may lead to a deficiency in cell membrane cholesterol with subsequent membrane dysfunction and/or a triggering of cell death (apoptosis).[35,36,38]

Figure 1. Fluorescent filipin staining of (A) normal and (B) NPC fibroblasts showing characteristic peri-nuclear accumulation of LDL-derived cholesterol *(images provided by J. Imrie)*



Cells are first cultured in conditions of cholesterol deprivation to create a baseline level of cholesterol and maximal expression of the NPC phenotype. Cells are then incubated in fresh medium with LDL, followed by washing, fixing and staining for cholesterol with filipin.[39]

The definitive biochemical diagnosis of NPC is currently based on the demonstration of abnormal intracellular cholesterol homeostasis in cultures of fibroblasts taken from the patient; cholesterol esterification assays (Figure 2) and filipin staining (Figure 1) are employed in tandem[14] (see *Diagnostic strategies, p. 21*). The large majority (approximately 85%) of *NPC1* mutations that have been identified have been associated with the 'classic biochemical phenotype' of severely impaired cellular cholesterol trafficking.[40] However, some patients display relatively mild alterations of intracellular cholesterol transport that do not show any strict correlation with their clinical NPC symptomatology. Such patients are termed as having the 'variant biochemical phenotype'.[12]

Patterns of inheritance

NPC is inherited in an autosomal recessive manner, i.e., two copies of a gene mutation at a particular locus on one of the 22 pairs of autosomes (non-sex chromosomes) must be present for the disease phenotype to manifest. The phenotype of a particular inherited mutation (i.e., age of onset and profile/severity of symptoms) usually runs consistently within families[34] (see *Mutations, p. 10*).

In each pregnancy of a carrier couple, there is a 25% chance that they will both pass their non-functional mutant NPC genes to a child, who would then be affected. There is a 50% chance that only one of them would pass a non-functional gene, making the child a heterozygote carrier like the parents. There is a 25% chance that both functional genes would be passed and the child would neither be a carrier nor affected. Overall, unaffected children have a two in three risk of carrying one abnormal *NPC1* or *NPC2* allele. Further, each sibling of an affected individual's parents is at a 50% risk of being a carrier.

Carrier detection and genetic counselling

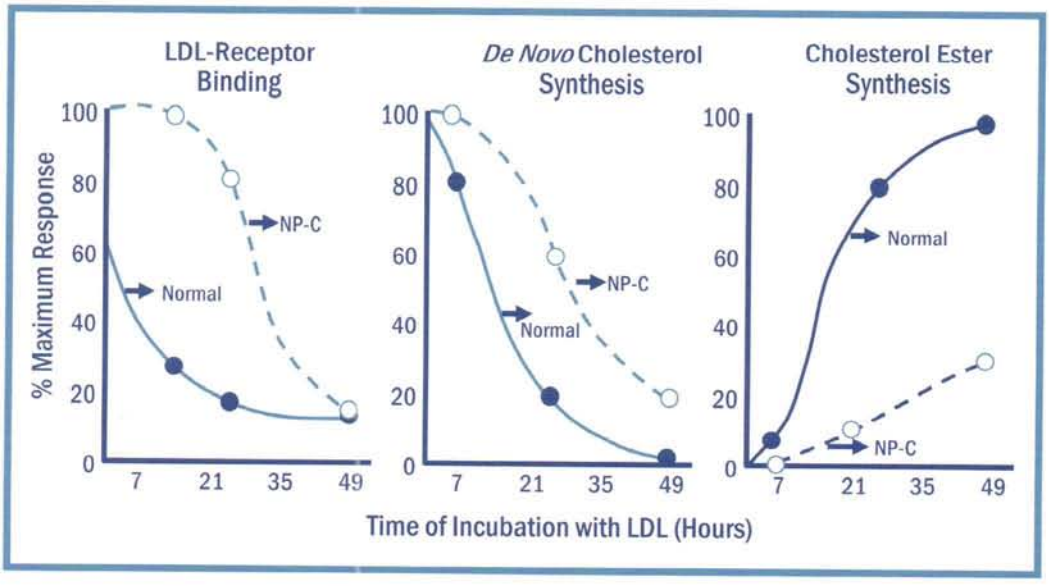
Biochemical testing cannot be relied upon to identify individuals who are heterozygous for NPC gene mutations (carriers), because test results for such individuals are similar to those seen in controls. Molecular analysis of the *NPC1* or *NPC2* genes can be used to identify NPC carriers if *NPC1* or *NPC2* mutations have been found in an identified, affected family member (see *Diagnostic strategies, p. 21*).

Genetic counselling provides individuals and families affected by NPC with information on the nature, inheritance, and implications of this genetic disorder in order to help them make informed medical and personal decisions. It is particularly relevant with regard to family planning. The optimal time to determine genetic risk, clarify carrier status and discuss the availability of prenatal testing is before pregnancy.

Prenatal testing is provided to pregnant women where there is a 25% risk of NPC in the foetus. It is performed in either of two ways:[12]

1. Biochemical testing performed on foetal cells obtained by amniocentesis at 15–18 weeks of gestation, or by chorionic villus sampling (CVS) at 10–12 weeks' gestation. This is only applicable if the affected individual from whom a family history has been ascertained (the proband) has a 'classic' biochemical phenotype, and is of no use when the proband has the 'variant biochemical phenotype' (see *Pathophysiology, p. 12*).
2. Genetic testing performed on DNA extracted from foetal cells obtained by amniocentesis at 15–18 weeks of gestation, or by CVS at 10–12 weeks' gestation. Molecular genetic analysis is only applicable if disease-causing mutations in *NPC1* or *NPC2* have been identified in the proband, or if a family study has indicated linked genetic markers.

Figure 2. Cholesterol trafficking defect in cultured NPC fibroblasts (*with permission from Pentchev et al.*[41])



Fibroblasts were cultured in lipoprotein-deficient serum to activate the LDL receptor pathway, and then LDL was added to measure their ability to respond to the endocytic uptake and accumulation of lipoprotein-derived cholesterol.[41]

NPC results in a complex pattern of intracellular lipid storage, with the profile of abnormal lipid levels varying between tissues. In the liver and spleen of NPC patients, unesterified cholesterol, sphingomyelin, phospholipids and glycosphingolipids are stored in excess, with no particular lipid moiety predominating.[1] Lipids accumulate to a greater extent in the spleen than in the liver, where alteration can be mild.[39] However, it has been shown that glucosylceramide accumulates in the foetal NPC liver to an even greater level than that seen in Gaucher disease.[1]

It is well established that there is no overt increase in cellular cholesterol or sphingomyelin levels in the brain in NPC.[1] However, studies have demonstrated a many-fold increase in levels of glucosylceramide (again, up to levels seen in Gaucher disease) and lactosylceramide.[42,43] G_{M2} and G_{M3} gangliosides are also significantly elevated in NPC brain.[39,44]

At the histopathologic level, all clinical forms of NPC are associated with two key pathologic features: foamy storage cells in the visceral organs and accumulation of storage materials in neurones and glial cells. Foamy cells (lipid-laden macrophages) and sea-blue histiocytes can be seen in the spleen, liver, lung, lymph nodes and bone marrow preparations.[1] Only subtle pathologic involvement of skin, skeletal muscle and the eyes is seen during routine histopathologic assessment.

Neuropathology

At the gross level, the brain often shows signs of atrophy, with severe changes seen in patients with slowly progressive forms of NPC. Neuropathologic changes associated with glycosphingolipid accumulation range from distortion of neurone shape (meganeurite formation) to extensive growth of new, ectopic dendrites, possibly linked to ganglioside sequestration (Figure 3).[44,45] Such changes can affect the larger basal ganglia and thalamic neurones.[1]

Neurofibrillary tangles (NFTs), possibly linked to dysregulation of cholesterol metabolism, are consistent findings in the CNS of patients with a prolonged clinical course. These NFTs are similar to those seen in Alzheimer's disease, but are distributed throughout different regions of the CNS. Whilst Alzheimer brain shows NFTs concentrated in certain regions of the cerebral cortex, NFTs in NPC brain are more numerous in the basal ganglia, hypothalamus, brain stem and spinal cord (Figure 4).[1,46]

Neuroaxonal dystrophy has also been observed, and as the disease progresses, neurodegeneration can become apparent in some brain regions, particularly in Purkinje fibres in the cerebellum.[45] The cerebral white matter is usually unaffected.[1] The basis of selective neuronal vulnerability is not yet known.

Figure 3. Ectopic dendritogenesis in NPC *(reproduced with permission from Walkley and Suzuki[45])*

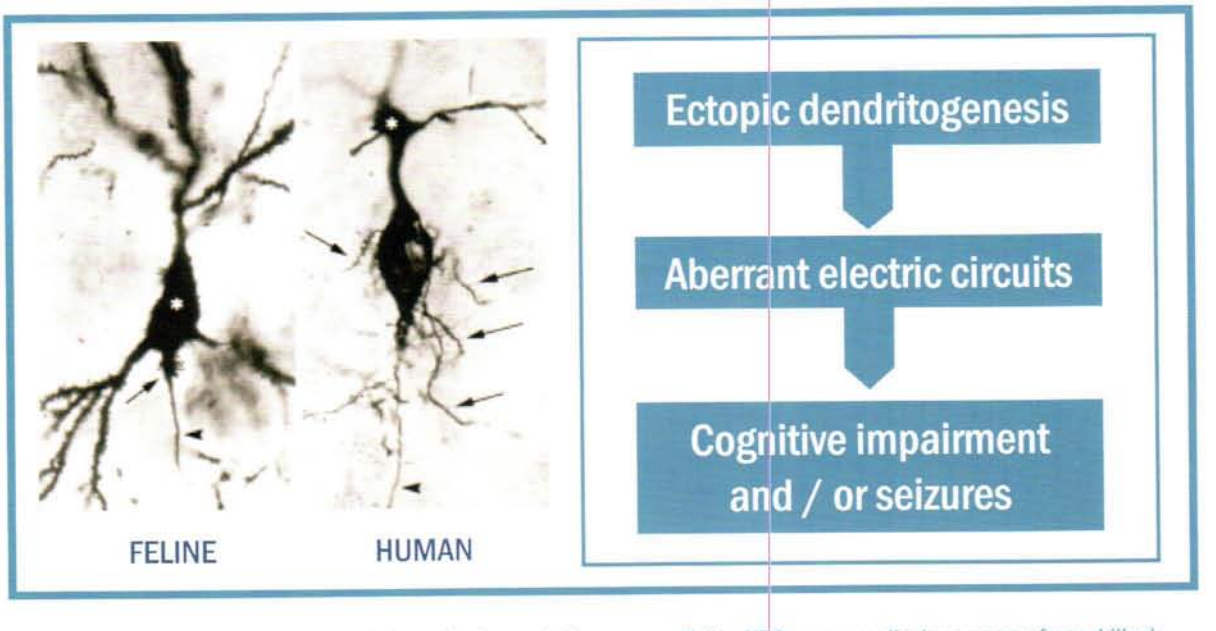
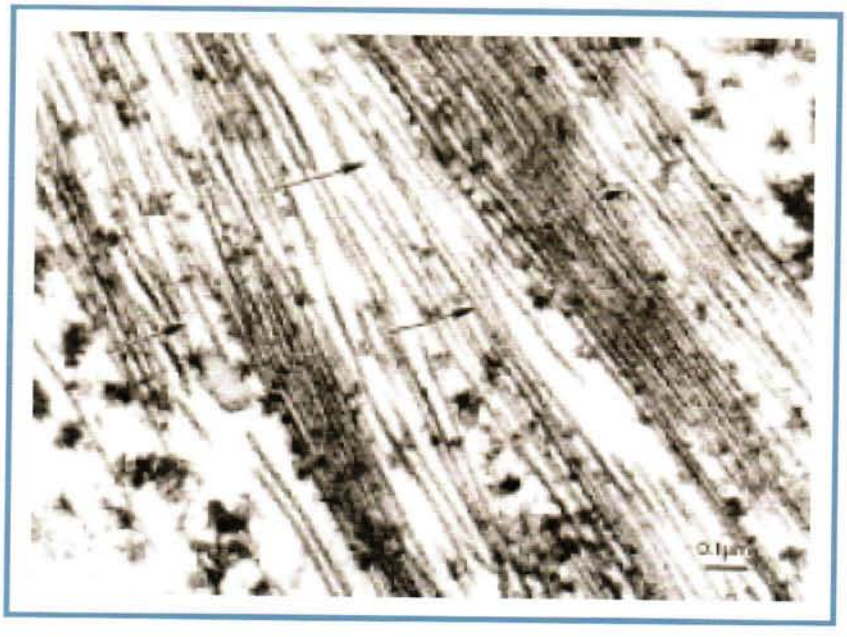


Figure showing ectopic dendritogenesis in cortical pyramidal neurones. Feline NPC neurones display a range of axon hillock changes associated with ectopic dendrites. Human NPC neurones display more severe morphological abnormalities; meganeurites can be 3–5 times larger that the soma and contain long, meandering ectopic dendrites. Such pathology can lead to aberrant neurotransmission that contributes to cognitive impairment and / or seizures.

Figure 4. Neurofibrillary tangles in NPC *(Reproduced with permission from Suzuki et al.[46])*



NFT distribution in parallel swollen storage neurons. Characteristic paired helical filaments are found in the swollen perikarya and in meganeurites, as well as in neurones without swollen perikarya.

Visceral pathology

In line with the heterogeneity of clinical symptoms, the severity and extent of visceral pathologic manifestations shows considerable variability. Splenomegaly with or without associated hepatomegaly is the most notable gross pathologic feature. Hepatic involvement may be prominent in early life, but hepatomegaly or lymphadenopathy are not generally seen in most juvenile and adult patients. Severe pulmonary involvement leading to death by respiratory failure has been seen in some infantile cases.

Roles of NPC gene products in pathogenesis

Research is ongoing to establish how *NPC1* and *NPC2* gene products are involved in the pathogenesis of lipid trafficking defects.[47] The *NPC1* gene product is a 1278 amino-acid protein (molecular weight 142 kDa) with 13 transmembrane domains.[1,48] It resides in the late-endosomal compartment, and is shuttled between this late-endosomal compartment and the plasma membrane, as well as other intracellular sites (Figure 5).[35,48] Its function is not yet clear, but it clearly plays an important role in the intracellular 'sorting' of cholesterol and glycosphingolipids (Figure 5).[47–51]

The NPC2 protein is a soluble 132-amino acid glycoprotein that is expressed in all tissues, with the highest concentrations being found in epididymal fluid. NPC2 protein binds cholesterol in the luminal space of the late-endosome/lysosome and transports it to the delimiting membrane.[31,32,35]

Figure 5. Alternative models for the role of NPC1 protein in cholesterol transport in NPC

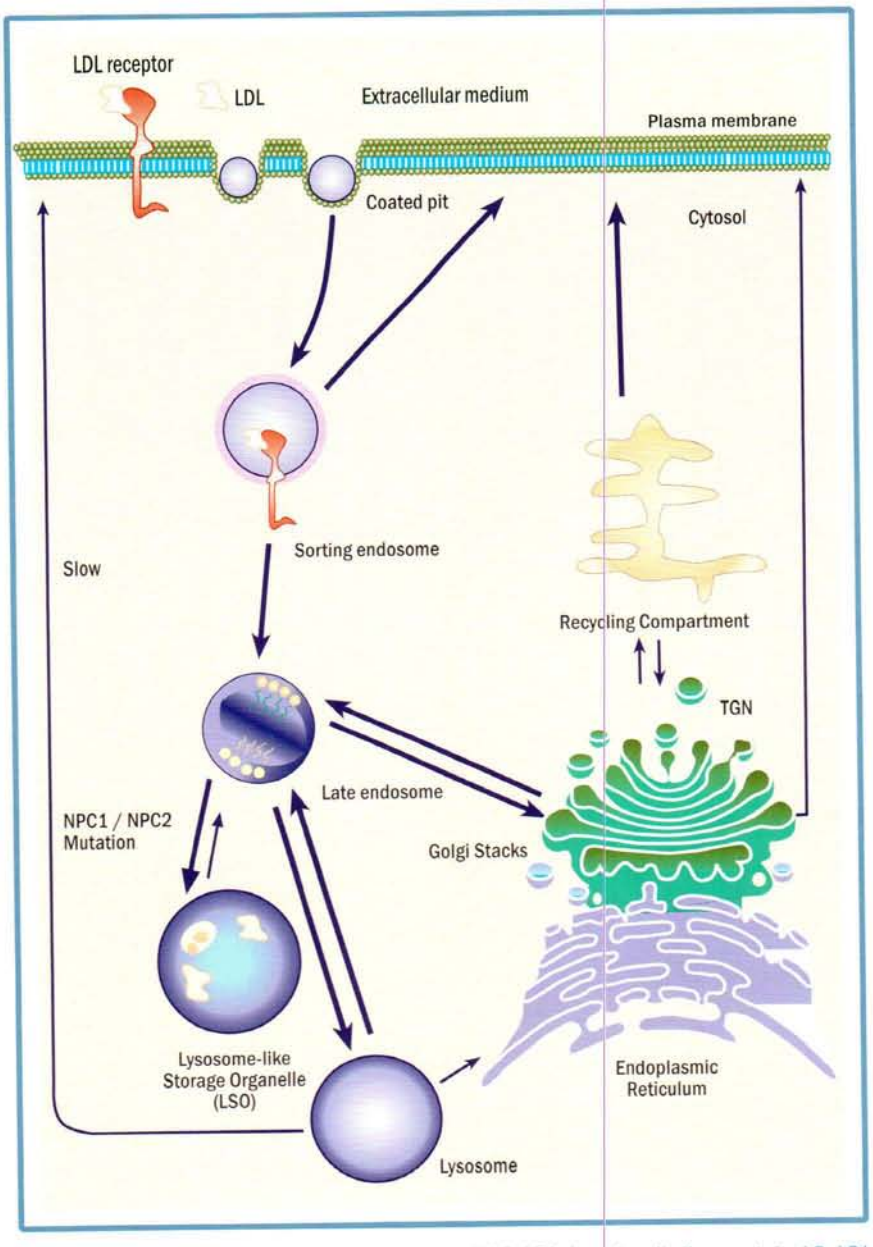


Illustration showing possible affected lipid trafficking pathways in NPC. LDL, low-density liproprotein; LR, LDL receptor; Tf, transferrin; TR, transferrin receptor.

A number of researchers have suggested that NPC1 and NPC2 proteins may act co-operatively within the scheme of cellular lipid trafficking, for instance in intracellular sterol homeostasis.[52-54] However, it is currently not clear how the activities of these two proteins are co-ordinated, or how their activity is regulated in relation to the presence and concentration of cellular lipids.[53]

Other possible pathogenetic mechanisms

Further to the Alzheimer-like neuropathologic features seen in NPC[46,55] (see Figure 4), links between pathogenetic mechanisms in NPC and Alzheimer's disease have been suggested by studies at the molecular-genetic level.[46,55-58] *In vitro* data indicate that endosomal abnormalities related to impaired lipid trafficking in NPC may contribute to abnormal processing of amyloid precursor protein (APP) and aggregation of amyloidogenic protein fragments (e.g., beta-amyloid) in certain areas of the brain.[56,57]

Analyses of genome-wide expression patterns have identified a number of changes in the expression of genes from NPC1 mutant fibroblasts that are also seen in Alzheimer's disease.[58] Many genes involved in the trafficking and processing of APP and the microtubule-associated protein, tau, were more highly expressed in NPC1 cells, as were a number of genes involved in membrane trafficking, intracellular regulation of calcium and metal ion levels and antioxidant capacity. While there seems to be a link between late-endosomal cholesterol accumulation and amyloid protein aggregation,[59] the precise details of this interaction are not yet known. Nevertheless, Alzheimer-associated protein aggregation is considered a likely contributor to neurodegeneration in NPC.

Most recently, *in vitro* studies have shown that cellular autophagy is increased in NPC.[60,61] Autophagy in NPC1 mutant cells has been linked to increased expression of beclin-1 which, in turn, is related to impaired cholesterol trafficking. Interestingly, similar findings have been shown in NPC2 mutant cells as well as cells from Sandhoff disease mice. Together, these findings suggest that autophagy may also be a relevant neurodegenerative process in NPC.[60,61]

Epidemiology

Incidence and prevalence

The prevalence of NPC has undoubtedly been underestimated in the past due to a mixture of factors including confusing terminology, prior lack of specific biochemical or genetic tests, varied pathology, and the many variant clinical manifestations of the disease. Epidemiologic data on NPC are very sparse, although prevalence has been estimated at 1:150,000 live births in Western Europe based on the number of cases identified over a 15-year period in France, Germany and the UK.[1] In particular, the prevalence of NPC in early life is probably underestimated, owing to its non-specific presentation and high fatality among infants.

Unlike NPA and NPB, which occur most commonly in people of Ashkenazi Jewish ancestry, NPC is considered to be pan-ethnic as it appears to occur with similar frequency across all populations, regardless of ethnic ancestry. Nevertheless, three genetic isolates have been identified that show a higher than average incidence: people of French Acadian descent in Nova Scotia, people of Hispanic descent in parts of Colorado and New Mexico, and a Bedouin group in Israel all show a degree of 'founder effect', i.e., a mutation traceable back to a single ancestor or small number of ancestors[4,62] (see *Mutations, p. 10*).

The importance of disease registries

National disease registries are important tools in the proper development of regional health policy/infrastructure and allocation of funding. Large collaborative registries collect vital data on the epidemiology (prevalence), symptomatology, and disease management of rare disorders such as NPC. An international NPC registry project is currently ongoing. It aims to enhance the understanding of the natural history of NPC by collecting data from patients irrespective of their treatment.

A recent survey of patients registered on the US National Niemann–Pick C1 database incorporated the largest population of NPC patients studied to date.[63] Data on clinical features and patient health problems in NPC1 were obtained from 87 respondents over a 1-year period. A similar patient review, covering a total of 94 patients (58 still alive) has also been conducted in the UK.[34] Database surveys such as these provide an overview of important clinical variables as well as the natural history of NPC (see *Clinical manifestations, p. 18*).

Clinical Manifestations

Signs and symptoms

In line with the high degree of genetic heterogeneity of NPC, clinical signs and symptoms of the disease are extremely varied, but can be broadly grouped into categories based on presenting symptoms and patients' age at onset (Table 3).

Table 3. Clinical presentation of NPC at different ages of onset (*adapted from Patterson et al.*[1])

Age of onset	Presentation
Perinatal period	Foetal ascites with persistence after birth Neonatal jaundice (benign, self-limiting or rapidly fatal) Hepatosplenomegaly VSGP usually absent
Early infantile period	Hypotonia Delayed developmental motor milestones Hepatosplenomegaly VSGP usually absent
Late infantile period	'Clumsy', frequent falls (ataxia) Isolated organomegaly VSGP may be present
Juvenile (classical)	School failure (impaired intellect and movement) Behavioural problems Ataxia, dysarthria, dystonia Seizures (partial and/or generalised) Gelastic cataplexy VSGP usually present
Adolescent and adult	Dementia Psychosis Progressive neurologic deterioration VSGP may be present

VSGP, vertical supranuclear gaze palsy, increased latency in initiation of vertical saccades with gradual slowing and eventual loss of saccadic velocity.

Neonatal and infantile presentations

The presentation of NPC in early life is non-specific and may go unrecognised by inexperienced clinicians. Severely affected babies may have ascites *in utero* that is detectable using ultrasonography,[64] with most found to have severe liver disease with hepatosplenomegaly, jaundice, and persistence of ascites after birth.[4] Although enlarged liver or spleen may be present in children presenting with symptomatic liver disease, many NPC cases never exhibit organomegaly. However, the absence of organomegaly does not eliminate a diagnosis of NPC.[4]

Extensive pulmonary infiltration with foam cells can occur as a presenting feature in NPC2, and is often fatal early on due to pulmonary failure secondary to impaired diffusion. Some children have early hypotonia and delayed psychomotor development with minimal or absent hepatic or pulmonary dysfunction, and usually do not exhibit vertical supranuclear gaze palsy (VSGP; impaired control of vertical gaze due to degeneration of supranuclear oculomotor pathways in the brainstem) at onset. However, VSGP often occurs eventually in such patients.

Childhood presentation

The classic form of NPC manifests in middle-to-late childhood with apparent clumsiness and gait disturbance that evolves into overt ataxia. Impairment of vertical gaze (up or down) is a frequent initial neurologic finding. Gelastic cataplexy, varying from head nods to complete collapse provoked by humorous stimuli, is characteristic of classic

NPC and has been estimated to occur in 20–50% of cases.[4,63,65] Partial and/or generalised seizures also develop in 33–54% of patients,[39,63] and can be difficult to control. Disturbed sleep, possibly related to reduced cerebrospinal fluid hypocretin levels in NPC, suggests effects on hypocretin-secreting cells of the hypothalamus.[66,67]

As the disease progresses, most children develop dystonia, typically beginning as action dystonia in one limb, and gradually spreading to involve all of the limbs and axial muscles. Progressive dysphagia and dysarthria also arise, with gradual disruption of speech and swallowing until eventually, oral feeding becomes impossible due to frequent aspiration on food (see *Classical NPC: the patient journey, p. 7*).

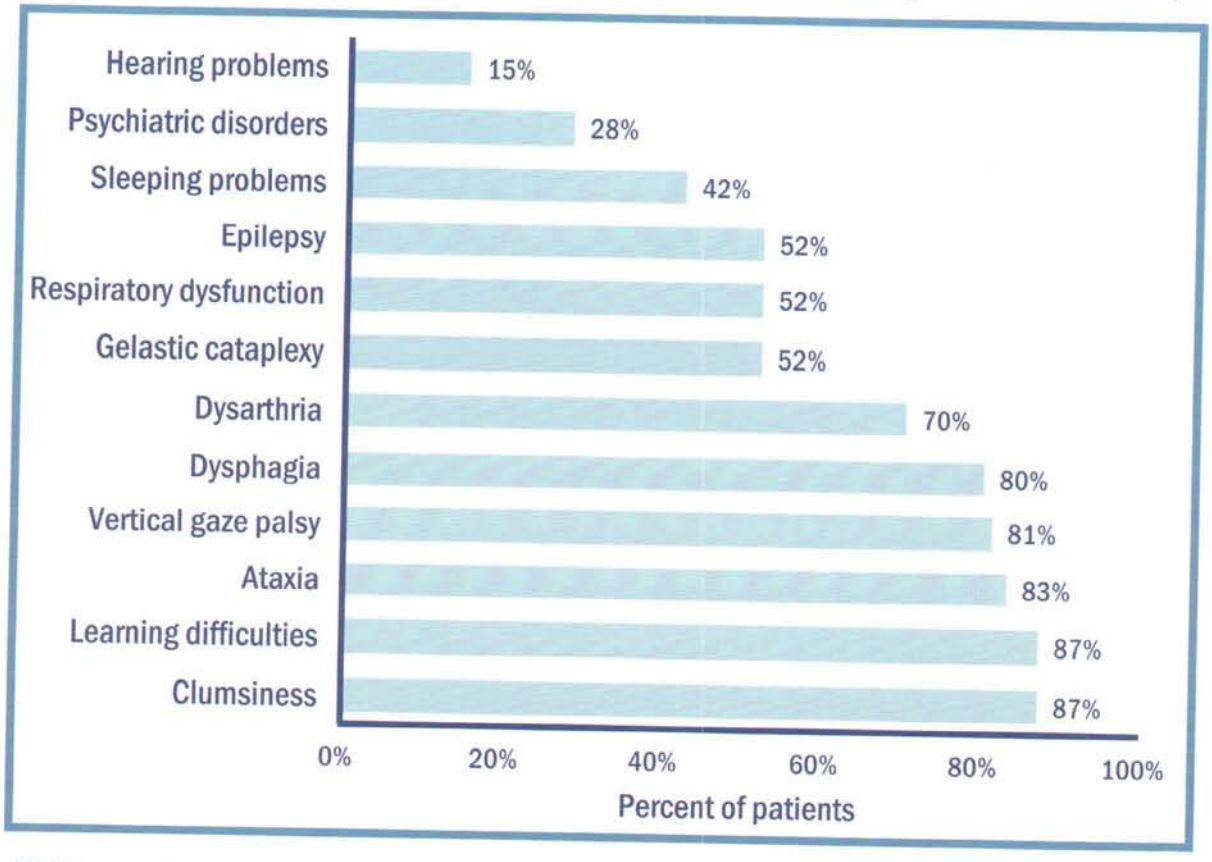
Adolescent and adult presentations

Late-onset presentations with partial phenotypes are now being recognised more often.[68,69] Adolescents and adults may present with subtle physical findings associated with various forms of apparent psychiatric illness (e.g., psychosis, depression, schizophreniform pathology).[68,70] Patients may present with neurologic deficits similar to those seen in childhood-onset NPC, but tend to show a much slower rate of progression and are often overshadowed by psychiatric problems.[33] In particular, the presence of vertical gaze palsy is an important clinical clue and can be useful in diagnosis.[68] There is frequently a lack of visceral symptoms in adult-onset patients.[4]

Overall symptom occurrence

Data from the recent large-scale US survey of NPC1 are possibly the best current source of information on what physicians can expect to encounter in the majority of cases. Common medical and developmental problems reported in this survey are listed in Figure 6.

Figure 6. Common* medical and developmental problems in NPC1 (*large-scale survey data from Garver et al.*[63])



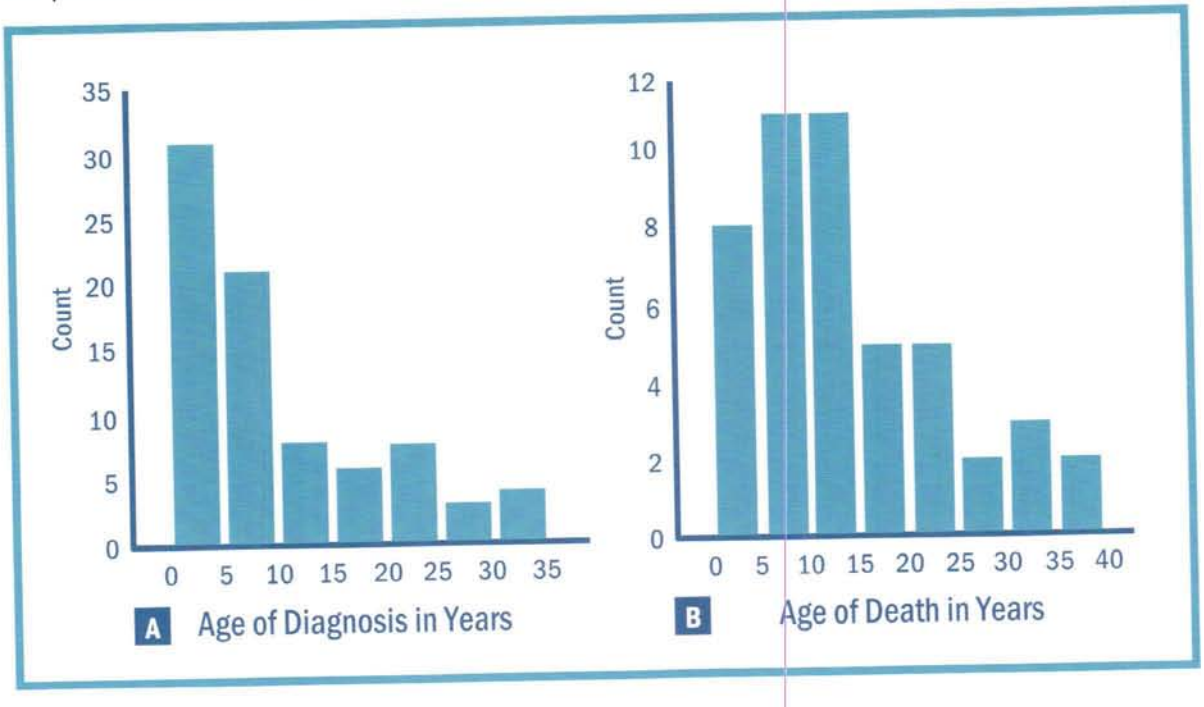
*Problems seen in > 10% of respondents.

Prognosis

The variability of NPC presentations provides a wide range of life expectancy. In general, early-onset patients tend to die during childhood or early adolescence, while patients with later-onset disease who appear to be less drastically affected can live into late adulthood. To establish a rough prognosis, physicians must take into account the age at onset, duration of disease (at presentation), risk of complications, probable outcomes, general death rates, and other outcome possibilities. Naturally, such forecasts can be unpredictable in NPC.

National patient databases such as that recently published by Garver *et al.*[63] are useful in this respect, as they provide a broad view of patient survival (Figure 7). Amongst the 87 respondents in the US database survey, the average age at diagnosis was 10.4 years, while the average age of death was 16.2 years; more than half of patients died before the age of 12.5 years.

Figure 7. Age of NPC1 patients (A) at diagnosis, (B) at death: data from a US national NPC1 database (*adapted with permission from Garver et al.*[63])



Diagnostic Strategies

Accurate diagnosis of NPC requires awareness of many clinical phenotypes, narrowing of differential diagnosis by ancillary testing and final confirmation by biochemical testing – the current mainstay of primary diagnosis in NPC. In some cases, supportive information can be gained by assessments of histopathology and ultrastructural changes in skin or rectal biopsies. Molecular genetic testing is used generally to confirm the diagnosis in individuals with variant biochemical findings.[4]

When to suspect

Diagnostic procedures for NPC should be considered in individuals presenting with the features listed in Table 4.

Table 4. Clinical features indicating a possible diagnosis of NPC[4]

- Foetal ascites or neonatal liver disease, particularly when the latter is accompanied by prolonged jaundice and/or pulmonary infiltrates
- Infantile hypotonia without evidence of progression for months to years, followed by features outlined below:
  - Vertical supranuclear gaze palsy, progressive ataxia, dysarthria, dystonia, and in some cases, seizures and gelastic cataplexy
  - Onset of these symptoms in middle childhood, with progression over a course of years; in rare cases such presentations begin later in childhood or in adult life
- Psychiatric presentations mimicking depression or schizophrenia, with few or subtle neurologic signs, starting during adolescence or adulthood
- Enlargement of the liver or spleen, particularly in early childhood

Diagnostic testing

Biochemical

Routine laboratory profiles including standard blood biochemistry, plasma lipids, urinalysis and cerebrospinal fluid metabolites are generally normal in NPC except in patients with hypersplenism or cholestatic jaundice. A definitive diagnosis of NPC requires demonstration of abnormal intracellular cholesterol homeostasis in cultures of fibroblasts taken from the patient.[14] In NPC-positive cultures, these cells show a reduced ability to esterify cholesterol after loading with exogenously derived LDL-cholesterol. Only certain specialist laboratories perform specific biochemical testing for NPC. These can be located/contacted through various NPC resources via specialist care centres.[4]

Filipin staining is employed to identify any fluorescence concentrated around the nucleus, which indicates the accumulation of unesterified cholesterol in perinuclear lysosomes. Most patients with a 'classic biochemical phenotype' of NPC have zero or very low esterification levels with a classic, intense dotted filipin staining pattern. However, approximately 15% of individuals display the 'variant biochemical phenotype', showing intermediate levels of cholesterol esterification.[40] Filipin staining is more sensitive in detecting such patients, although the variant phenotype is also associated with a less distinctive fibroblast staining pattern.[1,4] Alternative methods exist to characterise such patients more precisely,[73] but are not yet routinely available.

Studies suggest that measurements of plasma chitotriosidase activities could be useful as a marker for lysosomal storage diseases including Gaucher disease and Niemann–Pick disease.[74–76] Plasma chitotriosidase is markedly elevated in Gaucher disease, has consistently been shown to decrease with therapy,[77] and is recommended as a screening procedure in children in some countries.[78] Laboratory studies suggest that the same might also apply in NPC,[76] although patients typically show more biochemical variability in this disease.[79]

Histologic

Prior to the recognition of the primary biochemical defect in NPC[14] and the development of direct biochemical testing for abnormal intracellular cholesterol homeostasis, histopathologic analysis of tissue biopsies and/or tissue lipid analysis was essential to confirm a diagnosis of NPC. However, such invasive tests are now only required in rare cases.

Histologic analyses include examination of bone marrow, spleen and liver for the presence and extent of foam cells (lipid-laden macrophages) as well as the presence of sea-blue histiocytes, which may be seen in bone marrow. However, these features are considered non-specific, as failure to detect them does not rule out a diagnosis of NPC.[1] Electron microscopic analyses of skin, rectal neurones, liver or brain can also be employed, and can show polymorphous cytoplasmic bodies.[4,80]

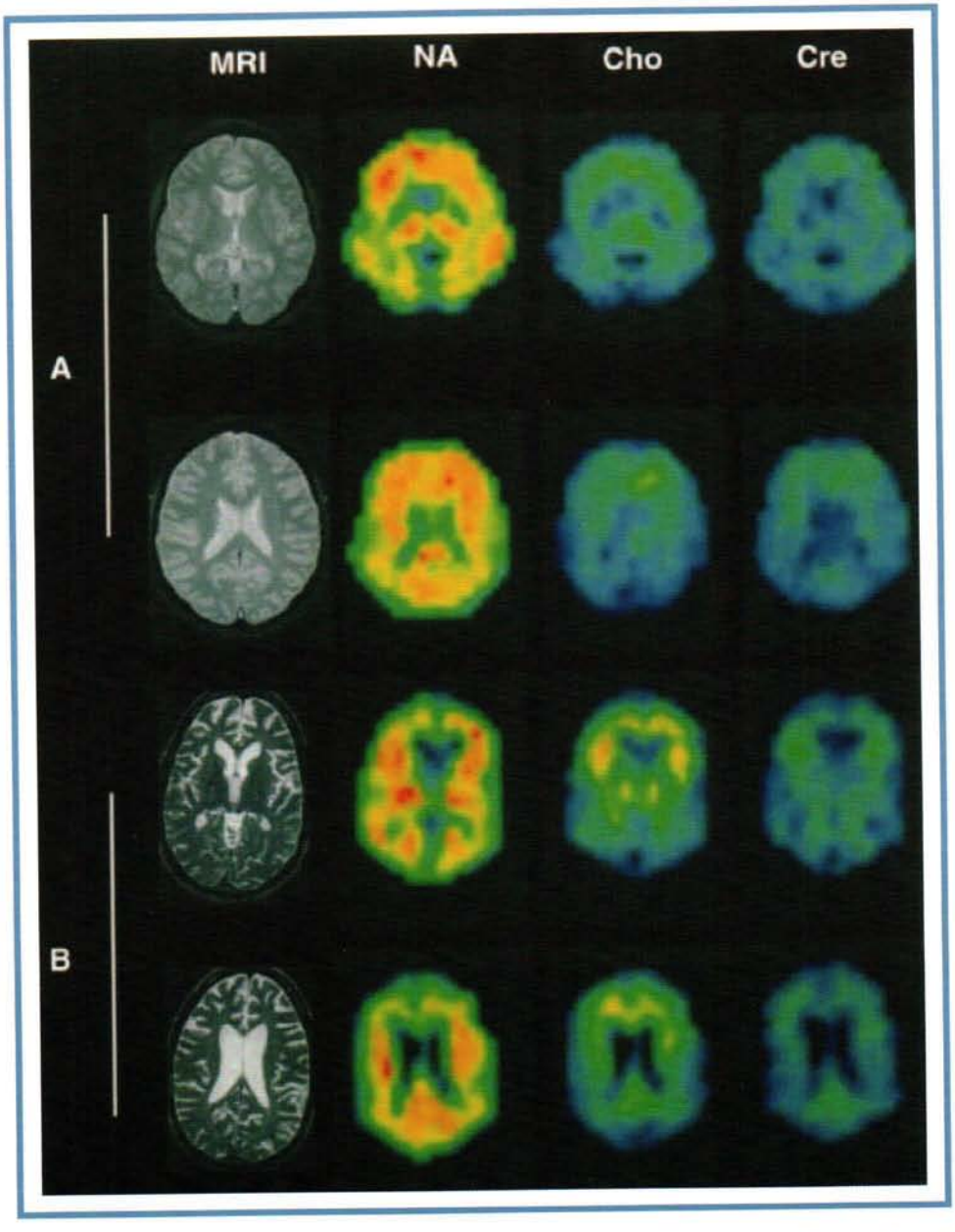
Genetic

Molecular diagnosis is not considered a suitable tool for primary diagnosis,[1] but is of use in prenatal diagnosis and the identification of heterozygotes in probands' families – especially as regards family planning (see *Patterns of inheritance, p. 11*), and in the confirmation of diagnosis in individuals with a variant biochemical phenotype. The majority of individuals with NPC harbour mutations in the *NPC1* gene while, it is assumed, the remaining individuals with NPC have mutations in the *NPC2* gene (see *Carrier detection and genetic counselling, p. 11*).

Imaging

Magnetic resonance imaging (MRI) or computed tomography (CT) scans of the brain are usually normal until the late stages of NPC, and imaging is considered non-specific.[1] However, cerebral atrophy and in particular, marked atrophy of the superior/anterior cerebellar vermis may be seen, usually in patients with slowly progressive disease. Other abnormalities can include thinning of the corpus callosum and increased signal in the periatrial white matter, reflecting secondary demyelination. Proton magnetic resonance spectroscopy (H-MRSI) may be a more sensitive imaging technique in NPC than standard MRI.[81]

Figure 8. MRI and H-MRSI in control and NPC brain (*reproduced with permission from Tedeschi et al.*[81])



Proton magnetic resonance spectroscopic imaging (H-MRSI) and corresponding MRI scans from (A) a control and (B) a patient with NPC. H-MRSI permits the simultaneous measurement of N-acetyl-aspartate (NA), choline-containing compounds (Cho), creatine plus phosphocreatine (Cre) and lactate (Lac) signal intensities, and identification of regions of interest. In patients with NPC, NA/Cre was significantly reduced in the frontal and parietal cortices, centrum semiovale and caudate nucleus; Cho/Cre was increased in the frontal cortex and centrum semiovale. These changes were correlated with clinical staging score.[81]

Evaluations following initial diagnosis

Patients should be monitored during clinic visits at least every 6 months. Initial evaluations should include assessment of the affected individual's ability to walk and transfer and to communicate, including assessments of language, speech and hearing.

General evaluations, with special attention to pulmonary function, swallowing, bowel habit and mood (for occult depression) should be performed in juvenile and adult patients. Electroencephalograms may be indicated if the patient history suggests seizures. Sleep disturbances are common in NPC, and patient sleep hygiene should be ascertained at each clinic visit.

Patients presenting with hepatosplenomegaly should have a complete blood count and tests of hepatic function to identify any risk of organ failure.

Misdiagnosis

NPC is an extremely rare and highly heterogeneous disease that affects multiple body systems and has variable onset and progression over a course of years. It is perhaps not surprising that in past decades it has frequently been misdiagnosed or even undetected at presentation in primary care. This was especially true before the characterisation of the primary molecular and genetic defects, when diagnosis relied on non-standardised histopathologic analysis, and has contributed to very varied terminology used to describe patients who are now known to have NPC: juvenile dystonic idiocy; juvenile dystonic lipidosis; neurovisceral lipidosis with vertical supranuclear gaze palsy, Neville–Lake disease, sea-blue histiocytosis, lactosylceramidosis; downgaze paralysis, ataxia and foam-cells syndrome (DAF).

Even now that direct biochemical and genetic tests are available, diagnosis can be challenging. This highlights the need for increased awareness of this disease, and for effective referral to specialist care for patients with suspected NPC. The following case history, adapted from the 2005 UK Department of Health report on the management of inherited metabolic diseases,[82] highlights some of the problems associated with non-specialist centres advising parents without reference to up-to-date information on NPC.

Child 1 – case history

Child 1 was a newborn baby girl who presented with severe neonatal jaundice and organomegaly. Initially it was thought that she had a severe infection and she was treated with antibiotics. However, there was no resolution and the liver disease persisted over the next few weeks, when it was thought that she had biliary atresia. Her parents were told that the liver disease was so severe she would die. A scan, followed by surgery, showed that this was not the case, and over the next 6 months she had a further four liver biopsies. Eventually, storage cells were found in the biopsies and a full metabolic work-up was performed, which eventually showed that she had NPC.

At this point, the parents were informed that Child 1 would not develop normally, would never walk or talk, and would die before the age of 2 years. By the time she was referred to the specialist team, the parents had already been referred to the local palliative care team. The first task for the specialist nurse was therefore to 'pick up the pieces', as the parents were in extreme despair.

The nurse informed the parents that the natural history of the disease is very variable, and they were introduced in the clinic to parents of children up to the age of 5 years who had no neurological problems, so that they could see that the outlook may not be as bad as they had originally been told. They were also informed about possible genetic counselling and the options available to them if they wished to have more children.

When Child 1 was seen in clinic, at age 9 months, she was still very jaundiced. However, the last report on her at age 17 months was that she was developing well, had learned to walk and was speaking 18 recognisable words.[82]

Where to Refer: Specialist Care Centres

Given the varied range of neurologic, hepatic and psychiatric symptoms, patients with NPC typically present to a wide range of health professionals including perinatologists, paediatricians, family practitioners, haematologists, gastroenterologists, neurologists, internists and psychiatrists. Both children and adults are usually already under specialist care when diagnosis is confirmed. However, patients with NPC should be referred to regional or national care centres that are specialised in the management of NPC specifically, or inherited metabolic disorders generally, for long-term management (see *Referral to specialist care centres, p. 27*).

The Impact of NPC

Effects on schooling and work

Children affected by NPC typically experience progressive intellectual, movement and behavioural problems that can severely limit their performance at school. Annual psychometric testing may be helpful in arranging appropriate school placement. Teenagers and adults with motor or sensory impairments who are driving should be monitored at 6–12 month intervals to ensure that neither they nor other road users are at risk. The progressive dementia and/or psychiatric symptoms that often feature in adult-onset NPC can place severe limits on patients' abilities to work effectively. Some of the schooling problems experienced in NPC are illustrated in the case history of 'Child 2', below.

Child 2 – case history

Child 2 was diagnosed as a neonate when intrauterine hepatosplenomegaly was detected. Her liver disease resolved and her development progress was relatively normal until puberty, at which point she deteriorated markedly with increasing ataxia, learning difficulties and slowing of speech. She was assessed and transferred to a residential special school with on-site health care facilities. She settled very well and progressed well at her own speed. At the age of 16 seizures became a major problem but did improve with medication. This happened to coincide with a long break from school, so the local children's' hospice was approached for respite care, although sadly room was not available. A meeting was arranged at her school soon after this, to discuss transitional arrangements for when Child 2 was too old for her current placement. This involved all members of the team, including the school head, her class teacher, the school nurse, the NPC nurse specialist, the physiotherapist, the speech therapist and her social worker, as well as representatives from the education authority and the family.

Normally children progress on to college at the same residential campus as her school. In Child 2's case however, it was felt that there would be safety risks because the college is not as confined as the school. She is very happy and settled where she is, and because she finds change very distressing, it was agreed that she should stay at the school until the age of nineteen; Child 2 is not upset by being in a class with children younger than her.

Impact on family members

Parents of children with NPC, as well as the patients themselves, face some of the biggest possible challenges related to a severe and chronic disease. Parents or other family caregivers learn to provide complex treatment regimes; they recognise and deal with acute crises that can occur at any time. Parents have to deal with a lot of

medical specialists, as their child has complications and problems affecting various organ systems. They may also need to understand and take difficult decisions over the familial aspects of the condition such as finance, time management and geographical location. Many parents experience financial problems or difficulties with getting financial support. On top of all this, their energies may be almost totally consumed in coping with the severe disability their child has in terms of everyday living, education and work opportunities.

In addition, while positive effects can occur in some non-affected siblings of children with NPC such as strengthening of parent–child and child–child relationships, negative outcomes can also arise. These can include excessive sibling worry about the ill child's condition, jealousy of the attention paid to the ill child, and restriction of family events. Chronic illness can be disruptive and stressful for non-affected siblings, and in some cases can interact with other factors to create an increased risk of psychological/emotional disturbance.[83] Devising ways of improving mothers' awareness and helping them to feel confident in managing their child's illness is important to aid in the optimal development of their ill child as well as any non-affected siblings.[84,85]

Impact on healthcare

Increased awareness of NPC across the medical community and within families of affected individuals, as well as improved diagnosis, have led to more patients being identified. However, healthcare for a rare genetic disorder such as NPC can be hampered by poor understanding of the disease by local heath services, social services and education. There is an increasing demand for specialist resources to enable the systematic management of patients and referral to specialist centres (see *Referral to specialist care centres, p. 27*). Looking ahead, advances in the detection and management of NPC may in future lead to more patients surviving into adolescence and adulthood. While most geographical regions have some provision for paediatric care, resources for older patients with NPC are currently not so common. The patient history below highlights the kind of co-ordinated, team-based support necessary in an adolescent case.

Child 3 – case history

Child 3 was diagnosed with NPC at the age of 16 following increasing difficulties in keeping up with peers at school. He had impaired speech, was ataxic and had problems looking down. In retrospect it was apparent that he had suffered with problems since the age of 4. However, his deterioration was very slow in the intervening time, and he had received good support from local social services to ensure that his problems were addressed. At age 18 he was having greater problems with swallowing, and the speech and language team and a dietician became involved. Child 3 also received physiotherapy because dystonia was affecting his limb positioning and walking, but 6 months later he was in a wheelchair at all times.

When Child 3 was just over 19 his mother contacted the nurse specialist as things seemed to be falling apart. An emergency home visit concluded that his problems were now very complex, but he was no longer covered by paediatric services and had not been allocated another consultant. He was attending a day care centre with in-patient facilities during the day, but his mother felt that he was not receiving enough stimulation as he just sat all day. With no consultant to contact, the nurse specialist spoke with both the social worker and day centre staff, and the first of many meetings was set up to co-ordinate the team that were in contact with Child 3 and his mother. His feeding was assessed and he was fed via a gastrostomy on bad days but allowed to eat if he wished. He was provided with facilities in the home for hoisting, as well as a special bed. A member of staff at the day centre acted as 'key worker' to ensure that his needs were all addressed and that everyone was aware of any changes.

For the last year of his life, Child 3 lived in his own flat with 24-hour carers, in line with his wishes at the time. There are times when his mother found it difficult to talk directly to the team, and in these situations the nurse specialist often liaised on the family's behalf.

Healthcare Support

The role of patient associations

NPC is a devastating condition that impacts upon the entire family. Medical therapy aside, support of both the patient and family throughout the course of NPC in terms of advice and education is vital. If possible, counselling services should be made available to the whole family. Patient-centred umbrella organisations offer information at the international level, while national patient associations now offer information and counselling within many countries. Patient associations provide information about specialist services to professionals, patients with NPC and the public, and most provide at least basic educational material. Many patient associations also actively participate in fund-raising, for the support of medical therapy and research in NPC. A list of support organisations is provided in the *Resources* section, *p. 31*.

Referral to specialist care centres

Specialist care centres can provide comprehensive, integrated, multidisciplinary care for patients, as well as information and support for family members, as they aim to incorporate networks of all relevant medical disciplines within the core team. They have effective links with national networks of testing laboratories and other care centres at the national and international level, and have important roles in disease auditing and the maintenance of geographical coverage. Metabolic nurses play vital roles in the day-to-day running of clinics, and deal with many of the familial aspects of work with patients and family members. Physical therapists, speech therapists, occupational therapists and disease counsellors should also all be involved in supportive care for patients.

A prime concern amongst voluntary, patient-focused organisations is the need for increased awareness among general health practitioners regarding the symptoms, diagnosis and management of NPC. In some cases, initial health services can do "more harm than good" if there is a lack of any specialist knowledge or expertise (see *Misdiagnosis, p. 24*). It has been estimated that less than half of patients with an inherited metabolic disease are currently being looked after through specialist care centres,[82] partially due to a general reluctance to refer, but also through a lack of local resources.

Medical research foundations

Private research foundations have been founded by a number of concerned individuals and family members of NPC patients, often with substantial funding donated by industry. Examples and contact details are provided in the '*Resources*' section, *p. 31*.

Treatment Strategies in NPC

There is no definitive therapy for NPC, and there is no cure. The majority of therapies are palliative, although recent progress in the characterisation and understanding of the disease has identified possible targets for specific therapies.

Symptomatic therapies

Current, non-specific treatments for NPC focus mainly on supportive care, aimed toward managing the symptoms of the disease. As such, these treatments have no effect on disease progression or long-term outcomes. Common problems and relevant supportive treatments are listed in Table 5.

Table 5. General symptomatic therapies provided in supportive care

Symptom	Treatment
Feeding problems	Most children with NPC eventually require gastrostomy feeding, with monitoring in case of aspiration or nutritional compromise
Cataplexy	Tricyclic antidepressants or CNS stimulants can be effective in controlling cataplexy
Dystonia and tremor	Some patients respond to anti-cholinergic drugs
Movement restriction	Physical therapy is indicated to maintain mobility as long as possible
Seizures	Anti-epileptic drugs can control or diminish the frequency of seizures
Sleep disorders	Melatonin or a nocturnal sedative may be indicated. In complex cases, formal evaluation by a sleep specialist should be considered
Constipation	Patients should have a regular bowel program to prevent severe constipation
Lung involvement	Chest physical therapy with aggressive bronchodilation appears beneficial. Antibiotic therapy should be provided in case of intercurrent pulmonary infection

Specific therapies

Specific therapies for the intended treatment of NPC are based on targeting known pathophysiological and/or biochemical defects involved in the pathogenesis of the disease. While earlier attempts proved largely ineffective, more recent efforts indicate possible hope for the future.

Bone marrow and liver transplantation

A number of studies have established that bone marrow transplantation or combined bone marrow and liver transplantation are ineffective in treating the neurological symptoms of NPC1.[1,4,86,87] In theory, bone marrow transplantation (BMT) may benefit patients with *NPC2* gene mutations as the NPC2 protein is a lysosomal glycoprotein and BMT has been used successfully in other lysosomal enzyme deficiency disorders. Liver transplantation in humans corrects hepatic dysfunction, but does not ameliorate the neurologic disease.

Replacement or repair of gene or gene product

The most common NPC gene product, NPC1 protein, is not suitable for transduction therapies, and *NPC1* gene replacement or repair is not yet practicable.

Cholesterol depletion

A trial with different combinations of cholesterol-lowering agents (cholestyramine, lovastatin, nicotinic acid and dimethyl sulfoxide [DMSO]) was performed in 25 patients to assess the effects of reducing tissue and plasma levels of free cholesterol in NPC.[88] Although all treatment regimens except DMSO reduced hepatic and plasma cholesterol levels, no effect on the neurologic symptom progression was identified.[1,88]

Cell-signalling targets

Studies have demonstrated that direct or indirect over-expression of the GTPase enzyme, Rab 9, can reverse the NPC phenotype (i.e., restore lipid trafficking) in tissue culture.[89,90] Although not yet tested in human trials, this suggests mobilisation of endosomal cargoes as a potential target for small-molecule therapies.

Laboratory studies of cellular and mouse NPC models have suggested interruption of apoptosis and related routes of cell death and dysfunction as a possible therapeutic target in NPC.[91] A putative compound (NP-27) has been identified that partially corrects the NPC biochemical phenotype in cell culture by stimulating cholesterol transport pathways and restoring LDL stimulation of cholesterol esterification in cultured cells from NPC mice.[92] Future plans for this compound are not known.

Neurosteroids

Neurosteroids are steroids synthesised in the brain that affect neuronal growth and differentiation during development, and which modulate a variety of neurotransmitter receptors. NPC1 mutant mice exhibit a normal capacity to synthesise neurosteroids during embryonic/foetal development, but lose this ability in the early neonatal period, prior to onset of neurological symptoms.[93,94] This suggests a possible role for neurosteroid replacement as a therapy in NPC.

Preliminary studies of neurosteroid replacement with allopregnanolone, given as a single injection or repetitively in mouse models of NPC, have indicated delayed demyelination and symptom onset, reduced lipid accumulation, and improvements in survival provided that treatment is initiated early in post-natal life.[95-97] The mechanisms by which these effects take place are not fully known, although $GABA_A$ receptors are believed to play some role, possibly in conjunction with pregnane X receptors.[94,97]

Glycosphingolipid synthesis inhibition

The use of inhibitors of glycosphingolipid synthesis is showing promise as a possible treatment for NPC, offering a means of breaking the lipid trafficking gridlock.[98] Inhibition of glycosphingolipid synthesis by miglustat has been shown to delay symptom onset and prolong survival in both murine and feline models of NPC.[99]

A recent case study of one year of miglustat therapy in two children with NPC (aged 9 and 14 years) reported improvements in CNS symptoms and stabilisation of systemic disease.[79] Improvements were seen in assessments of movement, swallowing ability and aspects of cognition.

A 24-month, randomised, controlled clinical trial of miglustat in 29 patients with juvenile/adult NPC (with a sub-study in 12 paediatric patients) has recently been completed. Preliminary data from the first 12 months on treatment indicate that miglustat improved or stabilised several clinically relevant markers of the disease; saccadic eye movements, cognition, auditory acuity and ambulation.[5] This is the first treatment studied in NPC for which there are clinical data supporting a disease-modifying benefit.[5]

Miglustat is still under investigation for the treatment of patients with NPC, and is currently approved in the European Union, the United States, Australia, Canada, Switzerland, Brazil and Israel for the treatment of patients with mild-to-moderate type 1 Gaucher disease who are unsuitable for enzyme replacement therapy.

Evaluating clinical treatment effects

Patients with NPC usually undergo long series of diagnostic tests and monitoring procedures throughout the course of disease (see *Diagnostic strategies, p. 21,* and *Evaluations following initial diagnosis, p. 24.* However, with the possibility of new methods of treatment comes the requirement for a rational evaluation of outcomes, allowing full assessments of treatment efficacy and safety. Therapeutic outcomes can be either qualitative or quantitative, based on their derivation and on how directly they reflect disease progression. Table 6 lists possible key parameters for determining treatment effectiveness in past and future NPC trials.

Table 6. Key parameters for establishing treatment outcomes in NPC

Quantitative measures	Detail
Saccade eye movement velocity	A specialised ophthalmic assessment based on video-recorded eye movement and subsequent computerised measurements of peak velocity, amplitude and duration of vertical and / or horizontal saccadic eye movements.[100] In particular, peak velocity has been shown to be far lower in patients with NPC compared with controls[101]
Kinematic analysis	Motoric control / impairment can be assessed using a variety of electrophysiologic parameters: accelerometry allows the measurement of tremor amplitude and frequency, and surface electromyography (sEMG) can monitor for abnormal patterns consistent with various movement disorders[102]
Ambulatory index	Evaluations of ambulation and gait can be performed using quantitative, categorical scales. A recent study evaluated patients across a range of scores from zero (asymptomatic or fully active) through 4 (requires unilateral support such as a cane or single crutch to walk > 80% of the time and walks 25 feet in 20 seconds) to 9 (restriction to wheelchair and unable to transfer independently)
Cognitive testing	Multiple cognitive deficits, often categorised collectively as dementia, occur as NPC progresses. The mini mental-status examination (MMSE) – a generic measure quantifying cognition across a wide range of domains including orientation, recall, verbal and written comments, writing and drawing – has been established as an appropriate measure for use in children and may therefore be particularly useful in NPC[103,104]
Qualitative measures	
Quality of life (QoL)	QoL is an important measure of therapeutic outcome in NPC, where there is invariably a substantial impact on quality of life for the whole family. There are no specific measures for QoL related to NPC, but a number of widely used and well validated generic measures are available, including the Short-Form 36-item QoL questionnaire (SF-36)[105]
Swallowing	Radiological videofluoroscopic (VFS) analysis of liquid barium swallowing can be used effectively to monitor treatment effects on swallowing ability and progression of dysphagia. Systematic evaluations can be achieved by sequential analyses using a sensitive and specific scoring system to assess all phases of the swallowing motion[79,106]

Conclusions

NPC is a rare but devastating inherited disorder arising from impaired cellular lipid trafficking. While the classical clinical phenotype is seen in infants and children – featuring chronic progression and death usually by the early teens – the disease can be detected much later with some patients surviving into early adulthood and, in extremely rare cases, well into middle age.

The extreme disabilities suffered by individuals affected by NPC, particularly during later disease stages, have a vast impact on the whole family and require highly specialised healthcare within a multidisciplinary, managed-care setting. Ideally this should be provided by specialist centres, requiring referral, as it is important that patients as well as family members receive adequate support in terms of counselling, advice and access to necessary

supportive treatments. It is therefore vital to raise awareness of the disease across the board in the healthcare setting.

Although there is currently no cure for NPC, knowledge on its pathogenesis has increased several-fold since the characterisation of the *NPC1* and *NPC2* genes. While the key cellular (and diagnostic) hallmark of NPC is considered to be impaired processing and utilisation of exogenous cholesterol, the disease process is now believed to involve a more general dysfunction in lipid trafficking. Profiles of lipid accumulation vary among tissues, with minimal cholesterol accumulation in neurones (in contrast to liver and spleen), but marked accumulation of several glycosphingolipids including gangliosides G_{M2} and G_{M3} and glucosylceramide. Increased knowledge on the natural history of NPC will help in the further development of much-needed treatments for this tragic disease.

Resources

Niemann–Pick Association (Italy)

Associazione Italiana Niemann Pick e Malattie Affini
Via Cafasse, 28
10074 LANZO T.SE
TO
Italy
Phone: +39 (0) 123 289 95
Fax: +39 (0) 123 320 149
E-mail: info@niemannpick.org
Website: www.niemannpick.org/

Niemann–Pick Self-help Group (Germany)

Niemann–Pick Selbsthilfegruppe e.V.
Hindenburgstrasse 25/2
71106 Magstadt
Germany
Phone: +49 (0) 6897 726 72
Fax: +49 (0) 6897 726 72
E-mail: info@Niemann–Pick.de
Website: www.Niemann–Pick.de/

Niemann–Pick Disease Group (UK)

11 Greenwood Close
Fatfield Washington
Tyne and Wear
NE38 8LR
United Kingdom
Phone: +44 0191 415 0693
E-mail: Niemann–Pick@zetnet.co.uk
Website: www.niemannpick.org.uk

Niemann-Pick Disease Resources

National Niemann-Pick Disease Foundation, Inc.
Providing Family Services to the U.S. and Canada)

Nadine M. Hill, Executive Director
P.O. Box 49; 401 Madison Avenue, Suite B
Fort Atkinson, WI 53538
Toll Free: 1-877-287-3672
Tele: (920) 563-0930; Fax: (920) 563-0931
Email: nnpdf@idcnet.com **Web Site:** www.nnpdf.org

Niemann-Pick Disease Type C (NPC)

tional Institute of Child Health & Human Development
National Institutes of Health

Dr. Forbes "Denny" Porter, M.D., Ph.D.
Senior Investigator, Sect. on Molecular Dysmorphology
Tele: (301) 435-4432
Email: fdporter@mail.nih.gov

Nicole Farhat, C.R.N.P.
Developmental Endocrinology & Genetics
Tele: (301) 594-1765
Email: nicole.farhat@nih.gov

Lee Ann Keener
Clinical Research Nurse
(301)-594-2005
Email: leeann.keener@nih.gov

Mailing Address for Dr. Porter, Nicole Farhat & Lee Ann Keener
National Institutes of Health
9000 Rockville Pike
Building 10, Room 1-3330
Bethesda, MD 20892

Mayo Clinic, Department of Neurology

Dr. Marc C. Patterson, M.D.
Chair, Div. of Child & Adolescent Neurology
200 First Street SW
Rochester, MN 55905
Tele: (507) 284-3351; Fax: (507) 284-0727
Email: patterson.marc@mayo.edu

Washington University School of Medicine

Dr. Daniel Ory, M.D.
Co-Dir., Diabetic Cardiovascular Disease Ctr.
Professor of Medicine, Cell Biology & Physiology
600 South Euclid Ave., Box 8086
St. Louis, MO 63110
Tele: 314-362-8737
Email: dory@dom.wustl.edu

Niemann-Pick Disease Types A & B/ASMD
(Acid Sphingomyelinase Deficiency)

Dr. Andrew Lieberman, MD PhD
Abrams Collegiate Professor of Pathology
Director of Neuropathology
Co-Director, Michigan Protein Folding Disease Initiative
University of Michigan Medical School
3510 MSRB 1, 1150 W. Medical Center Dr.
Ann Arbor, Michigan 48109-0605
Phone: 734-647-4624
Email: liebermn@med.umich.edu

Mount Sinai International Center for Types A and B Niemann-Pick Disease
Web site: www.mssm.edu/niemann-pick/

Dr. Edward H. Schuchman, Ph.D.
Director, Intl. Ctr. for Types A & B NPD
Mount Sinai School of Medicine
1425 Madison Ave., Rm. 14-20A
New York, NY 10029
Tele: (212) 659-6711; Fax: (212) 849-2447
Email: edward.schuchman@mssm.edu

Melissa Wasserstein, M.D.
Medical Director, Intl. Ctr. for Types A & B NPD
Mount Sinai School of Medicine
One Gustave L. Levy Place, Box 1497
New York, NY 10029
Tele: (212) 241-2478; Fax: (212) 860-3316
Email: melissa.wasserstein@mssm.edu

Canada – ASMD (Types A & B) and NPC

Hospital for Sick Children (SickKids Hospital)

Julian Raiman, M.B., B.S., M.Sc., M.R.C.P.
Staff Physician
Div. of Clinical & Metabolic Genetics
Tele: 416-813-5753

Margaret Mackrell, R.N., B.H.Sc.N.
Research Nurse Coordinator
Div. of Clinical & Metabolic Genetics
Tele: 416-813-8367
Email: margaret.mackrell@sickkids.ca

Mailing Address for Dr. Raiman & Ms. Mackrell
Division of Clinical & Metabolic Genetics
Hospital for Sick Children
555 University Avenue
Toronto, Ontario M5G 1X8

References

1. Patterson MC, Vanier MT, Suzuki K *et al.* Niemann-Pick disease, type C: a lipid trafficking disorder. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Vogelstein B (eds) *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed, 2001. New York: McGraw-Hill, Ch 145, pp 3611–33.
2. Brady RO, Filling-Katz MR, Barton NW. Niemann-Pick disease types C and D. Neurol Clin 1989;7:75–88.
3. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. JAMA 1999;281:249–54.
4. Patterson MC. Niemann-Pick disease Type C. Gene Reviews 2007a (updated 9 July). Accessible at: www.geneclinics.org. Accessed 10 July 2007.
5. Patterson MC, Vecchio D, Prady H *et al.* Miglustat in Niemann-Pick type C disease: Results of the first 12 months treatment. Lancet Neurol 2007b, July 30; [Epub ahead of print].
6. Niemann A. Ein unbekanntes krankheitsbild. Jahrb Kinderheilkd 1914;79:1.
7. Crocker AC, Farber S. Niemann-Pick disease: a review of eighteen patients. Medicine 1958;37:1.
8. Crocker AC. The cerebral defect in Tay-Sachs disease and Niemann-Pick disease. J Neurochem 1961;7:69.
9. Kanfer JN, Young OM, Shapiro D, Brady RO. The metabolism of sphingomyelin. I. Purification and properties of a sphingomyelin-cleaving enzyme from rat liver tissue. J Biol Chem 1966;241:1081–4.
10. Elleder M, Jirásek A. Niemann-Pick Disease. Report on a symposium held in Hlava's Institute of Pathology, Charles University, Prague 2nd-3rd September, 1982. Acta Univ Carol [Med] (Praha) 1983;29:259–67.
11. National Institute of Neurological Disorders and Stroke (NINDS). *Niemann-Pick disease.* Available at: www.ninds.nih.gov/disorders/niemann/niemann.htm. Accessed July 20, 2007.
12. Vanier MT. Prenatal diagnosis of Niemann-Pick diseases types A, B and C. Prenat Diagn 2002;22:630–2.
13. Pentchev PG, Boothe AD, Kruth HS *et al.* A genetic storage disorder in BALB/C mice with a metabolic block in esterification of exogenous cholesterol. J Biol Chem 1984;259:5784–91.
14. Pentchev PG, Comly ME, Kruth HS *et al.* A defect in cholesterol esterification in Niemann-Pick disease (type C) patients. Proc Natl Acad Sci USA 1985;82:8247–51.
15. Pentchev PG, Comly ME, Kruth HS *et al.* The cholesterol storage disorder of the mutant BALB/c mouse. A primary genetic lesion closely linked to defective esterification of exogenously derived cholesterol and its relationship to human type C Niemann-Pick disease. J Biol Chem 1986;261:2772–7.
16. Vanier MT, Rodriguez-Lafrasse C, Rousson R *et al.* Prenatal diagnosis of Niemann-Pick type C disease: current strategy from an experience of 37 pregnancies at risk. Am J Hum Genet 1992;51:111–22.
17. Carstea ED, Polymeropoulos MH, Parker CC *et al.* Linkage of Niemann-Pick disease type C to human chromosome 18. Proc Natl Acad Sci USA 1993;90:2002–4.
18. Carstea ED, Morris JA, Coleman KG *et al.* Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. Science 1997;277:228–31.
19. Vanier MT, Duthel S, Rodriguez-Lafrasse C *et al.* Genetic heterogeneity in Niemann-Pick C disease: a study using somatic cell hybridization and linkage analysis. Am J Hum Genet 1996;58:118–25.
20. Greer WL, Dobson MJ, Girouard GS *et al.* Mutations in NPC1 highlight a conserved NPC1-specific cysteine-rich domain. Am J Hum Genet 1999;65:1252–60.
21. Park WD, O'Brien JF, Lundquist PA *et al.* Identification of 58 novel mutations in Niemann-Pick disease type C: correlation with biochemical phenotype and importance of PTC1-like domains in NPC1. Hum Mutat 2003;22:313–25.
22. Naureckiene S, Sleat DE, Lackland H *et al.* Identification of HE1 as the second gene of Niemann-Pick C disease. Science 2000;290:2298–301.
23. Millat G, Chikh K, Naureckiene S *et al.* Niemann-Pick disease type C: spectrum of HE1 mutations and genotype/phenotype correlations in the NPC2 group. Am J Hum Genet 2001a;69:1013–21.
24. Morris JA, Zhang D, Coleman KG *et al.* The genomic organization and polymorphism analysis of the human Niemann-Pick C1 gene. Biochem Biophys Res Commun 1999;261:493–8.
25. Millat G, Baïlo N, Molinero S *et al.* Niemann-Pick C disease: use of denaturing high performance liquid chromatography for the detection of NPC1 and NPC2 genetic variations and impact on management of patients and families. Mol Genet Metab 2005;86:220–32.
26. Scott C, Ioannou YA. The NPC1 protein: structure implies function. Biochim Biophys Acta 2004;1685:8–13.

27. Fernandez-Valero EM, Ballart A, Iturriaga C *et al.* Identification of 25 new mutations in 40 unrelated Spanish Niemann-Pick type C patients: genotype-phenotype correlations. Clin Genet 2005;68:245-54.
28. Greer WL, Riddell DC, Gillan TL *et al.* The Nova Scotia (type D) form of Niemann-Pick disease is caused by a G3097-->T transversion in NPC1. Am J Hum Genet 1998;63:52-4.
29. Millat G, Marçais C, Rafi MA *et al.* Niemann-Pick C1 disease: the I1061T substitution is a frequent mutant allele in patients of Western European descent and correlates with a classic juvenile phenotype. Am J Hum Genet 1999;65:1321-9.
30. Millat G, Marçais C, Tomasetto C *et al.* Niemann-Pick C1 disease: correlations between NPC1 mutations, levels of NPC1 protein, and phenotypes emphasize the functional significance of the putative sterol-sensing domain and of the cysteine-rich luminal loop. Am J Hum Genet 2001b;68:1373-85.
31. Chikh K, Vey S, Simonot C *et al.* Niemann-Pick type C disease: importance of N-glycosylation sites for function and cellular location of the NPC2 protein. Mol Genet Metab 2004;83:220-30.
32. Chikh K, Rodriguez C, Vey S *et al.* Niemann-Pick type C disease: subcellular location and functional characterization of NPC2 proteins with naturally occurring missense mutations. Hum Mutat 2005;26:20-8.
33. Klünemann HH, Elleder M, Kaminski WE *et al.* Frontal lobe atrophy due to a mutation in the cholesterol binding protein HE1/NPC2. Ann Neurol 2002;52:743-9.
34. Imrie J, Dasgupta S, Besley GT *et al.* The natural history of Niemann-Pick disease type C in the UK. J Inherit Metab Dis 2007;30:51-9.
35. Mukherjee S, Maxfield FR. Lipid and cholesterol trafficking in NPC. Biochim Biophys Acta 2004;1685:28-37.
36. Sokol J, Blanchette-Mackie J, Kruth HS *et al.* Type C Niemann-Pick disease. Lysosomal accumulation and defective intracellular mobilization of low density lipoprotein cholesterol. J Biol Chem 1988;263:3411-7.
37. Shamburek RD, Pentchev PG, Zech LA *et al.* Intracellular trafficking of the free cholesterol derived from LDL cholesteryl ester is defective in vivo in Niemann-Pick C disease: insights on normal metabolism of HDL and LDL gained from the NP-C mutation. J Lipid Res 1997;38:2422-35.
38. Beltroy EP, Richardson JA, Horton JD *et al.* Cholesterol accumulation and liver cell death in mice with Niemann-Pick type C disease. Hepatology 2005;42:886-93.
39. Vanier MT, Wenger DA, Comly ME *et al.* Niemann-Pick disease group C: clinical variability and diagnosis based on defective cholesterol esterification. A collaborative study on 70 patients. Clin Genet 1988;33:331-48.
40. Vanier MT, Rodriguez-Lafrasse C, Rousson R *et al.* Type C Niemann-Pick disease: biochemical aspects and phenotypic heterogeneity. Dev Neurosci 1991;13:307-14.
41. Pentchev PG, Comly ME, Kruth HS *et al.* Group C Niemann-Pick disease: faulty regulation of low-density lipoprotein uptake and cholesterol storage in cultured fibroblasts. FASEB J 1987;1:40-5.
42. Dawson G, Matalon R, Stein AO. Lactosylceramidosis: lactosylceramide galactosyl hydrolase deficiency and accumulation of lactosylceramide in cultured skin fibroblasts. J Pediatr 1971;79:423-9.
43. Dawson G. Glycosphingolipid levels in an unusual neurovisceral storage disease characterized by lactosylceramide galactosyl hydrolase deficiency: lactosylceramidosis. J Lipid Res 1972;13:207-19.
44. Zervas M, Dobrenis K, Walkley SU. Neurons in Niemann-Pick disease type C accumulate gangliosides as well as unesterified cholesterol and undergo dendritic and axonal alterations. J Neuropathol Exp Neurol 2001a;60:49-64.
45. Walkley SU, Suzuki K. Consequences of NPC1 and NPC2 loss of function in mammalian neurons. Biochim Biophys Acta 2004;1685:48-62.
46. Suzuki K, Parker CC, Pentchev PG *et al.* Neurofibrillary tangles in Niemann-Pick disease type C. Acta Neuropathol (Berl) 1995;89:227-38.
47. Sturley SL, Patterson MC, Balch W, Liscum L. The pathophysiology and mechanisms of NP-C disease. Biochim Biophys Acta 2004;1685:83-7.
48. Neufeld EB, Wastney M, Patel S *et al.* The Niemann-Pick C1 protein resides in a vesicular compartment linked to retrograde transport of multiple lysosomal cargo. J Biol Chem 1999;274:9627-35.

49. Watari H, Blanchette-Mackie EJ, Dwyer NK *et al.* Niemann-Pick C1 protein: obligatory roles for N-terminal domains and lysosomal targeting in cholesterol mobilization. Proc Natl Acad Sci USA 1999;96:805-10.
50. Wojtanik KM, Liscum L. The transport of low density lipoprotein-derived cholesterol to the plasma membrane is defective in NPC1 cells. J Biol Chem 2003;278:14850-6.
51. Millard EE, Gale SE, Dudley N *et al.* The sterol-sensing domain of the Niemann-Pick C1 (NPC1) protein regulates trafficking of low density lipoprotein cholesterol. J Biol Chem 2005;280:28581-90.
52. Strauss JF, Liu P, Christenson LK, Watari H. Sterols and intracellular vesicular trafficking: lessons from the study of NPC1. Steroids 2002;67:947-51.
53. Liscum L, Sturley SL. Intracellular trafficking of Niemann-Pick C proteins 1 and 2: obligate components of subcellular lipid transport. Biochim Biophys Acta 2004;1685:22-7.
54. Frolov A, Zielinski SE, Crowley JR *et al.* NPC1 and NPC2 regulate cellular cholesterol homeostasis through generation of low density lipoprotein cholesterol-derived oxysterols. J Biol Chem 2003;278:25517-25.
55. Saito Y, Suzuki K, Nanba E *et al.* Niemann-Pick type C disease: accelerated neurofibrillary tangle formation and amyloid beta deposition associated with apolipoprotein E epsilon 4 homozygosity. Ann Neurol 2002;52:351-5.
56. Jin LW, Shie FS, Maezawa I *et al.* Intracellular accumulation of amyloidogenic fragments of amyloid-beta precursor protein in neurons with Niemann-Pick type C defects is associated with endosomal abnormalities. Am J Pathol 2004;164:975-85.
57. Nixon RA. Niemann-Pick Type C disease and Alzheimer's disease: the APP-endosome connection fattens up. Am J Pathol 2004;164:757-61.
58. Reddy JV, Ganley IG, Pfeffer SR. Clues to neuro-degeneration in Niemann-Pick type C disease from global gene expression profiling. PLoS ONE 2006;1:e19.
59. Yamazaki T, Chang TY, Haass C, Ihara Y. Accumulation and aggregation of amyloid beta-protein in late endosomes of Niemann-Pick type C cells. J Biol Chem 2001;276:4454-60.
60. Pacheco CD, Kunkel R, Lieberman AP. Autophagy in Niemann-Pick C disease is dependent upon Beclin-1 and responsive to lipid trafficking defects. Hum Mol Genet 2007;16:1495-503.
61. Pacheco CD, Lieberman AP. Lipid trafficking defects increase beclin-1 and activate autophagy in Niemann-Pick Type C disease. Autophagy 2007 Jun 14: [Epub ahead of print].
62. Winsor EJ, Welch JP. Genetic and demographic aspects of Nova Scotia Niemann-Pick disease (type D). Am J Hum Genet 1978;30:530-8.
63. Garver WS, Francis GA, Jelinek D *et al.* The National Niemann-Pick C1 database: report of clinical features and health problems. Amer J Med Genet Part A 2007;143A:1204-11.
64. Manning DJ, Price WI, Pearse RG. Foetal ascites: an unusual presentation of Niemann-Pick disease type C. Arch Dis Child 1990;65:335-6.
65. Philippart M, Engel J, Zimmerman EG. Gelastic cataplexy in Niemann-Pick disease group C and related variants without generalized sphingomyelinase deficiency. Ann Neurol 1983;14:492-3.
66. Kanbayashi T, Abe M, Fujimoto S *et al.* Hypocretin deficiency in Niemann-Pick type C with cataplexy. Neuropediatrics 2003;34:52-3.
67. Vankova J, Stepanova I, Jech R *et al.* Sleep disturbances and hypocretin deficiency in Niemann-Pick disease type C. Sleep 2003;26:427-30.
68. Imrie J, Vijayaraghaven S, Whitehouse C *et al.* Niemann-Pick disease type C in adults. J Inherit Metab Dis 2002;25:491-500.
69. Sévin M, Lesca G, Baumann N *et al.* The adult form of Niemann-Pick disease type C. Brain 2006;130:120-33.
70. Josephs KA, Van Gerpen MW, Van Gerpen JA. Adult onset Niemann-Pick disease type C presenting with psychosis. J Neurol Neurosurg Psychiatry 2003;74:528-9.
71. Vanier MT. Phenotypic and genetic heterogeneity in Niemann-Pick disease type C: current knowledge and practical implications. Wien Klin Wochenschr 1997;109:68-73.
72. Fink JK, Filling-Katz MR, Sokol J *et al.* Clinical spectrum of Niemann-Pick disease type C. Neurology 1989;39:1040-9.

73. Sun X, Marks DL, Park WD *et al.* Niemann-Pick C variant detection by altered sphingolipid trafficking and correlation with mutations within a specific domain of NPC1. Am J Hum Genet 2001;68:1361-72.
74. Hollak CE, van Weely S, van Oers MH, Aerts JM. Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. J Clin Invest 1994;93:1288-92.
75. Wajner A, Michelin K, Burin MG *et al.* Biochemical characterization of chitotriosidase enzyme: comparison between normal individuals and patients with Gaucher and with Niemann-Pick diseases. Clin Biochem 2004;37:893-7.
76. Ries M, Schaefer E, Lührs T *et al.* Critical assessment of chitotriosidase analysis in the rational laboratory diagnosis of children with Gaucher disease and Niemann-Pick disease type A/B and C. J Inherit Metab Dis 2006;29:647-52.
77. Cox T, Lachmann R, Hollak C *et al.* Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. Lancet 2000;355:1481-5.
78. Vellodi A, Wraith JE, McHugh K, Cooper A. Guidelines for the management of paediatric Gaucher disease in the United Kingdom - 2005. UK Department of Health (Crown Copyright). Available at: www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4118403. Accessed 20 July, 2007.
79. Chien YH, Lee NC, Tsai LK *et al.* Treatment of Niemann-Pick disease type C in two children with miglustat: Initial responses and maintenance of effects over 1 year. J Inherit Metab Dis 2007, June 21; [Epub ahead of print].
80. Boustany RN, Kaye E, Alroy J. Ultrastructural findings in skin from patients with Niemann-Pick disease, type C. Pediatr Neurol 1990;6:177-83.
81. Tedeschi G, Bonavita S, Barton NW *et al.* Proton magnetic resonance spectroscopic imaging in the clinical evaluation of patients with Niemann-Pick type C disease. J Neurol Neurosurg Psychiatry 1998;65:72-9.
82. Burton H. Metabolic pathways Networks of Care: A needs assessment and review of services for people with inherited metabolic disease in the United Kingdom. Public Health Genetics Unit, 2005: available at http://www.phgfoundation.org/. Accessed 20 July, 2007.
83. Drotar D, Crawford P. Psychological adaptation of siblings of chronically ill children: research and practice implications. J Dev Behav Pediatr 1985;6:355-62.
84. Derouin D, Jessee PO. Impact of a chronic illness in childhood: siblings' perceptions. Issues Compr Pediatr Nurs 1996;19:135-47.
85. Taylor, Fuggle P, Charman T. Well sibling psychological adjustment to chronic physical disorder in a sibling: how important is maternal awareness of their illness attitudes and perceptions? J Child Psychol Psychiatry 2001;42:953-62.
86. Sakiyama T, Tsuda M, Owada M *et al.* Bone marrow transplantation for Niemann-Pick mice. Biochem Biophys Res Commun 1983;113:605-10.
87. Yasumizu R, Miyawaki S, Sugiura K *et al.* Allogeneic bone marrow-plus-liver transplantation in the C57BL/KsJ spm/spm mouse, an animal model of Niemann-Pick disease. Transplantation 1990;49:759-64.
88. Patterson MC, Di Bisceglie AM, Higgins JJ *et al.* The effect of cholesterol-lowering agents on hepatic and plasma cholesterol in Niemann-Pick disease type C. Neurology 1993;43:61-4.
89. Choudhury A, Dominguez M, Puri V *et al.* Rab proteins mediate Golgi transport of caveola-internalized glycosphingolipids and correct lipid trafficking in Niemann-Pick C cells. J Clin Invest 2002;109:1541-50.
90. Walter M, Davies JP, Ioannou YA. Telomerase immortalization upregulates Rab9 expression and restores LDL cholesterol egress from Niemann-Pick C1 late endosomes. J Lipid Res 2003;4:243-53.
91. Patterson MC, Platt F. Therapy of Niemann-Pick disease, type C. Biochim Biophys Acta 2004;1685:77-82.
92. Liscum L, Arnio E, Anthony M *et al.* Identification of a pharmaceutical compound that partially corrects the Niemann-Pick C phenotype in cultured cells. J Lipid Res 2002;43:1708-17.
93. Griffin LD, Gong W, Verot L *et al.* Niemann-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. Nat Med 2004;10:704-11.
94. Mellon SH, Gong W, Schonemann MD. Endogenous and synthetic neurosteroids in treatment of Niemann-Pick Type C disease. Brain Res Rev 2007 Jun 12; [Epub ahead of print].

95. Mellon SH, Griffin LD. Neurosteroids: biochemistry and clinical significance. Trends Endocrinol Metab 2002;13:35–43.
96. Ahmad I, Lope-Piedrafita S, Bi X *et al.* Allopregnanolone treatment, both as a single injection or repetitively, delays demyelination and enhances survival of Niemann–Pick C mice. J Neurosci Res 2005;82:811–21.
97. Langmade SJ, Gale SE, Frolov A *et al.* Pregnane X receptor (PXR) activation: a mechanism for neuroprotection in a mouse model of Niemann–Pick C disease. Proc Natl Acad Sci USA 2006;103:13807–12.
98. Lachmann RH, te Vruchte D, Lloyd-Evans E *et al.* Treatment with miglustat reverses the lipid-trafficking defect in Niemann–Pick disease type C. Neurobiol Dis 2004;16:654–8.
99. Zervas M, Somers KL, Thrall MA, Walkley SU. Critical role for glycosphingolipids in Niemann–Pick disease type C. Curr Biol 2001b;11:1283–7.
100. Garbutt S, Harwood MR, Harris CM. Comparison of the main sequence of reflexive saccades and the quick phases of optokinetic nystagmus. Br J Ophthalmol 2001;85:1477–83.
101. Rottach KG, von Maydell RD, Das VE *et al.* Evidence for independent feedback control of horizontal and vertical saccades from Niemann–Pick type C disease. Vision Res 1997;37:3627–38.
102. Floyd AG, Yu QP, Piboolnurak P *et al.* Kinematic analysis of motor dysfunction in Niemann–Pick type C. Clin Neurophysiol 2007;118:1010–8.
103. Jain M, Passi GR. Assessment of a modified Mini-Mental Scale for cognitive functions in children. Indian Pediatr 2005;42:907–12.
104. Ouvrier RA, Goldsmith RF, Ouvrier S, Williams IC. The value of the Mini-Mental State Examination in childhood: a preliminary study. J Child Neurol 1993;8:145–8.
105. Ware JE, Sherbourne CD. The MOS 36–item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473–83.
106. Han TR, Paik NJ, Park JW. Quantifying swallowing function after stroke: A functional dysphagia scale based on videofluoroscopic studies. Arch Phys Med Rehabil 2001;82:677–82.