Efficient Investigation and Differential Diagnosis of Childhood Onset Niemann-Pick Type C

Alasdair Parker

Consultant Paediatric Neurologist, Department of Paediatric Neurology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

Abstract

Niemann-Pick disease type C (NPC) is a fatal, neurodegenerative, lysosomal storage disorder. It is rare with a broad phenotypic spectrum and variable age of onset. This complicates diagnosis, which is often delayed by several years after presentation of the first symptoms. It is a treatable condition if detected early, therefore reliable means of diagnosis are essential. Clinical diagnosis of NPC involves identifying characteristic neurological features, taking a detailed history of the patient's details, and must be confirmed by biochemical and/or genetic testing. The key laboratory diagnostic test for NPC is filipin staining of cultured skin fibroblasts, which shows free cholesterol accumulation in lysosomes resulting from impaired intracellular cholesterol transport. Genetic testing for mutations in the *NPC1* and *NPC2* genes is also important for confirmation of the diagnosis. However, there is an unmet need for cheaper diagnostic tests with greater specificity and sensitivity.

Keywords

Filipin, lysosomal storage disorder, Niemann-Pick type C (NPC), *NPC1*, *NPC2*, progressive intellectual, neurological deterioration, genetic analysis

Disclosure: Alasdair Parker is a member of the Niemann-Pick Type C advisory panel for which he receives an honorarium from Actelion. He has also received remuneration from Actelion for developing and running workshops and lectures.
Acknowledgement: Editorial assistance was provided by Touch Briefings and funded by Actelion.
Received: 10 September 2012 **Accepted:** 17 September 2012 **Citation:** *European Neurological Review*, 2012;7(3):153–9 *DOI:10.17925/ENR.2012.07.03.153*
Correspondence: Alasdair Parker, Consultant Paediatric Neurologist, Child Development Centre, Box 107, Addenbrooke's Hospital, Cambridge, CB2 0QQ, UK.
E: alasdair.parker@addenbrookes.nhs.uk

Support: The publication of this article was funded by Actelion. The views and opinions expressed are those of the author and not necessarily those of Actelion.

Niemann-Pick disease type C (NPC) is a rare, autosomal recessive, neurodegenerative disorder occurring in all ethnic groups, with an estimated minimal incidence of 0.82 per 100,000 live births.[1] This figure is likely to be an underestimation due to failure to recognise the clinical characteristics and initiate appropriate tests. NPC is a devastating disorder, characterised by a variety of progressive and disabling neurological/psychiatric symptoms and leading to premature death.[2] In the terminal phase, patients are immobile and require tube feeding.[3] The emotional and economic burden imposed by NPC on patients, families and society is disproportionate to the rarity of the disease.[3]

The wide clinical spectrum of NPC was not recognised until the early 1990s, particularly regarding rapidly fatal cases in infants, and no specific laboratory tests had been available making accurate diagnosis challenging. Delayed presentation of NPC in adolescents and adults has further added to the misdiagnosis of this disorder. Since awareness of the disease and diagnostic techniques have improved, what was once considered to be a childhood condition is now increasingly recognised as an illness affecting individuals of all ages.

The biochemistry behind NPC was elucidated in 1984 following studies on cholesterol metabolism.[4] It is a cellular lipid trafficking disorder characterised by lysosomal accumulation of low-density lipoprotein (LDL)-derived, unesterified cholesterol.[1] Genetic studies found that mutations in two genes, *NPC1* (reported in 90–95 % of patients) and *NPC2* (~5 % of patients), play a role in the disrupted transport of unesterified cholesterol, sphingolipids and glycosphingolipids. When there is mutation within one of these genes, accumulation of cholesterol in late endosomes and lysosomes within the spleen, liver and brain occurs.[3,5,6]

NPC is a heterogeneous condition with an age at onset ranging from the perinatal period to as late as 50 years of age or older; juvenile onset (ages 6–15 years) is the most common.[1] The age of onset has a major impact on the severity and the course of the disease although progression is linear and independent of this variable (see *Figure 1*).[2,7] The different ages of disease onset can be used to define the disorder: the generally accepted categories are pre/perinatal, early-infantile, late-infantile, juvenile, and adolescent/adult.[3] Apart from a subset of patients that die at birth, most patients follow a progressive and ultimately fatal neurological disease course. The lifespan of affected individuals can vary from days to years with many dying between 10 and 25 years of age.[1]

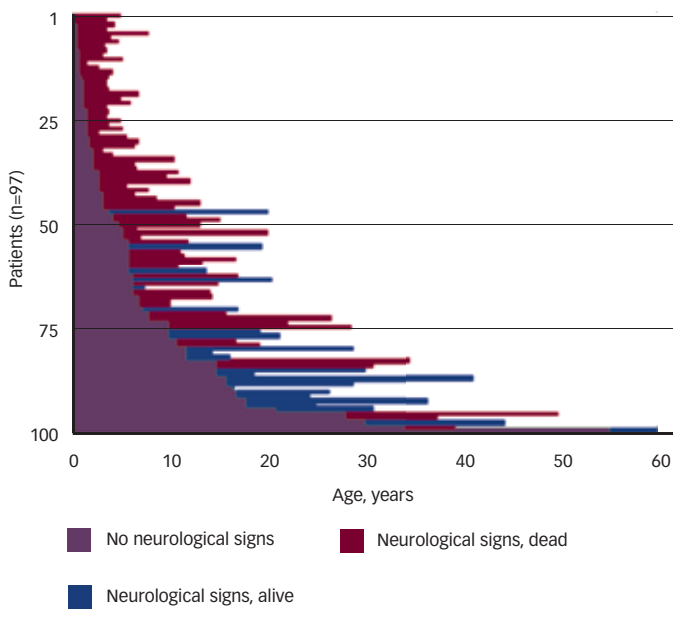
While diagnosis of this rare condition is not difficult *per se*, mis-/delayed diagnoses are common – possibly due to poor clinical examination and inappropriate tests being performed. Timely detection of NPC is

Table 1: List of Symptoms Associated with Age at Onset

Age at Onset	Systemic Manifestations	Neurological Manifestations
Pre/peri-natal period (≤3 months)	• Foetal hydrops • Hepatosplenomegaly • Foetal ascites with or without persistence after birth • Prolonged cholestasis (frequent) • Respiratory failure • Hepatic failure	• Usually not recognised
Early-infantile period (3 months to <2 years)	• Isolated hepatosplenomegaly or hepatosplenomegaly	• Delayed developmental motor milestones • Central hypotonia • Hearing loss • VSGP* (usually not recognised)
Late-infantile period (2 years to <6 years)	• Isolated organomegaly or organomegaly (usually present)	• Frequent falls, clumsiness • Progressive ataxia, dystonia, dysphagia, dysarthria • Central hypotonia • Hearing loss • Seizures (partial or generalised) • Cataplexy • VSPG* (usually present)
Juvenile (classical) (6–15 years)	• Isolated organomegaly or organomegaly (not always present)	• School failure, learning disability • Behavioural problems • Frequent falls, clumsiness • Progressive ataxia, dysarthria, dystonia, dysphagia • Myoclonus • Cataplexy • Seizures (partial and/or generalised) • VSPG* (usually present)
Adolescent and adult (>15 years)	• Organomegaly (not always present) or isolated splenomegaly in adults has been described in exceedingly rare cases	• Clumsiness • Cataplexy • Psychiatric signs† • Cognitive decline, dementia, learning disability • VSPG* (usually present) • Slowly progressing motor symptoms‡ • Myoclonus • Seizures (partial and/or generalised)

**VSGP = vertical, supranuclear gaze palsy – increased latency in initiation of vertical saccades, with gradual slowing and eventual loss of saccadic velocity; †schizophrenia (psychosis), depression; ‡ataxia, dystonia, dysarthria, dysphagia. Source: Wraith JE et al., 2009.[3]*

Figure 1: Relationship Between Age at Onset and Niemann-Pick Disease Type C Severity Based on the Natural History of the Disease Without any Treatment



Source: Vanier MT, 2010. [1]

essential, however, because this disorder responds better to disease-modifying pharmacological and supportive treatments when they are administered at an early stage.[8] This article will review the clinical manifestations of NPC as well as discuss current strategies for the efficient diagnosis of this rare disease.

Signs and Symptoms

The broad clinical manifestations of NPC and their association with age at onset are presented in *Table 1*, although there is considerable overlap between the different categories. The wide range of signs and symptoms includes cerebellar ataxia manifesting as language delay, gait problems, frequent falls and clumsiness; this leads to dysarthria (slurred speech). There is also dysphasia, and dysphagia is a pronounced symptom. Dystonia commonly begins with the turning inwards of one foot when walking, but hand posturing is also often evident early on. Later in the disease course, spasticity and progressive dementia become more obvious. Children of school age frequently exhibit learning and/or behavioural difficulties. Seizures are also common and psychiatric symptoms are typically seen in late-onset patients.[1]

Moreover, NPC is associated with some very specific signs. Vertical supranuclear gaze palsy (VSGP) beginning with subtle abnormal saccadic eye movements is more commonly detected from late infancy onwards. However, it is a sign that is mostly overlooked and

hard to diagnose in early childhood (<3 years of age), since physicians often do not specifically examine the patient for it. The child loses balance easily and shows learning difficulties, eventually being unable to interpret visual cues in the environment and in social situations. Gelastic cataplexy, a sudden loss of muscle tone due to a positive emotional stimulus such as laughter is another very specific sign of NPC.[9] This symptom is different from narcolepsy, as it occurs in full consciousness, and in the absence of excessive daytime sleepiness and short sleep latency.

Systemic, and in particular visceral symptoms, may also be present. NPC is a significant cause of neonatal cholestatic liver disease with associated splenomegaly and of isolated splenomegaly or hepatosplenomegaly in childhood. Prolonged, neonatal, conjugated hyperbilirubinaemia may be found in the medical records of patients of all ages.[2] Hepatosplenomegaly may be present in all age groups, but could remain asymptomatic and unrecognised in patients that develop the disease at an older age. Pulmonary infiltration with foam cells may also rarely be seen.[3,10,11]

Challenges in Diagnosing Niemann-Pick Disease Type C

For many neurometabolic conditions, no disease-modifying therapies are available. However, treatment is available for NPC, which can arrest and/or slow disease progression, although improvement of symptoms is unlikely. As a result, early diagnosis is imperative to allow for prompt application of management strategies. Unfortunately, NPC is often misdiagnosed for various reasons leading to a delay between initial symptoms and appropriate therapy.

Childhood-onset NPC is frequently missed as it tends to start insidiously with an inconspicuous steady progression that diverges increasingly from the normal population. Moreover, the rate of disease progression varies greatly, further confusing matters. Lack of awareness among clinicians and paediatricians (perhaps due to the rarity of NPC) also contributes to the mis/underdiagnosis of this disease.[1]

Furthermore, NPC is a multifaceted and heterogeneous condition, so not all classical signs may be present at any given time.[3] Symptoms might be quite common and/or spread over time (e.g. unexplained prolonged jaundice as neonates followed by developmental delay later in life) and there is often a 'wait and see' attitude among physicians when no obvious cause is found, which is not appropriate when NPC is a possible diagnosis.

As a result of these obstacles, the average time from initial symptoms to a definite diagnosis is approximately four years.[12] Routine tests (even for metabolic dysfunctions) do not normally include NPC. Consequently, it is often overlooked.

Clinical Investigation and Differential Diagnosis

The clinician is faced with a broad spectrum of symptoms in suspected NPC, as a consequence efficient and correct diagnosis is often not made. Paediatricians need to be able to effectively distinguish conditions such as NPC, which are fully treatable, from simple 'developmental delay' or static neuropsychiatic disorders in children. Moreover, they need to ensure efficient investigation and application of appropriate tests for degenerative disorders. While there remains no standard procedure to follow for NPC diagnosis, there are key symptoms that should signal the need for further examination. The following details the steps to take in the diagnostic work-up of patients who potentially have NPC.

Table 2: High Yield Information to Capture During History Taking

1. Is development progressing, plateauing or deteriorating?
2. Is there consanguinuity?
3. Was there anything remarkable in pregnancy or the peri-natal period?
4. Is there any significant past medical history, other than routine childhood illnesses and minor injuries?
5. Is there any relevant family history of degenerative disorders?
6. Are there any other objective symptoms of concern?

Table 3: Red Flag Signs to Note During Neurological Examination

Examination	Red Flag Signs*
Introduction and gait	Gait ataxia, spasticity and dystonia
Dynamic assessment	Gait ataxia, weakness and dystonia
Arms and legs	Dysdiadochokinesis Dysmetria Dystonia Spasticity
Eyes	Vertical supranuclear gaze palsy Nystagmus Blindness Retinal abnormality
Other cranial nerves	Dysphagia Dysarthria

**In static developmental delay, these signs need to be absent.*

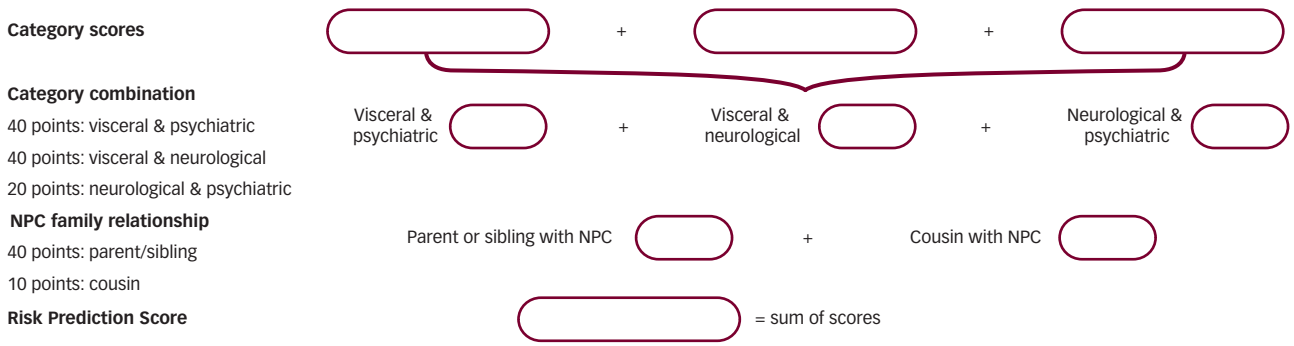
An essential part of the initial investigation is a thorough history taking of the patients' details, usually through the parent(s). This will reveal any major milestones experienced in the past and processes that may have affected the brain. Obtaining their family histories in terms of neurological disorders or early death, and discovering any previous significant illnesses such as prolonged neonatal jaundice and seizures should alert healthcare professionals to the possibility of an NPC diagnosis. In addition, specifically asking for consanguinity in the family history is essential at this stage. *Table 2* highlights the key information that should be elucidated during this history taking. A complete history of the patient is vital in determining the next steps of the assessment process.

With a clear understanding of the individual's history, a detailed examination can be performed to separate the developmental delay or dyspraxia from other neurological signs. *Table 3* summarises the signs that should raise red flags in the physician's mind regarding an NPC diagnosis. The clinical assessment must include ophthalmologic evaluation, with specific examination of spontaneous vertical saccadic eye movements. The impairment of these movements is specific for VSGP, a key sign of NPC. Neurological examination should include assessment of muscle strength, muscle tone, motor reflexes, movement, speech, swallowing, and cognition.[3] Psychometric testing is also advisable. When there are focal neurological signs and/or a suggestive history, additional specific testing using biochemical techniques and genetic analyses should be performed; these are discussed in the next section.

A frequent error is to base differential diagnoses on a deficient examination. Systemic signs such as neonatal jaundice/isolated splenomegaly, and neurological symptoms such as dystonia, ataxia,

Figure 2: The Niemann-Pick Disease Type C Suspicion Index Tool

Signs and Symptoms						
Indicators	Visceral	Score	Neurological	Score	Psychiatric	Score
Very Strong 40 points per item			• Vertical supranuclear gaze palsy • Gelastic cataplexy	☐ ☐		
Strong 20 points per item	• Prolonged unexplained neonatal jaundice or cholestatis • Isolated unexplained splenomegaly (historical and/or current) with/ without hepatomegaly	☐ ☐			• Pre-senile cognitive decline and/or dementia	☐
Moderate 10 points per item			• Ataxia, clumsiness or frequent falls • Dysarthria and/or dysphagia • Dystonia	☐ ☐ ☐	• Psychotic symptoms (hallucinations, delusions and/or thought disorder)	☐
Weak 5 points per item			• Acquired and progressive spasticity	☐	• Treatment-resistent psychiatric symptoms • Other psychiatric disorders	☐ ☐
Anciliary 1 point per item	• Hydrops fetalis • Siblings with foetal ascites	☐ ☐	• Hypotonia • Delayed developmental milestones • Seizure (partial or generalised) • Myoclonus	☐ ☐ ☐ ☐	• Disruptive or agressive behaviour in adolescence and childhood	☐



NPC = Niemann-Pick type C. Source: Patterson MC et al., 2012.[19]

spasticity and supranuclear gaze palsy can appear in other diseases.[3,13] Therefore other conditions that involve these symptoms need to be considered during diagnosis.

In neonatal and infantile presentation, differential diagnosis should be made between NPC and idiopathic neonatal hepatitis. It must be emphasised that isolated splenomegaly or hepatosplenomegaly can present long before neurological symptoms; the latter being seen in up to 50 % of cases of perinatal presentation.[1,14]

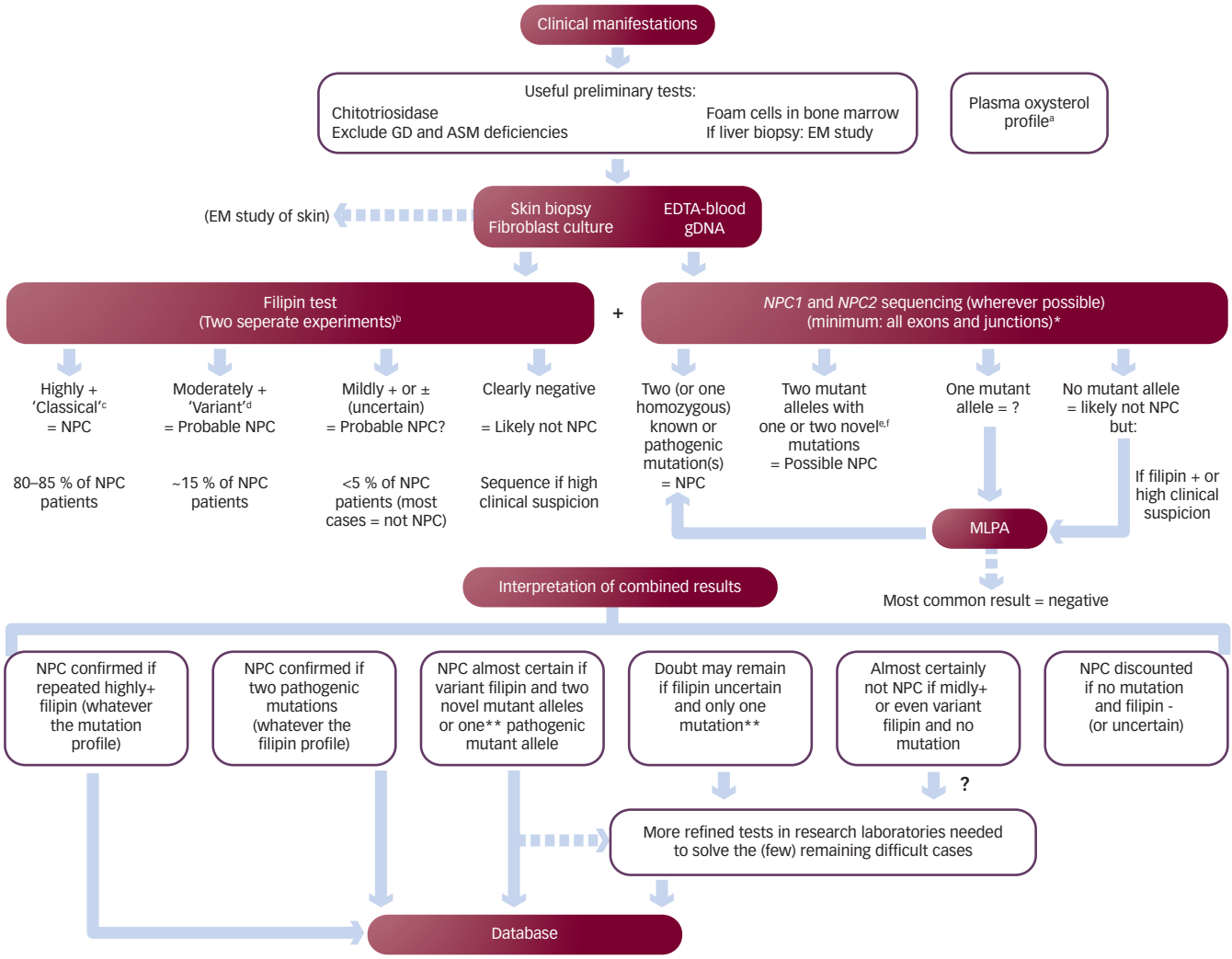
In juvenile NPC, all of the neurodegenerative diseases that present during teenage years, such as Wilson's and Huntington's, need to be excluded. In general with juvenile disease, because there are minimal psychiatric symptoms, NPC will tend not to be confused with other psychiatric diseases. Instead, NPC is more likely to be confused with diseases showing progressive intellectual and neurological deterioration (PIND). In adult-onset NPC, on the other hand, psychosis is often the initial manifestation of NPC and differential diagnosis includes schizophrenia[15] and multiple sclerosis.[16]

The other lysosomal storage disorders such as the gangliosidoses and lipofuscinoses, mitochondrial diseases, and dysmyelination syndromes such as Pelizaeus-Merzbacher, Rett's and organic acidaemias (particularly glutaric aciduria) should be taken into consideration for differential diagnosis. GM2 gangliosidiosis is probably the most frequently diagnosed similar disease, although it tends not to have such a slow deterioration. A study from the UK and reported by Verity et al, summarises the epidemiology of the majority of PIND disorders[17] and is a very good basis for consideration of differential diagnoses.

Niemann-Pick type B (NPB) results in similar foam cells in the bone marrow.[1] However, patients with NPC tend to have acid sphingomyelinase activity within the normal range in leucocytes. This is an important factor for excluding Niemann-Pick type A (NPA) and NPB.[18] Moreover, retinal pigment abnormalities, which are characteristic of certain other lysosomal storage disorders, are not associated with NPC and macular cherry-red spots are not seen in NPC, in contrast to NPA and NPB.[3]

The differentiation of NPC from static developmental disorders requires care. NPC is very slowly progressive and year on year assessment can give the impression that there is no significant deterioration. Attention is therefore needed to establish incontrovertible developmental steps and where evident – regression.

Figure 3: Laboratory Diagnostic Algorithm



**Numerous polymorphisms – check allele segregation from parental study; conclude quickly on NPC2 in children <8–10 months; **Profile also seen in heterozygote subjects. [a]New test under clinical development; [b]Two subsequent filipin tests (not duplicate); [c]i-cell disease gives a false positive result; [d]ASM deficiency can give a similar filipin pattern; [e]not certainly pathogenic; [f]cDNA is usually needed to study the effect of splice mutations. ASM = acid sphingomyelinase; EDTA = ethylene-diamine-tetra-acetic acid; EM = electron microscopy; GD = Gaucher disease; MLPA = lultiplex ligation-dependent probe aAmplification; NPC = Niemann-Pick type C. + and - signs denote positive and negative, respectively.*
Source: Patterson MC et al., 2012.[19]

Currently Available Diagnostic Tools

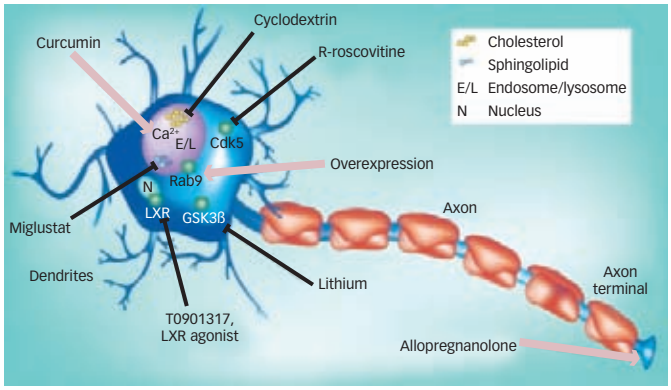
At present, there is a lack of a good non-invasive and definitive screening method for NPC. Most standard blood, urine and magnetic resonance imaging (MRI) tests employed for diagnosis of degenerative conditions are not useful in the diagnosis of NPC.

The NPC Guidelines Working Group comprises a panel of experts that has convened regularly since 2009, and most recently in 2011, to discuss best care practices for NPC.[19] Its current recommendations on the screening and diagnosis of NPC are an update to the original international guidelines published three ears ago.[3] They describe a new tool, the NPC suspicion index, which can be used to aid in diagnosing cases of suspected NPC, particularly within non-specialist centres where experience with the disease is limited (See *Figure 2*).[19] By assigning points to each symptom based on their association with NPC and combining these with the individual's family history, a final risk prediction score can be generated indicating whether the patient should be referred to an NPC centre (≥70 points), further follow-up is required (40 to 69 points) or the likelihood of NPC is low (<40 points).

Specific analyses (biochemical/histological and genetic testing) are the only means of a definitive diagnosis of NPC and should be performed according to the algorithm illustrated in *Figure 3*.[19] The key laboratory test is filipin staining of living fibroblasts to reveal any accumulation of cholesterol. For this technique, a skin biopsy is taken under local anaesthetic. Fibroblasts are cultured in an LDL-enriched medium, a process which takes four to six weeks, after which the cells are fixed and stained with filipin, a compound that binds specifically to unesterified cholesterol. Characteristic, strongly fluorescent cholesterol-filled perinuclear vesicles are detected by fluorescence microscopy in approximately 80–85 % of NPC cases[20] Although the filipin test is diagnostically valuable, the requirement for a skin biopsy excludes it from routine metabolic screening protocols. Furthermore, it is complex, expensive and should only be carried out at specialist centres with experienced staff, since assessment can be difficult in cell lines presenting only minor abnormalities.

Molecular genetic testing of *NPC1* and *NPC2* mutations is also important and provides confirmation of an NPC diagnosis.[21] It is offered at a number of specialist laboratories and has become

Figure 4: Mechanisms of Action of Therapeutic Interventions in Niemann-Pick Disease



Source: Madra M, Sturley SL, 2010.[24] © Future Science Group 2010

considerably less expensive over the last few years. Identification of *NPC1* mutations can be difficult, however, because of polymorphism. Still, genetic testing is considered essential for prenatal diagnosis at 10–12 weeks for families at risk and to identify carriers in blood relatives. In some patients mutations have only been identified in one allele and, in a few, no mutations at all have been detected, raising the possibility that a third gene may be involved in NPC.[1]

Chitotriosidase may also be normal or slightly elevated in NPC, although with poor sensitivity and specificity.[22] Other non-specific methods of analysis may include measurement of plasma lipid profiles.[23]

Following a diagnosis of NPC, patients may be started on treatment with the goal of stabilising disease or reducing the rate of disease progression.[19] Due to the nature of NPC, it can take six months to a year before clinical benefits are seen and in adults with slower progressive forms of NPC, these effects may take even longer to become evident.

Overview of Treatment and Management Options

At present, treatment strategies for NPC rely on limiting, rather than reversing, the damage. Several disease modification strategies are broadly discussed, although only one is currently approved. Their modes of action are illustrated in *Figure 4*.[24]

Miglustat, (N-butyldeoxynojirimycin, NB-DNJ, Zavesca®), inhibits glucosylceramide synthase, a key component of the glycosphingolipid biosynthetic pathway. It is currently the only approved disease-modifying therapy for NPC and slows progression with limited side effects.[25,26] Because the treatment stabilises rather than reverses disease progression, it is important to start treatment early. Once the disease is at an advanced stage, treatment with miglustat should be regularly re-evaluated, as the therapeutic effect on increasing quality of life may be limited.[8]

Various other potential therapeutic approaches are in clinical development. Pharmacological or molecular chaperone therapy is a relatively new therapeutic concept that may be applicable to NPC.[27,28] These include effective delivery, long-term expression, and avoidance of an immune response.[29] Further investigational treatments are in discussion, such as cyclodextrin that is hypothesised as promoting cholesterol efflux from the lysosomal compartments by an unknown mechanism, bypassing the defective NPC1 and NPC2 proteins.[30,31] Histone deacetylase inhibitors are another group of agents for which an effect on cholesterol accumulation in *NPC* mutant human fibroblasts could be observed.[32]

Besides pharmacological options, supportive strategies are also essential for minimising symptoms; physio, occupational and speech/language therapy, as well as psychological, family, social and educational support can be helpful.[3] There is no single approach for managing NPC and among the many issues to consider are mobility difficulties, fine motor control, pain, boredom, speech/swallowing ability, cognition and psychiatric signs. Symptomatic treatments are available for cataplexy, seizures and dystonia and should be employed when necessary.[3] Management of infections and feeding difficulties is also of importance.

Future Developments

There is a clear need for less-expensive, quicker, specific, and sensitive methods in NPC diagnosis. In future, there will likely be a trend towards wider genetic testing that will cover all the neurological disorders that present at certain ages. Genetic testing is advantageous in terms of turnaround time, cost, invasiveness and the number of different conditions that can be screened for simultaneously. However, a simple diagnostic blood test would represent a significant advance in the field of NPC.

At present there is considerable research into the development of screening tools, both with clinical algorithms and biochemical analyses. Several cholesterol oxidation products have been found to be elevated in mouse models, which were detectable before the onset of symptoms, and were associated with disease progression. Cholesterol oxidation products were similarly increased in the plasma of human NPC subjects and correlated with age of onset and disease severity. Recent data suggest that these plasma oxysterols may be sensitive and specific biomarkers for NPC. An LC-MS/MS assay has therefore been developed to quantify these oxysterols. This assay was able to discriminate with high sensitivity and specificity between control and NPC subjects and would offer clinicians a non-invasive, rapid, and highly effective method for diagnosing NPC.[33]

In addition, researchers at Oxford University, UK, have developed a microtitre-based assay utilising lysotracker as a measure of general lysosomal storage, rather than storage of a specific substrate, which can be adapted for use on any fluorescence plate reader. The assay has been validated using a macrophage model system as well as human NPC fibroblasts. An early report of it being applied to the screening of novel natural compounds for possible therapeutic benefits[34] shows that it may be valuable in diagnosing NPC.

Concluding Remarks

The need for prompt diagnosis of NPC is evident, but the current lack of a simple blood-based biochemical test with sufficient specificity to be used for screening is a major limitation. Physicians need an increased understanding of NPC, with better identification of sensitive and specific clinical symptoms, in order to make an early diagnosis, which would lead to appropriate treatment and avoid unnecessary tests. Current diagnostic techniques are relatively invasive, costly and time-consuming. However, new techniques are under development that should improve the range of diagnostic tests available. ■

1. Vanier MT, Niemann-Pick disease type C, *Orphanet J Rare Dis,* 2010;5:16.
2. 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.
3. Wraith JE, Baumgartner MR, Bembi B, et al., Recommendations on the diagnosis and management of Niemann-Pick disease type C, *Mol Genet Metab,* 2009;98:152–65.
4. 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.
5. Sleat DE, Wiseman JA, El-Banna M, et al., Genetic evidence for nonredundant functional cooperativity between NPC1 and NPC2 in lipid transport, *Proc Natl Acad Sci USA,* 2004;101:5886–91.
6. Kwon HJ, Abi-Mosleh L, Wang ML, et al., Structure of N-terminal domain of NPC1 reveals distinct subdomains for binding and transfer of cholesterol, *Cell*, 2009;137:1213–24.
7. Yanjanin NM, Velez JI, Gropman A, et al., Linear clinical progression, independent of age of onset, in Niemann-Pick disease, type C, *Am J Med Genet B Neuropsychiatr Genet,* 2010;153B:132–40.
8. Pineda M, Perez-Poyato MS, O'Callaghan M, et al., Clinical experience with miglustat therapy in pediatric patients with Niemann-Pick disease type C: a case series, *Mol Genet Metab*, 2010;99:358–66.
9. Philippart M, Engel J, Jr., Zimmerman EG, Gelastic cataplexy in Niemann-Pick disease group C and related variants without generalized sphingomyelinase deficiency, *Ann Neurol,* 1983;14:492–3.
10. Kovesi TA, Lee J, Shuckett B, et al., Pulmonary infiltration in Niemann-Pick disease type C, *J Inherit Metab Dis,* 1996;19:792–3.
11. Nicholson AG, Florio R, Hansell DM, et al., Pulmonary involvement by Niemann-Pick disease. A report of six cases, *Histopathology*, 2006;48:596–603.
12. Shin J, Epperson K, Yanjanin NM, et al., Defining natural history: assessment of the ability of college students to aid in characterizing clinical progression of Niemann-Pick disease, type C, *PLoS One,* 2011;6:e23666.
13. Schiffmann R, Niemann-Pick disease type C. From bench to bedside, *JAMA*, 1996;276:561–4.
14. Imrie J, Wraith JE, Isolated splenomegaly as the presenting feature of Niemann-Pick disease type C, *Arch Dis Child,* 2001;84:427–9.
15. Shulman LM, David NJ, Weiner WJ, Psychosis as the initial manifestation of adult-onset Niemann-Pick disease type C, *Neurology*, 1995;45:1739–43.
16. Grau AJ, Brandt T, Weisbrod M, et al., Adult Niemann-Pick disease type C mimicking features of multiple sclerosis, *J Neurol Neurosurg Psychiatry,* 1997;63:552.
17. Verity C, Winstone AM, Stellitano L, et al., The epidemiology of progressive intellectual and neurological deterioration in childhood, *Arch Dis Child,* 2010;95:361–4.
18. Tang Y, Li H, Liu JP, Niemann-Pick Disease Type C: from molecule to clinic, *Clin Exp Pharmacol Physiol,* 2010;37:132–40.
19. Patterson MC, Hendriksz CJ, Walterfang M, et al., Recommendations for the diagnosis and management of Niemann-Pick disease type C: an update, *Mol Genet Metab,* 2012;106:330–44.
20. Vanier MT, Rodriguez-Lafrasse C, Rousson R, et al., Type C Niemann-Pick disease: spectrum of phenotypic variation in disruption of intracellular LDL-derived cholesterol processing, *Biochim Biophys Acta,* 1991;1096:328–37.
21. Millat G, Bailo 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.
22. Ries M, Schaefer E, Luhrs 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.
23. Tangemo C, Weber D, Theiss S, et al., Niemann-Pick Type C disease: characterizing lipid levels in patients with variant lysosomal cholesterol storage, *J Lipid Res,* 2011;52:813–25.
24. Madra M, Sturley SL, Niemann-Pick type C pathogenesis and treatment: from statins to sugars, *Clin Lipidol,* 2010;5:387–95.
25. Patterson MC, Vecchio D, Jacklin E, et al., Long-term miglustat therapy in children with Niemann-Pick disease type C, *J Child Neurol,* 2010;25:300–5.
26. Patterson MC, Vecchio D, Prady H, et al., Miglustat for treatment of Niemann-Pick C disease: a randomised controlled study, *Lancet Neurol,* 2007;6:765–72.
27. Rosenbaum AI, Maxfield FR, Niemann-Pick type C disease: molecular mechanisms and potential therapeutic approaches, *J Neurochem,* 2011;116:789–95.
28. Gelsthorpe ME, Baumann N, Millard E, et al., Niemann-Pick type C1 I1061T mutant encodes a functional protein that is selected for endoplasmic reticulum-associated degradation due to protein misfolding, *J Biol Chem,* 2008;283:8229–36.
29. Patterson MC, Platt F, Therapy of Niemann-Pick disease, type C, *Biochim Biophys Acta,* 2004;1685:77–82.
30. Ramirez CM, Liu B, Taylor AM, et al., Weekly cyclodextrin administration normalizes cholesterol metabolism in nearly every organ of the Niemann-Pick type C1 mouse and markedly prolongs life, *Pediatr Res,* 2010;68:309–15.
31. Davidson CD, Ali NF, Micsenyi MC, et al., Chronic cyclodextrin treatment of murine Niemann-Pick C disease ameliorates neuronal cholesterol and glycosphingolipid storage and disease progression, *PLoS One,* 2009;4:e6951.
32. Pipalia NH, Cosner CC, Huang A, et al., Histone deacetylase inhibitor treatment dramatically reduces cholesterol accumulation in Niemann-Pick type C1 mutant human fibroblasts, *Proc Natl Acad Sci U S A,* 2011;108:5620–5.
33. Jiang X, Sidhu R, Porter FD, et al., A sensitive and specific LC-MS/MS method for rapid diagnosis of Niemann-Pick C1 disease from human plasma, *J Lipid Res,* 2011;52:1435–45.
34. Zhang J, Absence of cholesterol biosynthesis and exogenous uptake does not prevent induction of a Niemann-Pick C1 phenotype in normal cells, 2009. Available at: www.niemannpick.org.uk/research/documents/JinzhiZhang.doc (accessed 29 March 2012).