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The adult form of Niemann–Pick disease type C

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Niemann–Pick disease type C (NPC) is a fatal neurovisceral lipid storage disease of autosomal inheritance resulting from mutations in either the *NPC1* (95% of families) or *NPC2* gene. The encoded proteins appear to be involved in lysosomal/late endosomal transport of cholesterol, glycolipids and other molecules but their exact function is still unknown. The clinical spectrum of the disease ranges from a neonatal rapidly fatal disorder to an adult-onset chronic neurodegenerative disease. Based upon a comprehensive study of 13 unrelated adult patients diagnosed in France over the past 20 years as well as the analysis of the 55 other cases published since 1969, we have attempted to delineate the major clinical, radiological, biochemical and genotypic characteristics of adult NPC. Overall, mean age at onset (±SD) of neuropsychiatric symptoms was 25 ± 9.7 years. The diagnosis of NPC was established after a mean delay of 6.2 ± 6.4 years and the mean age at death (calculated from 20 cases) was 38 ± 10.2 years. Major clinical features included cerebellar ataxia (76%), vertical supranuclear ophthalmoplegia (VSO, 75%), dysarthria, (63%), cognitive troubles (61%), movement disorders (58%), splenomegaly (54%), psychiatric disorders (45%) and dysphagia (37%). Less frequent signs were epilepsy and cataplexy. During the course of the disease, clinical features could be subdivided into (i) visceral signs (hepatomegaly or splenomegaly), (ii) cortical signs (psychiatric cognitive disorders and epilepsy); and (iii) deep brain signs (VSO, ataxia, movement disorders, dysarthria, dysphagia, cataplexy) which exhibited different evolution patterns. Asymptomatic and non-evolutive visceral signs were often noticed since early childhood (38.5% of our patients), followed by mild cortical signs in childhood (learning difficulties) and early adulthood (62% of cases among which 38% were psychiatric disorders). Deep brain signs were observed in 96% of patients and were usually responsible for death. In general, there was a good correlation between clinical signs and the localization of brain atrophy on MRI. The 'variant' biochemical phenotype characterized by mild abnormalities of the cellular trafficking of endocytosed cholesterol was over-represented in the adult form of NPC and seemed associated with less frequent splenomegaly in childhood and lesser psychiatric signs. Involvement of the *NPC1* gene was shown in 33 families and of the *NPC2* gene in one. Improving the knowledge of the disease among psychiatrists and neurologists appears essential since emerging treatments should be more efficient at the visceral or cognitive/psychiatric stages of the disease, before the occurrence of widespread deep brain neurological lesions.

Keywords: adult; inborn errors of metabolism; lysosome; Niemann–Pick C

Abbreviations: NPC = Niemann–Pick disease type C; VSO = vertical supranuclear ophthalmoplegia

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Introduction

Niemann–Pick disease type C (NPC, MIM 257220) is a neurovisceral lysosomal lipid storage disorder of autosomal recessive inheritance characterized at the cellular level by accumulation of unesterified cholesterol and glycolipids in the endosomal/lysosomal system (Patterson *et al.*, 2001; Vanier and Millat, 2003). The pattern of accumulating lipids, however, is different in the brain and in non-neural organs. Approximately 95% of patients have mutations in the *NPC1* gene (mapped at 18q11; MIM 607623) which encodes a large membrane glycoprotein with late endosomal

localization. The remainder have mutations in the *NPC2* gene (mapped at 14q24.3) (MIM 601015), encoding a small soluble lysosomal protein that binds cholesterol with high affinity. The finding of a non-redundant functional cooperativity between NPC1 and NPC2 confirmed the early concept that the two proteins function in tandem or in sequence (Vanier *et al.*, 1996; Sleat *et al.*, 2004). They appear to be involved in post-lysosomal/late endosomal transport of cholesterol, glycolipids and other molecules, but their precise functions and relationship remain unclear, and their primary substrate unknown (for review see Vanier and Millat, 2003; Vincent *et al.*, 2003; Vanier and Millat, 2004). The biochemical diagnosis of NPC is currently based on the demonstration of impaired LDL-cholesterol trafficking in cultured fibroblasts of patients, by cytochemical visualization of accumulated free cholesterol after filipin staining and study of LDL-induced cholesterol ester formation (Vanier *et al.*, 1991*a*).

NPC shows an extreme clinical heterogeneity (Vanier and Suzuki, 1996; Patterson *et al.*, 2001; Patterson, 2003; Vanier and Millat 2003). The age of presentation is ranging from the perinatal period to adults over 50 years. The initial manifestations may also vary, being hepatic, pulmonary, neurological or psychiatric in nature. The systemic (including hepatic, splenic and pulmonary) and neurological diseases follow independent courses. NPC is now recognized as a relatively common cause of liver disease in early life, including foetal hydrops, and in nearly half of the cases, a neonatal cholestatic icterus with hepatosplenomegaly. In childhood, isolated hepatosplenomegaly is often the presenting symptom. Nevertheless, apart from infants with early death from liver or respiratory failure and exceedingly rare adults with isolated splenomegaly, all NPC patients develop neurological or psychiatric symptoms. Indeed, the most common classification of the disease is based on the age of onset of the neuropsychiatric symptoms, which to a large extent, correlates with the life span of the patients. In the severe infantile neurological form, delayed motor development and hypotonia become apparent between 1 and 2 years of age and most of these patients die before the age of 5 years. In the classical phenotype (60–70% of all cases), which includes the late infantile and the juvenile forms, neurological symptoms appear between 3 and 15 years, with death occurring in most cases between 8 and 25 years. Cerebellar ataxia, dysarthria, dysphagia, cataplexy, seizures, dystonia, progressive dementia and a characteristic vertical supranuclear ophthalmoplegia (VSO) are prominent signs. The adult neurological onset form is infrequent and the majority of publications have reported single cases. Previous attempts to delineate the major clinical features of the adult form of NPC were based on the review of a limited number of published cases (i.e. 16 cases in Schulman *et al.*, 1995 and 27 cases in Lossos *et al.*, 1997) and data concerning genotypes, biochemical phenotypes, brain imaging and disease course were lacking. Based on the detailed study of 13 adult patients diagnosed in France during the past 25 years and a literature review of 55 previously published cases, the aim of the present study is to provide an updated description of the adult form of the disease. This clarification is now becoming particularly important, since emerging therapies are in sight.

Patients and methods

Between 1985 and 2005, from the data of a national reference biochemical diagnostic centre (M.T.V.), 133 patients, belonging to 99 unrelated families, were definitively diagnosed as having NPC in France. Thirteen unrelated patients from this cohort with an age at onset of neurological or psychiatric signs at or after 15 years were included in the present study. Age at onset was defined here as the age of first obvious neurological or psychiatric symptoms, which excluded clumsiness, learning difficulties in childhood or reading difficulties which could retrospectively be related to mild VSO. For eight patients (numbered 1, 2, 4, 7, 8, 11, 12 and 13), neurological examination and medical data recording were performed by one of us (F.S.). In other cases, the clinical history was based on the detailed patient's medical records. MRI analysis was included in the present study only when recent data were available, which excluded patients for whom MRI was performed only at the beginning of the disease, or patients for whom only MRI reports were available. The diagnosis of NPC was biochemically confirmed in cultured skin fibroblasts by combined studies of filipin staining and LDL-induced cholesteryl ester formation, and patients were classified into either a classic or a variant biochemical phenotype (Vanier *et al.*, 1991*a*). The classic phenotype refers to patients whose fibroblasts show a striking cholesterol accumulation and a severe block in LDL-induced cholesteryl ester formation. In cells from biochemically variant patients, demonstration of cholesterol storage requires challenge with pure LDL, and the rate of cholesteryl ester formation may not be affected. Methods used for mutation analysis have been described previously (Millat *et al.*, 2001, 2005).

Dependence was defined as one or more of the following outcomes: (i) unable to walk alone; (ii) assistance required for simple activities of daily living; (iii) institutionalization; or (iv) gastrostomy tube required for feeding. Cognitive disorder was defined as any cognitive dysfunction (excluding psychiatric troubles) interfering with activities of daily living. Cases number 4 and 5 have been previously published as single case reports (Philit *et al.*, 2002; Tyvaert *et al.*, 2005), but were included in our 13 cases since additional clinical data and longer follow-up were available. These two cases were excluded from the literature review. Review of the literature was performed using the NIH Pubmed database. Several cases published more than once were excluded by careful perusal of publications or personal communications. Fifty-five cases of NPC disease in adult life were finally selected from 27 reports. Only cases for which detailed clinical data were available were included in the statistical analysis. For MRI analysis, only publications including MRI pictures of sufficient quality were included.

Results

Case reports (Table 1)

Cases with prominent psychiatric or cognitive signs

Case 1: This male patient had no familial or personal history. Childhood acquisitions were normal, but school performances were noted to be poor, because of slow learning,

Table 1 Summary of clinical, biochemical and genetic data in 13 French patients with adult neurological onset of NPC

Case	Gender	Premonitory signs in childhood	Age at neurol. onset (years)	Diagnosis delay (years)	First neuropsy. sign in adulthood	Major clinical signs during disease course	Dependence delay (years)	MRI (atrophy)	Age at death (years)	Age at last follow-up (years)	Bioch. phenotype	NPC1 mutations
Mean ± SD			20.7 ± 6.7	5.2 ± 4.8			8.9 ± 4		38 ± 10.2	30.3 ± 7		
1	M	Cog	16	9	Psy	Psy, Cog, At, VSO, Md	7	Cortical, subcortical		26	CI	R615L/?[a]
2	F	none	30	10	Psy	Psy, Cog, At, Md, VSO, Sm	10	Cortical, subcortical, corpus callosum		42	CI	D1097N/F283fs[a]
3	M	hm, Sm	15	8	Psy	Psy, Cog, At, VSO, Sm	9	Corpus callosum, subcortical, cortical	31	31	CI	Y871C/F763L[a]
4	F	none	18	13	Psy	Psy, Cog, At, VSO, Sm	11	NA		36	CI	R372W/?
5	M	Sm, hea	34	1	Psy	Psy, Cog, Sm	4	Normal	39	39	CI	Y825C/?
6	M	Sm, Cog	28	NA	Cog	Cog, At	4	Cortical and cerebellar	38	32	CI	I1061T/A605V[b]
7	M	VSO?	16	12	Md	Md, At, VSO, Sm	16	Cerebellar and brainstem, mild WM high intensity		36	Var	G992R/N452fs
8	F		17	2	At	At, Md, VSO, Sm	none	NA		23	Var	G992A/G993fs[a]
9	F	hea	28	0	At	At, VSO, Sm	none	NA	49	28	Var	V950M/V950M[b]
10	M	hm, Sm	17	1	Md	Md, At, VSO, Sm	13	Cortical and cerebellar	32	32	CI	R1059Q/L724P
11	F	VSO?, Cog,	18	2	At	Psy, Cog, At, Md, VSO, Sm	n	Cerebellar and brainstem, Mild WM high intensity		22	Var	V950M/I1061T
12	M	Cog, ca	15	2	VSO	Psy, Cog, At, VSO, Md, Sm	6	NA		27	Var	V950M/I1061T
13	M	sm	17	2	VSO	Cog, Md, VSO, Sm	n	Mild WM high intensity		20	CI	I1061T/G538R

At = ataxia; biochem. = biochemical; ca = cataplexy; CI = classical biochemical phenotype (severe cholesterol trafficking abnormalities); Cog = cognitive disorder (excluding psychiatric symptoms); F = female; hm = hepatomegaly; hea = hearing loss; M = male; Md = movement disorders (including dystonia, chorea or parkinsonism); NA = not available; neurol. = neurological; Psy = psychiatric troubles; sm = splenomegaly; Var = variant biochemical phenotype (mild cholesterol trafficking abnormalities); WM = white matter.

[a]Millat *et al.* (2005).

[b]Millat *et al.* (2001).

mild difficulties in writing, reading and speaking. However, he could follow a normal educational training. When he was 16 years old, he was hospitalized for an acute paranoid delirium, which resolved after 2 months. Three years later he experienced another acute episode but with persistence of psychotic features suggestive of paranoid schizophrenia. During the following years, he became clumsy with poor upper limbs coordination. Cognitive troubles appeared, including apathy, memory impairment and a dysexecutive syndrome which progressively worsened. At 21 years of age, neurological examination revealed a tetra pyramidal syndrome, gait and limbs cerebellar ataxia, dysarthric speech, dysphagia and VSO. At the age of 24 years, he had lost gait and speech. Dystonic postures involving hands and legs and myclonic jerks appeared. Gastrostomy was performed at 26 years because of severe deglutition problems. Mild cortical and subcortical atrophy were reported on brain MRI. Electroneuromyography, electroretinogram, visual evoked potentials and abdominal ultrasonography were normal. EEG performed at 23 years of age revealed generalized slowing.

Case 2: This 40-year-old female had normal psychomotor development, with normal schooling, and worked as an employee in a supermarket. She had two normal pregnancies. When 30 years old, she suffered from an acute persecutive delusion with acoustic hallucinations. Persistent delusion led to the diagnosis of dysthymic schizophrenia. Despite chronic therapy with neuroleptics and mood stabilizers, she experienced eight delusion relapses in 6 years requiring hospitalizations at each time. She refused medical follow-up for the next 4 years. During this period, in addition to psychotic features, she developed slow dysarthric speech, attention and memory impairment, dysphagia and ataxic gait. At the age of 40 years, neurological examination revealed VSO, gait cerebellar ataxia, pyramidal signs, left upper limb rest tremor and rigidity. Neuropsychological evaluation showed dementia with non-fluent aphasia, apragmatism, anosognosia, perseverations, memory impairment, visuoconstructive apraxia and prosopagnosia. Her gait progressively worsened and at the age of 42 years, she was unable to walk alone. Abdominal ultrasonography disclosed hepatomegaly and splenomegaly. Brain MRI revealed moderate atrophy affecting the cerebral cortex, the cerebellum and the corpus callosum, without signal abnormalities. EEG showed generalized slowing.

Case 3: This patient had no familial history. His personal history was remarkable for thalassaemia and thrombopenia with recurrent epistaxis in early childhood. At 24 months of age, hepatosplenomegaly was found and bone marrow aspiration revealed foam cells accumulation suggestive of NPC. Partial splenectomy was performed at 12 years because of an acute abdominal pain. Lipid analysis of frozen spleen tissue performed by one of us (M.T.V.) revealed a typical NPC pattern and the diagnosis was definitely confirmed later on skin fibroblasts. His psychomotor development was normal and he attended normal school until 15 years old, when his parents and teachers first noticed behavioural disturbances including social isolation, self mutilations, aggressiveness and hyperphagia. A psychiatric evaluation revealed a depressive syndrome. An insulin dependent diabetes mellitus developed at the age of 16 years. Gait abnormalities with falls appeared when he was 19 years old and progressively worsened. At the age of 24 years, behavioural disturbances led to institutionalization. Neurological examination found gait and upper limbs cerebellar syndrome and VSO. Neuropsychological evaluation was not possible. Brain MRI showed mild cortical, subcortical and corpus callosum atrophy with relative sparing of the posterior fossae. He experienced one generalized tonic–clonic seizure and frequent sudden falls without loss of consciousness, suggestive of cataplexy. He died at 30 years of age in a bedridden state. Electroneuromyography, EEG and brain MRI were normal.

Case 4: This patient was born premature at 32 weeks, but her psychomotor development was normal. She attended normal school until the age of 16, but stayed unemployed thereafter. Dissociative symptoms progressively appeared. When 29 years old, she was not autonomous anymore, because of disorientation in time and space and severe behaviour disturbances. She refused neuropsychological evaluation and brain MRI. Clinical examination revealed cerebellar ataxia, dysarthric speech, VSO and hepatosplenomegaly. Her motor skills progressively worsened and increasing dysphagia led to gastrostomy at the age of 35. At 36, she was not able to walk alone anymore, she was mute, with episodes of agitation and had tonico–clonic generalized seizures.

Case 5: This male patient had no familial history. He attended normal school and was employed in a gas company. Splenomegaly was noticed at the age of 3 years, for which a spleen biopsy revealed foam cells. A diagnosis of probable NPC was then strongly suspected. He was also treated for hypercholesterolaemia and, depression and presented deafness which was considered as a sequel of head trauma. He had been followed up for a transient glomerular nephropathy (membranoproliferative glomerulonephritis type II) with renal failure. At the age of 34 years, he developed acute delirium with paranoid delusion and dissociative features. Neuroleptic therapy led to partial improvement. On examination, he was alert and well oriented but a pyramidal syndrome was noted. Over the next 4 years, attention deficit and difficulties in finding words worsened. Neuropsychological evaluation, at the age of 38, showed diffuse cognitive alterations, with predominant frontal lobe dysfunction, memory impairment and visuoconstructive apraxia. He rapidly deteriorated within the next months with acute renal failure, acute delirium, dysphagia, cachexia and dehydration, requiring a combination of dialysis, sedative drugs and gastrostomy. He subsequently became catatonic and bedridden with total dependence. Routine laboratory tests, chest radiography and brain MRI were reported to be normal. He died at 39 years of age.

Case 6: This male patient had no familial history. Isolated splenomegaly was found during the first days of life, and a splenectomy was performed at 10 years of age revealing a storage process consistent with the diagnosis of NPC. Learning difficulties were noticed during late childhood. At 28 years of age he became slow, apathic and had difficulties in finding words. He had to stop working the same year. From the age of 30, he exhibited an unsteady gait and falls. Neurological examination 2 years later revealed manual grasping, cerebellar ataxia, pyramidal syndrome, severe dysarthria and dysphagia. No VSO was noticed. EEG revealed diffuse slow waves. On brain MRI, cerebral and cerebellar atrophy was reported. He died at the age of 38.

Cases with predominant movement and gait impairment

Case 7: This male patient had no personal or familial history. His psychomotor development was normal and schooling was unremarkable except that he reported difficulties to direct his gaze downward when reading. At 16 years of age, he noticed difficulties to perform fine left hand movements. Neurological examination, at 25 years, revealed VSO and mild generalized dystonia involving the four limbs, associated with myoclonic jerks that were both spontaneous and evoked by nociceptive stimulations. He became progressively ataxic and dysarthric, with a pyramidal syndrome including brisk tendon reflexes, legs spasticity and a bilateral Babinski sign. A neuropsychological evaluation disclosed a mild dysexecutive syndrome. Abdominal ultrasonography revealed a homogeneous splenomegaly. At 32 years, he could not walk alone anymore and from the age of 35, he became dependent for most daily activities. Brain MRI showed marked atrophy of the pons and cerebellum, mild atrophy of cortical and subcortical areas. Electroneuromyography showed only a bilateral carpal tunnel syndrome and EEG revealed generalized slowing.

Case 8: This female patient with no personal or familial medical history was noticed to be mildly clumsy since the age of 14, often breaking glasses. However, she attended normal school, obtained the French 'baccalaureat' and started a professional training course. When 17 years old, neurological abnormalities were first seen including dysarthric speech, gait and limb cerebellar ataxia and mild dysphagia. At 19 years, VSO was also found, together with brisk deep tendon reflexes and dystonia of the right lower and upper limbs. She also experienced several sudden falls while standing, without loss of consciousness, that were described as a tonus loss and were suggestive of cataplexy. General examination did not show splenomegaly but this was found with ultrasonography. Gait difficulties progressively increased but when 23 years old, she was still autonomous for activities of daily living. CT-scan of the brain was normal. She refused MRI. Electroneuromyography was normal and EEG showed non-specific bilateral slow waves.

Case 9: Age at onset could not be determined with precision in this female patient. She had hearing loss since the age of 3, and her familial history was remarkable for deafness also affecting two of her brothers as well as three nephews. Her physical, motor and intellectual developments were otherwise normal, but she was sometimes clumsy. She attended normal school until the age of 17. She worked successfully in several offices and had two normal pregnancies. When 28 years old, she consulted for deafness but did not complain of other symptoms. A careful neurological evaluation revealed cerebellar dysfunction with ataxic gait, dysmetria, dysarthric speech and VSO. Abdominal ultrasonography showed hepatosplenomegaly. No MRI of the brain was performed. With time, ataxia and dysarthria became more and more invalidating, but her mental capacities remained quite well preserved. Progressive dysphagia led to gastrostomy at the age of 45. She died from septicaemia at the age of 49.

Case 10: This male patient had no familial history. Following a transient documented episode of neonatal cholestatic icterus, hepatosplenomegaly was found on systematic clinical examination when he was 5 weeks old. During childhood, he presented numerous, spontaneously resolutive infections affecting skin and parotid glands, associated with interstitial neuropathy and persistent elevation of sedimentation rate, suggestive of immunodeficiency. A spleen biopsy revealed sea blue histiocytes but no definite diagnosis was made. When 17 years old, he exhibited abnormal, fixed postures of his hands, progressively followed by increasing gait difficulties. Neurological examination at the age of 18 showed generalized cervical and upper limbs dystonia, gait cerebellar ataxia and VSO. Splenomegaly was present. Cortical and cerebellar atrophy were noticed on a brain CT-scan (no MRI was performed). EEG showed diffuse slow waves. The biochemical examination of a liver biopsy (M.T.V.) showed a lipid storage profile typical of NPC and the diagnosis was confirmed on fibroblasts. Chorea and myoclonus affecting all body parts subsequently appeared, leading to a progressive deterioration of his motor skills. In addition, the cerebellar syndrome increased, and finally he became unable to walk alone at the age of 30. A gastrostomy tube was placed because of increasing dysphagia. He died when 32 years old after a post-traumatic cerebral haemorrhage.

Case 11: This female patient had learning difficulties since childhood because of poor concentration capacities. She also had a tendency to flex her head rapidly to see downward which was interpreted as a tic but which can in retrospect be interpreted as a sign of VSO. At 12 years, she benefited from orthoptic re-education. She could achieve a sales person professional position but with difficulties because of calculation impairment. From the age of 19 years she progressively exhibited dysarthria, cerebellar ataxia, dystonic postures involving fingers and VSO. Abdominal ultrasonography disclosed an isolated splenomegaly. At 20 years of age, the diagnosis of NPC was made. She was hospitalized

in a psychiatric department for 6 months because of social withdrawal, aggressiveness, persecutive delusion and suicide attempts. These psychiatric troubles were controlled with neuroleptics and at the age of 22 years, the patient is still autonomous for most activities of daily living. A brain MRI showed mild atrophy of the pons and cerebellum but without discernible cortical atrophy. Electroneuromyography was considered normal.

Case 12: This male patient experienced reading difficulties in childhood. These were not due to VSO, but rather to a problem in understanding the syllabus system. He could follow normal schooling with help and a professional training course in floriculture. When 14 years old, he had falls due to abrupt loss of muscular tone without loss of consciousness, which were retrospectively suggestive of cataplexy. When 15 years old, a teacher noticed he had difficulties to look downwards. At 17 years he first visited a neurologist who noted a cerebellar syndrome and VSO. Abdominal echography showed splenomegaly. The diagnosis of NPC was made. Ataxia worsened and at 21 years, he had to use a wheelchair. From 23 years, he also exhibited dysarthria, hand dystonic postures and choreic movements. From 20 years, he started to present acute psychiatric troubles with agitation, aggressiveness, visual and auditive hallucinations and delusion. These episodes occurred at an average rate of once per year, lasting ~15 days each time and were well controlled with treatment. MRI could not be performed because of choreic movements. Electroneuromyography did not show any abnormalities.

Case 13: This male patient had an asymptomatic splenomegaly which was first noticed at 2 years of age. He did not exhibit any intellectual or motor impairment until 17 years old when he started to experience learning difficulties together with oculomotor troubles. Progressively, dysarthria and dystonic postures involving hands and feet appeared. He also complained of mild hypoacousia. Neurological examination found dystonic hands and feet movements while walking, vertical and horizontal VSO, mild cerebellar ataxia and hypoacousia. Neuropsychological evaluation disclosed a mild dysexecutive syndrome. At 20 years of age, the patient is still autonomous in most activities and he currently undergoes a professional training course in geothermics. Brain MRI did not show abnormalities and electroneuromyography was normal.

Global analysis of our case reports and review of the literature

Data collected from our 13 patients are summarized in Tables 1, 3 and 4. Clinical, biochemical and genetic characteristics of published cases are summarized in Tables 2, 3 and 4. From the compilation of our cases and 55 case reports for which sufficient data were available, first presenting signs in adulthood were psychiatric troubles in 38% of cases; cognitive troubles (excluding psychiatric features) in 23%; ataxia in 20%; movement disorders (dystonia) in 11%; VSO in 8%; dysarthria in 8% and epilepsy in 3% of cases. Overall, the mean age at onset (±SD) of neuropsychiatric symptoms was 25 ± 9.7 years. The diagnosis of NPC was established after a mean delay of 6.2 ± 6.4 years and the mean age at death (calculated from 20 cases) was 38 ± 10.2 years.

Discussion

NPC is pan ethnic, with an estimated incidence in Western European countries of ~1/120 000 to 1/150 000 living births (Vanier and Millat, 2003). From published data, it is not possible to estimate the exact proportion of adult forms among NPC patients in general. This may also vary according to the ethnic background, due to different mutation patterns. From our study, a first evaluation can be made at the scale of one country. The adult form accounted for 10% of the patients diagnosed in France between 1985 and 2005. Among these patients, two were diagnosed between 1985 and 1995 and the remaining between 1995 and 2005, with an approximate constant rate of one patient per year during the past 10 years in a population of 60 million inhabitants. It is however difficult to provide a reliable estimation of the incidence of NPC in adults, as an unknown proportion of these patients is probably undiagnosed or misdiagnosed.

Two main explanations can be given to this situation. On one hand, most neurologists are not aware of this infrequent diagnosis as suggested by the main delay for diagnosis, which is ~6 years. On the other hand, NPC is particularly difficult to diagnose when presenting as pure psychiatric disorder, frontal dementia, ataxia or dystonia. In addition, the wide range of neurological signs seen in NPC can mimic other, more familiar, neurological and psychiatric diseases. Thus, in adults with NPC reported in the literature, some initial diagnoses were Alzheimer's disease, Parkinson's disease, schizophrenia, Wilson's disease, multiple sclerosis, Creutzfeldt–Jakob disease or Gayet–Wernicke encephalopathy. Finally, the biological confirmation of NPC requires a skin fibroblast culture, and only a limited number of laboratories offer reliable diagnosis tests. From data collected from our own experience and the exhaustive review of published cases, the major clinical, biological and molecular aspects of adult NPC can be defined as follows.

Disease course

First neurological symptoms occurred within the second or third decades in most patients, but onset as late as 54 years has been reported (Vanier *et al.*, 1991*b*; Trendelenburg, 2006). We defined here the onset of the adult disease as the age at onset of obvious neurological or psychiatric signs. Overall, age at onset was 25 years, but 11 of our 13 patients exhibited premonitory signs or symptoms in childhood that preceded neurological deterioration in adulthood. These signs included splenomegaly (5 cases), hepatomegaly

Table 2 Summary of clinical, biochemical and genetic data of adult-onset cases of NPC reported in the literature

Case	Sex	Age at onset (years)	Diagnosis delay (years)	Age at death (years)	Initial sign	VSO	Ataxia	Cognitive	Dysarthria	Splenomegaly	Dystonia	Psychiatric signs	Dysphagia	Chorea	Epilepsy	Hepatomegaly	Parkinsonism	Other signs	Atrophy (MRI)	Biochemical phenotype	Genotype **	Reference
		***26 ± 10**	***6.6 ± 6.9**	***38 ± 11.2**		**72%**	**72%**	**61%**	**59%**	**44%**	**41%**	**43%**	**30%**	**20%**	**17%**	**11%**	**10%**					
1	M	25	NA		Psy	+	–	+	+	+	+	+	–	–	–	+	–	–		NA	11061T/11061T	Sullivan *et al.* (2005)
2	M	25	8	38	Psy	+	+	+	+	–	+	+	–	–	–	–	–	–		Cl	11061T/P1007A	Battisti *et al.* (2003)
3	F	49	12	62	Psy	–	+	+	+	–	+	+	+	–	+	–	+	my	Frontal	Cl	NA	Josephs *et al.* (2003)
4	F	27	5		Psy	+	+	–	+	–	+	+	–	–	–	–	–	Pyr	Normal	Va	NA	Josephs *et al.* (2003)
5	F	40	2	43	Cog	+	+	+	+	–	–	–	+	+	–	–	–	–		NA	NPC2 V39M/ V39M*	Klünemann *et al.* (2002)
6	F	35	8		Cog	+	+	+	+	–	–	–	–	–	–	–	+	–		Va	NPC2 V39M/ V39M*	Klünemann *et al.* (2002)
7	M	22	NA		Ep	+	–	–	–	–	+	–	+	+	+	–	–	–		Cl	11061T/11061T	Imrie *et al.* (2002)
8	F	20	3	23	At	–	+	–	+	–	–	+	–	–	–	–	–	–		NA	NA	Imrie *et al.* (2002)
9	M	21	1		Psy	+	–	+	+	–	–	+	–	–	–	–	–	–		**Cl**	11061T/?	Imrie *et al.* (2002)
10	F	18	NA		Psy	+	+	–	–	–	+	+	+	–	+	–	–	Ca		**Cl**	11061T/?	Imrie *et al.* (2002)
11	F	18	NA		At	+	+	–	–	+	–	–	–	–	–	–	–	–		**Va**	NA	Imrie *et al.* (2002)
12	F	24	0		Dys	+	+	+	+	–	+	–	+	–	–	–	–	–		**Va**	NA	Imrie *et al.* (2002)
13	F	19	NA		Ep	+	+	–	+	–	+	–	+	–	+	–	–	–		**Va**	NA	Imrie *et al.* (2002)
14	M	16	NA		Psy	+	+	–	+	–	+	–	–	–	–	–	–	–		**Va**	11061T/P1007A	Imrie *et al.* (2002)
15	F	15	12		VSO	+	–	–	–	+	–	–	+	–	+	–	–	–		**Cl**	NA	Imrie *et al.* (2002)
16	M	17	0		Psy	–	+	–	+	+	–	+	+	+	+	–	–	–		**Cl**	NA	Imrie *et al.* (2002)
17	M	18	NA	29	At	–	+	–	–	+	+	+	–	–	–	–	–	–		NA	NA	Imrie *et al.* (2002)
18	F	22	NA		At	+	+	–	–	–	+	–	–	–	–	–	–	–		NA	NA	Imrie *et al.* (2002)
19	M	18	NA		Cog	+	–	+	–	–	–	–	–	–	–	–	–	–		NA	NA	Imrie *et al.* (2002)
20	M	25	1		Psy	+	+	–	–	+	–	+	+	–	+	–	–	–		**Va**	NA	Imrie *et al.* (2002)
21	F	38	0		Dys	+	+	–	+	–	–	–	–	–	–	–	–	–		NA	NA	Imrie *et al.* (2002)
22	F	33	0		Dys	NA	NA	NA	NA	+	NA	**NA**	NA	NA	NA	+	NA	NA		NA	NA	Schneider *et al.* (2001)
23	M	25	NA	42	NA	+	+	+	–	+	+	–	–	–	+	–	–	–		**Va**	V889M/ 1015–1032 del	Yamamoto *et al.* (2000)
24	M	16	0		Psy	+	+	+	+	–	–	+	–	–	–	–	–	–		NA	NA	Campo *et al.* (1998)
25	F	28	0		At	+	+	–	+	+	–	–	–	–	–	+	–	–	Cereb, brainstem	**Va**	A972V/A972V	Lossos *et al.* (1997)
26	M	NA	NA		VSO	+	–	–	–	+	–	–	–	–	–	–	–	–		Va	NA	Lossos *et al.* (1997)
27	M	NA	NA		VSO	+	–	–	–	–	–	–	–	–	–	–	–	–		Va	NA	Lossos *et al.* (1997)
28	F	30	4		Cog	–	+	+	–	+	–	–	–	–	–	–	–	–	HS WM	**Va**	D874V/D948N	Grau *et al.* (1997)
29	M	18	7	25	Cog	–	+	+	+	+	–	–	+	+	–	–	–	–		NA	NA	Love *et al.* (1995)
30	M	17	5	33	Psy	–	–	–	–	–	+	+	–	–	–	–	–	Pyr		NA	NA	Love *et al.* (1995)
31	M	18	5	33	Psy	NA	–	+	–	–	–	+	–	+	–	–	–	–		NA	NA	Love *et al.* (1995)
32	M	18	NA	25	Cog	+	–	+	–	–	–	–	–	+	–	–	–	–		NA	NA	Love *et al.* (1995)
33	M	30	8		Psy	+	+	+	+	–	+	+	+	+	+	–	+	–		NA	NA	Schulman *et al.* (1995)
34	M	40	0		Cog	–	–	+	–	+	–	–	+	–	–	–	–	–		NA	NA	Lanska and Lanska (1993)

Table 2 *Continued*

Case	Sex	Age at onset (years)	Diagnosis delay (years)	Age at death (years)	Initial sign	VSO	Ataxia	Cognitive	Dysarthria	Splenomegaly	Dystonia	Psychiatric signs	Dysphagia	Chorea	Epilepsy	Hepatomegaly	Parkinsonism	Other signs	Atrophy (MRI)	Biochemical phenotype	Genotype **	Reference
35	F	32	6		Cog	+	+	+	+	−	−	−	−	−	−	−	+	−		NA	NA	Hulette *et al.* (1992)
36	M	19	32	51	Psy	−	−	−	−	−	−	+	−	−	−	−	−	−		NA	NA	Vanier *et al.* (1991*b*)
37	M	45	0	45	Psy	−	−	+	−	−	−	+	−	−	−	−	−	−		NA	NA	Vanier *et al.* (1991*b*)
38	F	34	NA		Psy	−	−	−	−	−	−	+	−	−	−	−	−	−		Va	NA	Vanier *et al.* (1991*b*)
39	F	34	9		Cog	−	+	+	+	−	−	−	+	+	−	−	−	Pyr		Va	P1007A/P1007A	Vanier *et al.* (1991*b*)
40	F	36	NA		Cog	−	+	+	+	+	−	−	−	−	−	−	−	Pyr		NA	NA	Vanier *et al.* (1991*b*)
41	M	18	NA		Dys	+	+	+	+	+	+	−	+	−	−	−	−	−		Cl	NA	Fink *et al.* (1989)
42	NA	28	NA	47	At	−	+	+	+	−	−	+	−	−	−	−	−	Pyr		Va	NA	Vanier *et al.* (1988)
43	NA	59	NA		At	+	+	+	+	−	−	−	−	−	−	−	−	−		**Va**	NA	Vanier *et al.* (1988)
44	F	18	3		Dys	+	+	+	+	+	−	+	−	+	−	+	+	Pyr		NA	NA	Yan-Go *et al.* (1984)
45	F	26	20	46	Cog	+	+	+	−	+	+	−	−	+	−	−	+	−		NA	NA	Elleder *et al.* (1983)
46	M	23	20		At	+	+	+	+	+	+	−	−	−	−	+	−	−		NA	NA	Longstreth *et al.* (1982)
47	M	20	3		At	+	+	−	+	+	+	−	−	−	−	+	−	−		NA	NA	Breen *et al.* (1981)
48	F	18	11	29	Cog	+	+	+	+	+	−	−	+	−	−	−	−	Pyr		NA	NA	Horoupian and Yang (1978)
49	M	±30	10		NA	+	+	+	−	+	−	−	−	−	−	−	−	−		NA	NA	Wherett and Rewcastle (1969)
50	M	±30	10		NA	+	+	+	−	+	−	−	−	−	−	−	−	−		NA	NA	Wherett and Rewcastle (1969)
51	F	±25	NA		At	+	+	−	+	−	+	−	−	−	−	−	−	−		**Va**	11061T/?	Lachmann *et al.* (2004)
52	F	53	15		Psy	+	+	+	+	−	+	+	+	+	−	−	−	Pyr	Normal	**Va**	K1206fs/?	Trendelenburg *et al.* (2006)
53	M	15	8		Psy	+	+	+	+	+	+	+	−	−	−	−	−	−	Frontal, CC	Va	S940L/S954L	Walterfang *et al.* (2006)
54	M	16	8		Psy	+	+	+	+	−	−	+	−	−	−	−	−	−		Va	NA	Walterfang *et al.* (2006)
55	M	18	0		Cog	+	−	+	+	+	+	+	−	−	−	+	−	−	Normal	Cl	11061T/11061T	Walterfang *et al.* (2006)

At = ataxia; Ca = cataplexy; CC = corpus callosum; Cl = classical biochemical phenotype (bold: confirmed in our laboratory (MTV); Cog = cognitive disorders; Dys = dysarthria; my = myoclonus; Ep = epilepsy; HS WM = high signal of the white matter; NA = not available; Psy = psychiatric troubles; Pyr = pyramidal syndrome; VSO = vertical supranuclear ophthalmoplegia; Va = variant biochemical phenotype [bold: confirmed by one of us (MTV)]. *Mean ± SD. **All patients in whom the genotype was studied had mutations in the *NPC1* gene, except Patients 5 and 6 who had a mutation of the *NPC2* gene; *NPC1* complementation group was further determined by one of us (MTV) in Cases 11, 13, 15, 18 and 20 . Note that Cases 5 and 6, 8 and 9, 17 and 18, 36–38, 39 and 40, respectively, are siblings. Cases 25–27 are related.

(2 cases), learning difficulties (4 cases), deafness (2 cases) and difficulties to look downward (2 cases). However, even if these patients could not be considered as completely 'normal' during the early phases of the illness, they all followed normal schooling, and most of them had professional training or had started to work, meaning that their neurological involvement was not significant until adulthood. Except acute psychotic episodes, the disease was progressive. Our patients became dependent within a mean delay of 8.9 years after the beginning of neurological signs. In these and previous published cases, the age at death was 38 years which is 13 years after the beginning of main neurological signs. Clinical signs of the disease could be categorized into three categories: visceral signs (including hepatomegaly and splenomegaly), cortical signs (including psychiatric disorders, cognitive troubles and epilepsy) and deep brain signs [including movement disorders (dystonia, chorea or parkinsonism), cerebellar ataxia, VSO, dysarthria, dysphagia, cataplexy and deafness]. Each of these three categories of symptoms or signs exhibited sequential and distinct courses: (i) visceral signs were sometimes present since early childhood and generally remained stable or regressed, (ii) cortical signs sometimes present in childhood constituted the most frequent presenting features in early adulthood but were rarely a late complication thereafter, (iii) deep brain signs appeared quite late in the course of the disease but constituted the major cause of disability and death. In addition, from our personal series, some patients displayed a relatively milder clinical picture characterized by the predominance of deep brain signs without major cortical signs and absence of symptomatic visceral signs in childhood. As discussed later, these patients correspond mostly with the variant biochemical phenotype.

Table 3 Clinical signs of NPC in adulthood

	Clinical signs (%)		
	This series $n = 13$	Review $n = 54$*	Compilation $N = 67$**
Ataxia	92	72	76
VSO	85	72	75
Cognitive disorder	62	61	61
Dysarthria	77	59	63
Movement disorder	62	56	58
Splenomegaly	92	44	54
Psychiatric	53	43	45
Dysphagia	69	30	37
Pyramidal	39	15	19
Hepatomegaly	54	11	19
Epilepsy	15	17	16
Deafness	23	0	4
Cataplexy	23	0	4

*$n = 55$ for splenomegaly and hepatomegaly. **$n = 68$ for splenomegaly and hepatomegaly.

Visceral signs

Hepatomegaly and splenomegaly were present in 53.8 and 92.3% of our 13 patients, respectively. These frequencies are significantly higher than those calculated from previously published cases (10.7% for hepatomegaly and 44.6% for splenomegaly). In most of our cases, however, splenomegaly was asymptomatic and was found only after abdominal ultrasonography. Therefore, this sign should easily be missed, possibly explaining the low frequencies reported in the literature. As in other forms of NPC, visceral involvement always preceded neurological involvement. This 'visceral period' could last for decades as illustrated by 38.5% of our cases in whom organomegaly was noticed from early childhood and by published reports of adult patients presenting with an isolated splenomegaly (Fensom *et al.*, 1999; Harzer *et al.*, 2003; Dvorakova *et al.*, 2006). Better awareness that NPC is one of the possible diagnoses for isolated splenomegaly or hepatosplenomegaly should allow an earlier detection of patients with this presentation, including some who will not develop neurological symptoms until adulthood. The fact that visceral and neurological signs follow independent courses suggests that the nervous system involvement is caused by a different pathophysiological mechanism. Although neonatal cholestatic jaundice is frequent in classic NPC, this feature was apparently very rare in the adult form, both in our cases (1 case) or in the literature (none reported). Nevertheless, patient notes concerning the neonatal period were probably seldom available, and transient episodes might have been overlooked.

Table 4 Proportion (%) of different clinical signs during disease course

	Premonitory signs in childhood	First neuropsychiatric signs in adulthood			Clinical signs during disease course		
	This series $n = 13$	This series $n = 13$	Review $n = 52$	Compilation $n = 65$	This series $n = 13$	Review $n = 54$*	Compilation $n = 67$**
Visceral	39				92	44	**53**
Cortical	31	46	65	**62**	69	78	**76**
Deep brain	0	54	35	**38**	100	94	**96**

Visceral: visceral signs (include hepatomegaly, splenomegaly); cortical: cortical signs (include psychiatric, cognitive signs and epilepsy); deep brain signs include ataxia, VSO, dystonia. *$n = 55$ for splenomegaly and hepatomegaly. **$n = 68$ for splenomegaly and hepatomegaly.

'Cortical' signs

Overall, psychiatric, cognitive troubles or epilepsy constituted the presenting feature in early adulthood in 62% of cases, with 38% of patients exhibiting psychiatric disorders, 23% cognitive troubles and 3% epilepsy.

Psychiatric signs

These could remain isolated for several years and were usually consistent with psychosis, including paranoid delusions, auditory or visual hallucinations, interpretative thoughts, behavioural disturbances with aggressiveness, self-mutilation or social isolation. Other types of psychiatric disturbances included depressive syndrome, transient isolated visual hallucinations, bipolar disorders and obsessive-compulsive disorders (Imrie *et al.*, 2002; Battisti *et al.*, 2003; Sullivan *et al.*, 2005). Onset could be progressive or acute, with spontaneous remissions and relapses. Most of patients who presented with psychosis as the initial manifestation of the disease did not have abnormalities at neurological examination, and therefore were diagnosed as having schizophrenia or other forms of psychosis. Importantly, psychiatric features rarely constituted a late complication, with only two of our patients presenting psychiatric troubles after an initial motor presentation.

Cognitive dysfunction

This was highly variable, from patients affected by a moderate dysexecutive syndrome, only detectable by specific psychometric testing, to severely demented patients with major apathy and mutism, requiring institutionalization. The commonest feature was the presence of a frontal syndrome. However, some patients exhibited other dysfunctions such as aphasia, apraxia and memory impairment, consistent with a widespread cortical dysfunction. Such cortical dysfunction is in accordance with the Alzheimer-like lesions that have been described in NPC (Horoupian and Yang, 1978; Love *et al.*, 1995; Yamazaki *et al.*, 2001; Saito *et al.*, 2002).

Epilepsy

This is frequent in late infantile or juvenile forms of the disease but was rarely present in adults. Only two of our patients experienced tonico–clonic generalized fits but this was not a major feature of their neurological disease. Epilepsy was found in 18% of published cases, but was disabling, presenting as progressive myoclonic epilepsy, in only two cases (Imrie *et al.*, 2002).

'Deep brain' signs

These signs could be classified into three main categories according to the rostro-caudal gradient of involved cerebral structures: basal ganglia dysfunction (dystonia, chorea and parkinsonism), cerebellar dysfunction (cerebellar ataxia) and brainstem dysfunction (VSO, dysarthria, dysphagia, cataplexy and deafness). They were presenting signs in adulthood in 38% of cases. Thereafter, deep brain signs constituted an almost constant feature during the course of the disease (96% of patients) and represented the major cause of death.

Cerebellar ataxia

This was the most common sign during the course of the disease (76% of all cases). It usually consisted of both a static and kinetic cerebellar syndrome, which could involve the trunk and the four limbs.

VSO

This was present in 75% of cases. However, it was rarely a presenting sign (8% of cases). At the beginning, gaze disturbances resulted in mild difficulties in reading or going downstairs. Downward gaze was usually more affected than upward gaze, and dissociation could be observed between early impairment of saccades, and relative preservation of pursuit movements. Most patients displayed a quite specific pattern of abnormalities, consisting of (i) abolition of all vertical voluntary saccades, (ii) paresis of downward pursuit movements and (iii) preservation of full vertical oculocephalic reflexes. This pattern did not differ from what has been observed in juvenile cases (Vanier *et al.*, 1988, 1991*b*; Fink *et al.*, 1989; Patterson *et al.*, 2001). In later stages of the disease, both vertical and horizontal movements became impaired, with limitation of both voluntary and pursuit movements but preservation of oculocephalic reflexes. In addition, VSO is seen in a limited number of neurodegenerative diseases (such as progressive supranuclear palsy, corticobasal degeneration, Huntington disease, diffuse Lewy body disease, Creutzfeld–Jacob disease). It is also observed in Whipple's disease, mesencephalic focal lesions or paraneoplastic syndromes. This sign has also been reported in adults with inborn errors of metabolism including GM2 gangliosidosis, non-ketotic hyperglycinaemia, glutaric aciduria type 1 and horizontal supranuclear ophthalmoplegia, which is a common feature of type III Gaucher disease. Thus, the finding of a supranuclear ophthalmoplegia in a patient with an unclassified neurological affection rapidly narrows the field of possible diagnoses.

Movement disorders

Movement disorders (58%) included dystonia (40%), chorea (19%) or parkinsonism (10%). Focal onset dystonia affecting either upper or lower limbs, the trunk or the orofacial region with secondary generalization, as well as an initial generalized presentation, were observed. Parkinsonism when noted was mild, consisting in bradykinesia, axial rigidity, hypomimia or isolated rest tremor. It was frequently found only after systematic neurological examination, and did not represent a key diagnostic feature. Chorea has been described either as focal or generalized, sometimes leading to severe functional impairment (Love *et al.*, 1995; Shulman *et al.*, 1995; Imrie *et al.*, 2002; Klünemann *et al.*, 2002).

Dysarthria and dysphagia

During the course of the disease, dysarthria (63% of all cases) and dysphagia (37% of cases) constituted major

causes of disability in patients. They were of mixed origin, due to combined cerebellar dysfunction, dystonia and brainstem involvement.

Cataplexy

This was observed in ~20% of cases in the classical form but has been described only in one case with adult onset (Imrie *et al.*, 2002). We found in our cases three patients who actually had frequent falls without loss of consciousness, which were compatible with cataplexy. Since cataplexy is not a frequently recognized neurological sign in adults, it might be underreported, and falls due to cataplexy may be misdiagnosed as a consequence of cerebellar ataxia.

Perceptive deafness

This was found in three of our patients. In one of them, it had initially been regarded as a sequel of mild head trauma. The second patient had a familial history of deafness, compatible with a recessive mode of inheritance. The third patient had unexplained perceptive hypoacousia. To our knowledge, deafness has not been reported either in adults or children with NPC. However, Fink *et al.* (1989) in a series of 22 patients with various clinical forms of NPC, found alterations of both central and peripheral auditory pathways in more than half.

Other signs

Few cases of documented peripheral neuropathy have been reported in children (Hahn *et al.*, 1994; Alvelius *et al.*, 2001; Zafeiriou *et al.*, 2003). However, electroneuromyography was considered normal in all our patients in whom it was performed (Cases 1, 3, 7, 8, 11, 12 and 13) and peripheral neuropathy has never been described in the 55 previously published cases of adult NPC. Thus, peripheral nervous system involvement is not a usual feature of adult NPC. One of our patients developed diabetes mellitus when 16 years old and another a severe nephropathy but this seems to be coincidental.

Neuroradiology

Detailed MRI data were available for four of our patients and eight published cases. MRI was normal or in most cases at the beginning of the disease. Thereafter, there was a good correlation between predominant clinical signs and radiological abnormalities. Patients exhibiting predominant psychiatric or cognitive signs displayed cortical atrophy predominating in the frontal lobes, sometimes with corpus callosum atrophy. In contrast, patients with predominant gait and movement disorders had more pronounced brainstem and cerebellar atrophy with relative sparing of the cortical and subcortical areas. At late stages of the disease, atrophy was diffuse, involving cortical, subcortical areas and the posterior fossae. In addition, recent reports of functional imaging in NPC, using different techniques such as magnetic resonance spectroscopy, single-photon emission computed tomography (SPECT) or PET, revealed more diffuse abnormalities in thalamic and caudate nuclei, and frontal and parieto-occipital regions (Tedeschi *et al.*, 1998; Battisti *et al.*, 2003; Trendelenburg *et al.*, 2006).

Correlations between biochemical and clinical phenotypes and genotype

To-date ~230 disease-causing mutations of the *NPC1* gene have been reported, with a majority (~70%) of missense mutations. About one-third of the mutations are located in a particular domain of the protein, the cysteine-rich luminal loop (codons 855–1098) (Vanier and Millat, 2003; Millat *et al.*, 2005). From the available data (Tables 1 and 2), mutations in this loop appear more frequently involved in adult patients than in the global population of NPC patients. I1061T, systematically screened for, in our 13 patients and in 19 unrelated patients of the literature (Table 2 and own unpublished data), constituted globally 23% of the mutated alleles and was the most recurrent mutation. Homozygous I1061T is typically associated with a slowly progressive juvenile neurological onset form (Millat *et al.*, 1999), but this genotype was also reported in three adult-onset cases (Table 2, Cases 1, 7 and 55). It is now well established that certain other mutations located in the cysteine-rich luminal loop, among which is P1007A, underlie the so called 'variant' biochemical phenotype, characterized by lesser alterations of cellular LDL-cholesterol trafficking than observed in the 'classic' phenotype (Millat *et al.*, 2001; Vanier and Millat, 2003). The biochemical phenotype was precisely defined in our 13 cases and 26 additional unrelated cases of the literature, among which 16 were also studied by one of us (M.T.V.). The variant phenotype, which is observed in 15–20% of the NPC1 families, was clearly over-represented in adult-onset NPC, being found in 5 of our 13 patients and in 17 of the 26 published cases, i.e. globally in 59% of the families. Although the finding of severe cholesterol trafficking alterations in culture fibroblasts (classic phenotype) does not exclude a late neurological onset of the disease, these data indicate that a 'variant' phenotype tends to be more often associated with a later onset form, which is in good agreement with our previous statements (Vanier *et al.*, 1991*b*; Vanier and Suzuki, 1996; Vanier and Millat, 2003). Since biochemical testing of the variant phenotype requires specific and complex conditions (Vanier *et al.*, 1991*a*), the frequent occurrence of this phenotype may be a further cause for under diagnosis of late-onset patients.

Interestingly, a classic phenotype was observed in all our five patients who exhibited a symptomatic splenomegaly in early childhood, as well as all our six patients with a psychiatric or cognitive onset. In contrast, all our five patients with a variant phenotype exhibited a more restricted clinical picture consistent with an involvement of deep brain structures, but without splenomegaly in childhood and no major cognitive or psychiatric troubles. Overall (Tables 1

and 2), among patients with a psychiatric onset biochemically studied by us, 75% (9 out of 12) showed a classical phenotype while only 15% (3 out of 20) patients displayed a variant phenotype. Although the limited number of patients precludes further extrapolations, the variant phenotype might represent a milder form of the disease relatively limited to deep brain structures.

Mutation P1007A, the second most common *NPC1* mutation, although not found in our series, is a 'variant' allele often associated with late-onset forms (literature Cases 2, 14 and 39). The V950M mutation was in our series as frequent as I1061T (4 out of 26 alleles). It clearly appears as an 'adult-onset' mutation, either in the homozygous state (Case 9) but also in combination with I1061T (Cases 11 and 12). Interestingly, the three unrelated patients carrying this allele are all from the French region of Brittany. Finally, several mutations have been reported to affect codon 992. G992W, characteristic of Nova Scotia patients (former type D) has not been reported so far in adult-onset patients, but G992R and G992A should be added to the list of *NPC1* mutations correlated with an adult-onset form of the disease. Only one family with *NPC2* mutations has been associated with an adult-onset form (Klünemann *et al.*, 2002).

Within a family, there is homogeneity in subtypes of neurological presentation, with relatively good genotype–phenotype correlations. On the other hand, a large variability of visceral manifestation between sibs can occur, especially regarding perinatal liver disease (Millat *et al.*, 2001; Vanier and Millat, 2003). Thus, the pathogenesis of visceral involvement may be related to additional yet unknown modifying factors.

Physiopathology and emerging treatments

Neurological signs of NPC arise from both neurodegeneration and neuronal dysfunction (Walkley and Suzuki, 2004). Restoring cellular homeostasis by any therapeutic approach could potentially reverse cellular dysfunction and then provide significant clinical improvement. Since brain cholesterol is essentially acquired from the endogenous pathway, the pathogenesis of the neuronal dysfunction cannot be explained by the impairment in processing and utilization of endocytosed cholesterol, which constitutes the cellular hallmark of the disease in fibroblasts. In neurons, cholesterol accumulation is indeed minimal (Karten *et al.*, 2002; Gondre-Lewis *et al.*, 2003), in contrast to what is observed in peripheral tissues such as liver and spleen. Indeed, early treatment strategies aiming at reducing cholesterol showed no clinical benefit on the neurological disease in patients (Schiffmann, 1996) or in the mouse model (Ericksson *et al.*, 2000). There is instead an obvious accumulation of several glycolipids, essentially gangliosides GM2 and GM3, lactosylceramide and glucosylceramide (Elleder *et al.*, 1985; Vanier, 1999; Zervas *et al.*, 2001*a*; Gondre-Lewis *et al.*, 2003). Since the glycolipid storage appears to contribute to some of the neuropathological features, substrate reduction therapy using *N*-butyl-deoxynojirimycin (NB-DNJ, OGT 918), an inhibitor of glucosylceramide synthase, was tried in the *NPC1* mouse and cat mutants. It resulted in delayed onset of neurological symptoms, and a 20% longer survival of the mice (Zervas *et al.*, 2001*b*). This compound has recently been approved in Europe, USA and Israel for treatment of type 1 Gaucher disease under the name of miglustat. A controlled clinical trial was thus initiated in neurologically symptomatic adult and juvenile patients with encouraging interim results (Patterson *et al.*, 2006). Other approaches such as allopregnanolone tested on animal models are promising (Griffin *et al.*, 2004; Ahmad *et al.*, 2005). An early diagnosis of the disease, before the occurrence of irreversible neurological lesions, is a challenge for future years as these prospective therapies might prove more efficient at early stages of the disease particularly in late-onset, slowly evolutive forms.

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