

Original Article

The clinical and genetic epidemiology of neuronal ceroid lipofuscinosis in Newfoundland

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The neuronal ceroid lipofuscinoses (NCLs) are the commonest neurodegenerative disorders of children. The aims of this study were to determine the incidence of NCL in Newfoundland, identify the causative genes, and analyze the relationship between phenotype and genotype. Patients with NCL diagnosed between 1960 and 2005 were ascertained through the provincial genetics and pediatric neurology clinics. Fifty-two patients from 34 families were identified. DNA was obtained from 28/34 (82%) families; 18 families had mutations in the *CLN2* gene, comprising five different mutations of which two were novel. One family had a *CLN3* mutation, another had a novel mutation in *CLN5*, and five families shared the same mutation in *CLN6*. One family was misdiagnosed, and in two, molecular testing was inconclusive. Disease from *CLN2* mutations had an earlier presentation ($p = 0.003$) and seizure onset ($p < 0.001$) compared with *CLN6* mutation. There was a slower clinical course for those with *CLN5* mutation compared with *CLN2* mutation. NCL in Newfoundland has a high incidence, 1 in 7353 live births, and shows extensive genetic heterogeneity. The incidence of late infantile NCL, 9.0 per 100,000 (or 1 in 11,161) live births, is the highest reported in the world.

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The neuronal ceroid lipofuscinoses (NCLs) are the most common group of progressive neurodegenerative disorders of children. They are usually recessively inherited and are characterized by developmental regression, seizures and visual failure culminating in premature death. There is an accumulation of autofluorescent storage material, or lipopigment, in lysosomes of neural and non-neural tissues, the morphology being characteristic

for each form of NCL (1). The disease has a worldwide distribution with an incidence of 1 in 8000 to 1 in 100,000, highest in Finland (2, 3).

Eight disease genes causing human NCL have now been identified: *CLN1* (4), *CLN2* (5), *CLN3* (6), *CLN5* (7), *CLN6* (8), *CLN8* (9), *CLN10* (also known as *CTSD*) (10, 11) and *MFSD8*, or *CLN7* (12). *CLN1* and *CLN2* encode lysosomal enzymes palmitoyl-protein thioesterase-1 (PPT1) and

tripeptidyl peptidase (TPP1), respectively (5, 13). *CLN10* encodes the lysosomal proteinase cathepsin D (*CTSD*) and is involved in proteolytic degradation, cell invasion and apoptosis (11). *CLN3* encodes an integral membrane protein of the lysosome, and *MFSD8* encodes a putative lysosomal transporter, although the function of these proteins has not been fully elucidated (12, 14). Mouse models implicate genes encoding neuronal chloride transport proteins as candidates for human NCL; the phenotype of *Cln6*^{-/-} mice resembles mild human NCL, and mutations in *CLCN6* were found in 2 of 75 patients with late-onset NCL, although confirmatory functional studies were lacking (15).

The NCLs are classified clinically based on age of onset, clinical course and ultrastructural morphology. Infantile NCL (INCL; MIM 256730) is associated with mutations in *CLN1*, classical late infantile NCL (LINCL; MIM204500) with mutations in *CLN2*, and juvenile NCL (JNCL; MIM204200) with mutations in *CLN3*. However, mutations in *CLN1* can cause LINCL, JNCL and adult-onset disease, and *CLN2* mutations can also result in a much later onset and slower disease progression than classical LINCL (16). INCL is the commonest type in Finland, while JNCL predominates in Western Germany, Norway, Western Scotland, the Netherlands and the USA (17–21).

Atypical, or variant, LINCL results from mutations in *CLN5* (MIM256731), which is most prevalent in Finland, and *CLN6* (MIM606725), which has a worldwide distribution. Mutations in *CLN8* (MIM 607837) and *MFSD8* (MIM 611124) underlie a subset of Turkish variant LINCL (9, 12). Mutations in *CLN8* can also result in progressive epilepsy with mental retardation (9). *CTSD* mutations can cause congenital NCL or a later-onset NCL-like disorder (10, 11).

Newfoundland is a large island in the North Atlantic, where the current population of 510,000 is descended from 20,000 to 30,000 founders who immigrated to the island from southwest England and southeast Ireland in the late 18th century and early 19th century (22). Its peopling has been characterized by little in or out migration, large families over many generations, settlement near the core community, and multiple genetic isolates in small outpost communities distributed along the shoreline of the island who have a high coefficient of kinship (23).

Other researchers noted the clustering of NCL patients in Newfoundland in the 1980s (24). In this population-based study, we identified 52 patients from 34 families who were diagnosed with NCL between 1960 and 2005. We describe the molecular genetic and clinical findings in these patients.

Materials and methods

We identified all children diagnosed with neurodegenerative conditions between 1960 and 2005 through the provincial genetics program, the provincial pediatric neurology clinic and a search of hospital discharge codes at the Janeway Child Health Centre, the only pediatric hospital in the province. Clinical data and biopsy reports were obtained from medical chart review, and patients diagnosed with NCL were enrolled in the study ($n = 52$). Pedigrees were constructed with data from family interviews and Provincial Genealogy Archives.

DNA was extracted from venous lymphocytes for molecular genetic testing using a proprietary (Gentra, Qiagen Corp., Valencia, CA) salting-out procedure. A two-staged analysis of *CLN* genes was used. The first stage involved recurrent mutation analysis of the *CLN1* [c.451C>T (p.R151X), c.364A>T (p.R122W), and c.223A>C (p.T75P)], *CLN2* [c.622C>T (p.R208X), c.509-1G>C, and c.851G>T (p.G284V)], *CLN3* (g.5373_6338 del965), *CLN5* [c.1175_1176del(p.Y392X)], and *CLN8* [c.70C>G (p.R24G)] genes. If a common mutation was identified in one allele, the second stage of testing involved sequence analysis of the entire coding region and intron/exon boundaries of the other allele for that particular gene. If no mutation was identified, *CLN* genes were sequenced in an order determined by the clinical features of the affected individual. For sequence analysis, primers were designed for polymerase chain reaction (PCR) amplification of all exons and flanking intron sequences of the *CLN1*, *CLN2*, *CLN3*, *CLN5*, *CLN6*, *CLN8* and *CLN10* (*CTSD*) genes. Primer sequences are available from the authors upon request. PCR reactions were performed using 100 ng of genomic DNA in a volume of 25 μ l containing 1 \times PCR buffer, 1.5 mM MgCl₂, 250 μ M of dNTPs, 0.200 μ M of forward and reverse primer, and 1 U of AmpliTaq Gold Polymerase (Applied Biosystems, Foster City, CA). In addition, PCR reactions for amplification of the *CLN10* gene contained 0.5 M betaine (Sigma-Aldrich, Saint Louis, MO). Touchdown PCR conditions were as follows: 10 min at 95°C, followed by 15 cycles at 95°C for 30 s, annealing at 64.5°C for 30 s (decreasing by 0.5°C/cycle), and extension at 72°C for 30 s. This step was followed by 20 cycles at 95°C for 30 s, 57°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. PCR products were purified using exonuclease I/shrimp alkaline phosphatase treatment (USB Corporation, Cleveland, OH) and sequenced using BigDye Terminator v.3.1 Cycle Sequencing Kit and an ABI 3730

DNA Analyzer (Applied Biosystems). Sequences were analyzed with SEQSCAPE v.2.5 software (Applied Biosystems).

The Kaplan–Meier survival analysis and log rank test with Bonferroni adjustment for multiple comparisons were used to compare the ages of presentation, onset of seizure, visual failure (defined as first abnormal eye examination or documented decrease in visual acuity) and death between genotype groups. A ‘genotype group’ refers to all patients who have mutations identified in the same *CLN* gene, regardless of the nature of the mutation. If DNA was unavailable from a patient, but relatives had a known mutation, then the patient was assumed to have that mutation for the purpose of the genotype–phenotype analysis. A result was regarded as significant if $p < 0.05$.

Ethical approval was given by the Human Investigation Committee of Memorial University of Newfoundland. Informed consent to participate was obtained from parents or guardians of affected individuals.

Results

Fifty-two patients from 34 families with a diagnosis of NCL from 1960 through 2005 were identified. The incidence of NCL in Newfoundland is

13.6 per 100,000 (or 1 in 7353) live births. This derives from 38 new cases in 278,961 live births from 1971 to 2005 inclusive. Parental consanguinity was present in 10/34 (29%) families. In six of the families, there were two affected siblings, and in one family, there were three affected siblings. In all other families with multiple affected individuals, the affected people were first cousins or more distantly related. Five patients were alive at completion of the study.

Molecular genetic tests

DNA was obtained from 28/34 (82%) families. In 13 families, DNA was obtained from affected individuals ($n = 19$). In a further 15 families, DNA was obtained from the parents of affected individuals, in three of whom DNA was available from only one parent. In six families, DNA was unavailable.

Mutations in *CLN* genes were identified in 25/28 (89%) families from whom DNA was available. Table 1 shows the mutations identified in the cohort. Figure 1 shows the location of the families and their respective *CLN* mutations. The incidence of LINCL caused by mutations in *CLN2* was 9.0 per 100,000 (or 1 in 11,161) live births. This is calculated from 24 cases with a mutation in the *CLN2* gene and one case with clinical

Table 1. Mutations identified in the Newfoundland neuronal ceroid lipofuscinoses families^a

Gene	Number of patients (number of families)	Allele 1			Allele 2		
		Mutation type	Nucleotide change	Amino acid change	Mutation type	Nucleotide change	Amino acid change
CLN1	None						
CLN2	3 (2)	Frameshift	c.1424delC	p.S475WfsX13	Frameshift	c.1424delC	p.S475WfsX13
CLN2	6 (5)	Missense	c.851G>T	p.G284V	Missense	c.851G>T	p.G284V
CLN2	4 (4)	Splicing	c.509-1G>C	Premature termination	Splicing	c.509-1G>C	Premature termination
CLN2	1 (1)	Nonsense	c.311T>A	p.L104X	Missense	c.851G>T	p.G284V
CLN2	3 (1)	Nonsense	c.622C>T	p.A208X	Missense	c.851G>T	p.G284V
CLN2	2 (2)	Splicing	c.509-1G>C	Premature termination	Missense	c.851G>T	p.G284V
CLN2	2 (2)	Splicing	c.509-1G>C	Premature termination	Nonsense	c.622C>T	p.A208X
CLN2	2 (1)	Missense	c.851G>T	p.G284V		None	
CLN3	3 (1)	Deletion	g.5373_6338del965	Premature termination	Deletion	g.5373_6338del965	Premature termination
CLN5	2 (1)	Nonsense	c.1054G>T	p.E352X	Nonsense	c.1054G>T	p.E352X
CLN6	7 (5) ^b	Frameshift	c.268_271dup	p.V91EfsX42	Frameshift	c.268_271dup	p.V91EfsX42
CLN8	None						
CTSD	None						
	3 (2) ^c						
	1 (1) ^d						

^aNovel mutations are highlighted in bold.

^bA parent for one family is also a carrier of a mutation (c.311T>A) in the CLN2 gene.

^cNo mutations identified in any CLN genes.

^dSubsequently diagnosed with pantothenate kinase-associated neurodegeneration.

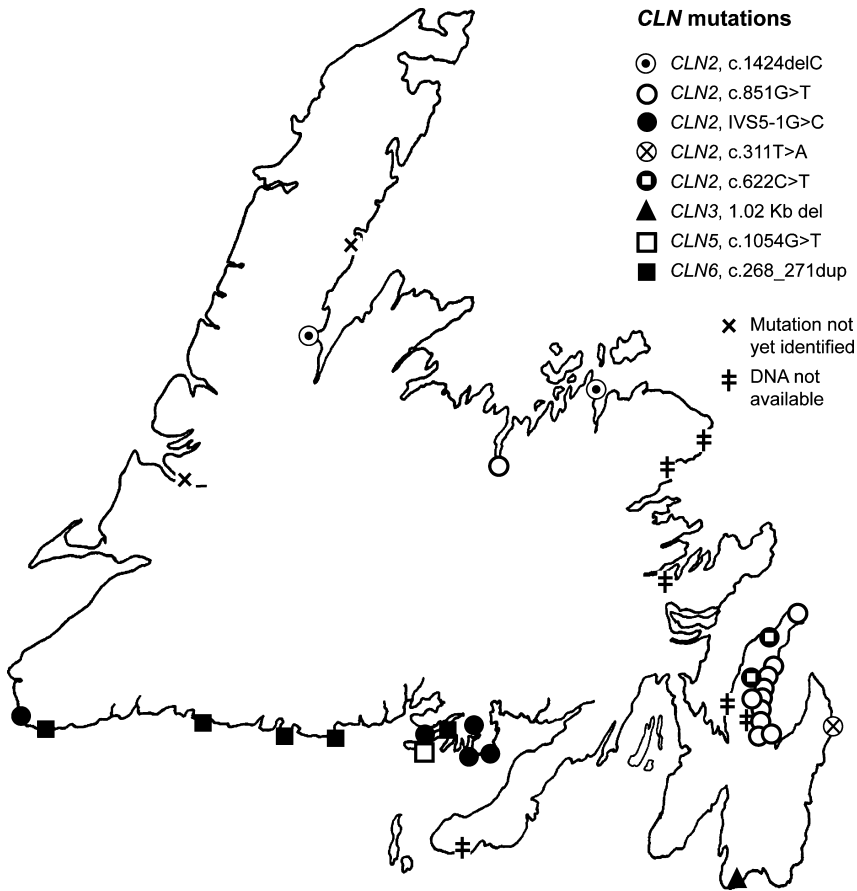


Fig. 1. Map of Newfoundland showing the location of families by mutations identified.

findings typical of classical LINCL (patient number 18.01 in Table 2) occurring from 1971 to 2005 inclusive in 278,961 live births.

Five different mutations in the *CLN2* gene were identified in 18 families, including two novel mutations (c.311T>A and c.1424delC). One of the *CLN2* mutations in this population (c.851G>T) has been previously reported (25). One family had the common deletion in *CLN3*. One family, which originated from the southwest of England, had a novel *CLN5* mutation. Five families, all located on the south coast of the island, had the same mutation in *CLN6*. In one of these families, a parent of an affected child was heterozygous for both the *CLN6* mutation and a mutation in *CLN2* (c.311T>A, p.L104X).

Patients without an identified CLN mutation

The clinical features of patients who have no identified mutation in a *CLN* gene, either because DNA was unavailable or because mutation testing was negative, are shown in Table 2. Clinical data are available for six of the seven patients from whom DNA is unavailable.

In three families (number 4, 12, and 34 in Table 2), no mutation in a *CLN* gene was identified

despite extensive molecular genetic tests. One of these patients (number 12.01) was found to have pantothenate kinase-associated neurodegeneration on further molecular genetic testing. This person was excluded from the analyses of clinical features.

In the other two families, sequencing of coding and flanking regions of *CLN1*, *CLN2*, *CLN3*, *CLN5*, *CLN6*, *CLN8* and *CLN10* genes in affected children was normal. Patient numbers 34.01 and 34.02 are sisters from a consanguineous marriage. Both have a history of developmental regression and seizures; patient number 34.01 now has reached a developmental plateau with well-controlled seizures, autistic behavioral features and chronic diarrhea. Her TPP1 level was normal. Her sister (patient number 34.02) has ongoing developmental regression, seizures, spasticity and chronic diarrhea. Retinal examination, visual evoked potential, MRI head scan and skin biopsy were normal in both affected sisters. The diagnostic work-up is ongoing. Patient number 4.01 presented at 8 months of age with global developmental delay and developed dystonia by 2 years of age. He had refractory seizures, visual failure, developmental regression, cerebral atrophy and an abnormal skin biopsy, consistent with NCL.

Table 2. Clinical features of 10 Newfoundland neuronal ceroid lipofuscinoses patients with no molecular genetic diagnosis

Patient ID number	Presenting symptom	Age at presentation (years)	Age at first seizure (years)	Age at visual failure (years)	Biopsy findings	Age at death (years)	Clinical diagnosis
3.01 ^a +++	Hyperactivity and short attention span	5.8	6.7	5.8	FP + RL	12.6	vLINCL
4.01 9.01 ^a	Global developmental delay No clinical data available	0.7	2.4	4.1	FP + GROD	9.1	?vLINCL
12.01	Motor delay, hypotonia, and tremor	1.5	Never had seizures	8.2	FP ^b	17	Other diagnosis – PKAN ^c
18.01 ^a	Seizure	2.6	2.6	2.9	CL	5.5	LINCL
21.01 ^a	Short concentration span in kindergarten	6.8	7.4	10	CL + FP + GROD	11	vLINCL
24.01 ^a	Seizure	2.0	2.0	3.0	Lipid storage	4.6	LINCL
24.02 ^a	Seizure	2.7	2.7	3.9	Gangliosidosis	6.9	LINCL
31.01 ^a	Change in personality	15	20	18	N/A	20.4	?JNCL
34.01 (alive)	Developmental regression	0.7	6.3, now well controlled on tegretol	Normal eye examination	Normal	Ambulatory some speech, delayed but static development at 10 years	Atypical – diagnostic work-up ongoing
34.02 (alive)	Developmental regression	3.5	3.7	Normal eye examination	Normal	Non-ambulatory, no speech at 9 years	Atypical – diagnostic work-up ongoing

CL, curvilinear; FP, fingerprint; GROD, granular osmiophilic deposits; JNCL, juvenile NCL; LINCL, late infantile NCL; N/A, not available; RL, rectilinear; vLINCL, variant late infantile NCL.

^aDNA not available.

^bDetected in only one skin cell.

^cPantothenate kinase-associated neurodegeneration.

Clinical data for these three patients are included in the 'unknown' genotype group.

Clinical course

Seizure was the presenting feature in the majority of patients (29/51, 57%). Other patients presented with developmental delay (10/51, 20%), developmental regression (5/51, 10%), unsteady gait (4/51, 8%) and visual failure (3/51, 5%). The median age at presentation was 3 years (range 0.7–15 years), and median age at death was 9.5 years (range 4.6–41 years). The median time to event derived from survival analysis and range of ages at onset for presentation, first seizure, visual failure, and death by genotype group is shown in Table 3. Presenting symptoms by genotype group are shown in Fig. 2.

Patients with mutations in *CLN2* had an earlier onset of symptoms compared with those with mutations in *CLN6*, although the age at death was not significantly different. Disease resulting from *CLN2* mutations was associated with an earlier presentation (median ages 3 and 4 years for the *CLN2* and *CLN6* groups, respectively, $p = 0.003$) and earlier onset of seizures (median age 3.3 years compared with 4.9 years, $p < 0.001$) (Fig. 3).

There was no significant difference in clinical course between patients with different *CLN2* mutations, although the numbers are small. Five patients in the *CLN2* genotype group and one patient in the *CLN6* group from whom DNA was unavailable were assumed to have mutations in the same *CLN* gene as their relatives who had identified mutations. Analysis without including these cases did not significantly change the results.

Patients with a mutation in *CLN5* also showed a later clinical course compared with those with *CLN2* mutations. There was a later age at first seizure for the *CLN5* group (5.0 years compared with 3.0 years) (Fig. 3), although this did not reach significance ($p = 0.06$). The median age of death was significantly later for the *CLN5* group compared with the *CLN2* and *CLN6* groups (14.9 years compared with 8.6 and 11 years, respectively, $p < 0.001$ for both comparisons) (Fig. 4). However, the analysis is limited by the small number of patients with *CLN5* mutations, one of whom had an unusually late age of death (41 years). There was no significant difference between the *CLN5* and the *CLN6* groups for age at presentation or age at first seizure.

The *CLN3* mutation was associated with a classic JNCL clinical course with visual failure as the

Table 3. Median time to clinical events for the Newfoundland neuronal ceroid lipofuscinoses patients with range for ages at onset (years)^a

Genotype group	Age to presentation (years)	Age to first seizure (years)	Age to visual failure ^b (years)	Age to death (years)
Whole group ($n = 51$)				
Median time to event (n)	3.0 (50)	3.3 (49)	4.2 (42)	9.5 (48)
Range of ages with event	0.7–15.0	1.8–12.1	2.3–18.0	4.6–41.0
<i>CLN2</i> ($n = 28$)				
Median time to event (n)	2.9 (28)	3.0 (28)	4.0 (22)	8.6 (27)
Range of ages with event	1.8–3.9	1.8–4.0	2.9–5.5	4.0–12.9
<i>CLN3</i> ($n = 3$)				
Median time to event (n)	6.7 (3)	10.0 (3)	6.7 (3)	18.9 (3)
Range of ages with event	6.5–7.1	9.4–12.1	6.5–7.1	15.1–23.3
<i>CLN5</i> ($n = 2$)				
Median time to event (n)	3.5 (2)	5.0 (2)	3.5 (1)	14.9 (2)
Range of ages with event	3.5–5.0	5.0–6.6		14.9–41.0
<i>CLN6</i> ($n = 8$)				
Median time to event (n)	4.0 (7)	4.9 (7)	4.9 (7)	11.0 (7)
Ranges of ages with event	3.0–4.9	3.0–7.2	4.0–6.0	8.0–14.9

^a n refers to the number of people for whom data are available. Data for group with unknown genotypes ($n = 10$) and the patient who was given a diagnosis of pantothenate kinase-associated neurodegeneration during this study are not included.

^bDefined as first abnormal eye examination or first documented decrease in visual acuity.

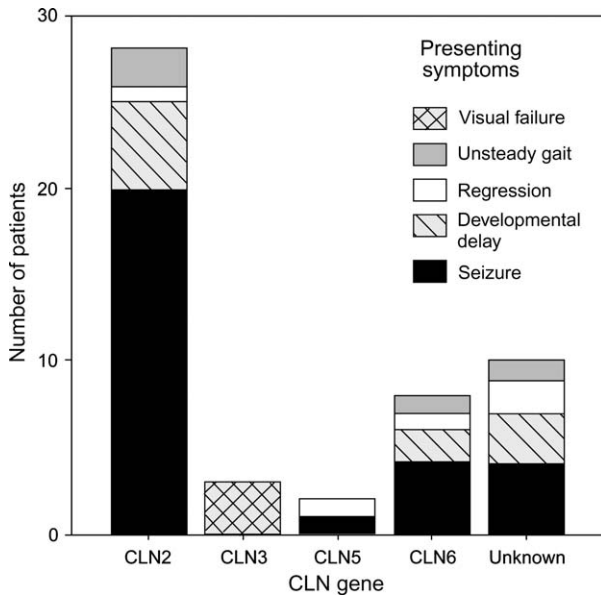


Fig. 2. Presenting symptom by genotype group for the Newfoundland NCL patients.

presenting symptom (Fig. 2) and significantly later median ages at presentation (6.7 years), first seizure (10.0 years) (Fig. 3), visual failure (6.7 years) and death (18.9 years) (Fig. 4) than the other genotype groups.

Pathology findings

Skin biopsies were performed in 39/51 (76%) patients. These were reported by different pathologists as part of the diagnostic process. Eighty-seven percent (20/23) of patients with a *CLN2* mutation who had a skin biopsy had curvilinear (CL) inclusions, two biopsies were described as lipodosis, and one showed mixed inclusions that

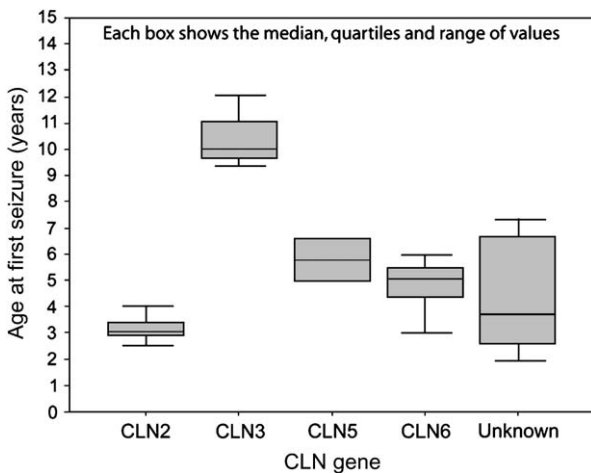


Fig. 3. Age at first seizure by genotype group for the Newfoundland NCL patients.

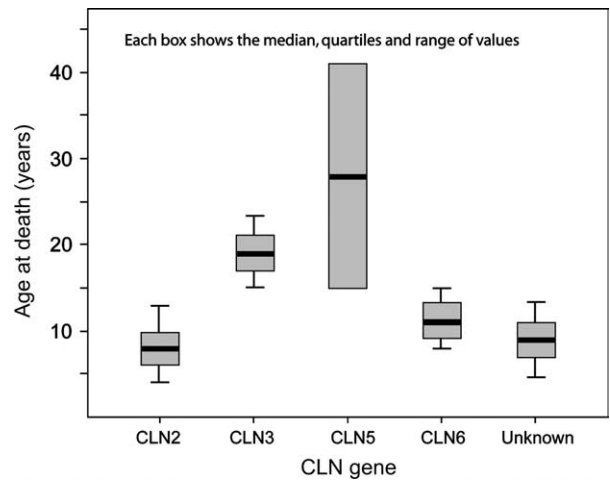


Fig. 4. Age at death by genotype group for the Newfoundland NCL patients.

were CL and granular osmiophilic deposits (GRODs). All three patients with a *CLN3* mutation had skin biopsies. One showed CL inclusions, one showed both CL and fingerprint (FP) profiles and the other had FP, CL, rectilinear (RL) inclusions and GRODs. Both patients with a *CLN5* mutation had mixed inclusions, one had CL and FP profiles and the other had RL and FP profiles. Skin biopsies from patients with a *CLN6* mutation showed FP profiles alone in one, CL and RL profiles in one and mixed CL and FP inclusions in two patients.

Discussion

Incidence of NCL in Newfoundland

Newfoundland has a high incidence of NCL, 13.6 per 100,000 live births. This incidence is similar to the figure in Finland (13 per 100,000 live births). Social isolation and founder effects have enriched this disease in both populations (7). These figures are considerably higher than the incidence of NCL in the USA, Norway, the Netherlands, Western Scotland, Western Germany and Italy (4, 2.8, 1.95, 1.61, 1.28 and 0.56 per 100,000 live births, respectively) (17–21, 26). There is a predominance of LINCL in Newfoundland, which has the highest incidence reported in the world, 9.0 per 100,000 live births compared with 0.5, 0.46 and 0.36 per 100,000 live births in the Netherlands, Western Germany and Italy, respectively (17, 20, 26).

Genetic basis of NCL in the Newfoundland population

There is extensive genetic heterogeneity of NCL in Newfoundland with mutations identified in four

different *CLN* genes and multiple mutations in the *CLN2* gene, implying that NCL in Newfoundland is a result of multiple genetic isolates rather than a single founder. This phenomenon has been seen in other recessive disorders in this population, such as Bardet–Biedl syndrome (27). Three patients from two families remain without a genetic diagnosis. It is possible that a mutation exists but was not detected in the genes that were screened, for example affecting promoter function. Genes causing NCL in mouse models, such as *CLCN6*, remain candidates for further testing but are beyond the scope of this study (15).

CLN2

Two mutations, c.622C>T (p.R208X) and c.509-1G>C, account for approximately two thirds of mutations in the *CLN2* gene in European Caucasian populations (28). In Newfoundland, the most prevalent mutation is c.851G>T (p.G284V). This missense mutation occurs in a highly conserved region and is predicted to result in a major conformational change in the enzyme TPP1 (25). Families with this mutation are clustered on the east coast of the island consistent with a founder effect, likely from migrants from the southwest of England. Two other mutations are novel to this population, c.311T>A (p.L104X) and c.1424delC (p.S475WfsX13). In each case, there is no known genealogical link between the two families in which the mutation is identified, although they are located in regions with common fishing grounds and trade routes. The splice site mutation, c.509-1G>C, is found in families located along the south coast of the island, suggesting another founder effect. The 28 patients with mutations in *CLN2* had a very similar clinical course, regardless of the nature of the mutation.

CLN5

The c.1054G>T mutation in *CLN5* identified in one family in this cohort is a novel mutation and makes the 15th disease-causing mutation in the *CLN5* gene reported to date (29). Mutations in *CLN5* have been identified in patients from Finland, Sweden, Columbia, Portugal, the Netherlands, Italy and the UK (30). This is the first clinical report of patients with *CLN5* mutations originating from England (30).

The clinical features of Finnish patients with *CLN5* mutations are well described (29, 31, 32). Initial clinical symptoms are clumsiness and concentration disturbances, with presentation between 2 and 7 years of age, followed by mental decline and progressive visual loss. Epileptic seizures start between 7 and 11 years of age. There was no significant difference between the pheno-

types of Finnish, Dutch and Swedish patients with different mutations in the *CLN5* gene (29). The Columbian patients had a later onset, presenting at 9 years with visual failure, with rapid disease progression (33). In the Newfoundland patients with a *CLN5* mutation, seizures occurred at an earlier age (5 and 6.6 years) than other reported cases and were the presenting symptom in one case. The other child had a more typical clinical course, with regression of gait as the initial symptom and seizures developing 3 years later. One of the patients died at 41 years of age, which to our knowledge is the oldest age of death reported for a patient with a *CLN5* mutation. Interestingly, an affected cousin died at 14 years of age. Likely, environmental factors and genetic background contributed to the large difference in ages of death between these two patients.

CLN6

There are 27 disease-causing mutations in *CLN6* reported to date. These mutations have a worldwide distribution (30). The c.268_271dup mutation identified in five families in Newfoundland has been previously reported in one patient (34). This patient was of Irish/Irish-French Canadian/Native American descent and was heterozygous for the c.268_271dup mutation. The mutation causes truncation of the protein creating a stop codon in exon 4 (34).

The families in Newfoundland with a *CLN6* mutation are located in small communities on the south coast of the island, which was settled in the 18th century by migration mainly from the Channel Islands and the southwest of England along a coastal trade route. These communities remain isolated today, with some accessible only by boat.

There is variability reported in the age of onset of disease associated with *CLN6* mutation, although the order in which symptoms develop is characteristic, with seizures and motor difficulties presenting early and visual impairment later. Disease onset may occur earlier or later than in classical LINCL (16). In the Newfoundland population, *CLN6* mutation was associated with a significantly later onset of disease and seizures than *CLN2* mutation. Limited data are available to compare the clinical course in Newfoundland patients with other populations. The disease in Newfoundland patients is slightly more protracted than in patients from Pakistan and Portugal (35). The age of onset of disease and seizures is similar in Newfoundland and Costa Rican patients, but the age of death is younger in the Newfoundland patients (range 8.0–14.9 years compared with 14.5–16.8 years) (35).

Genotype–phenotype relationship

The differences in clinical phenotype observed for mutations in different *CLN* genes will be helpful in prioritizing molecular genetic testing for an individual but are not sufficiently large to accurately predict the causative gene in all cases. Similarly, pathology findings are not unique to genotype group. Enzyme testing can exclude patients from PPT1, TPP1 and CTSD loci. Molecular genetic diagnosis remains an essential part of the work-up for children with neurodegenerative disease consistent with NCL, providing affected families with important prognostic information and enabling carrier testing of relatives.

Conclusions

NCL in Newfoundland shows extensive genetic heterogeneity. This population has the highest reported incidence in the world of LINCL, 9.0 per 100,000 live births. Two novel mutations in the *CLN2* gene and one novel mutation in the *CLN5* gene have been identified. Disease associated with *CLN2* mutation is more aggressive than *CLN6*- or *CLN5*-associated disease in this population. The identification of the molecular basis of NCL in Newfoundland enables appropriate mutation screening for individuals at risk in this population. Molecular genetic diagnosis remains an essential component in the diagnostic work-up of children with possible NCL.

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