J Inherit Metab Dis (2011) 34:1083–1093
DOI 10.1007/s10545-011-9323-7

ORIGINAL ARTICLE

Juvenile neuronal ceroid lipofuscinosis: clinical course and genetic studies in Spanish patients

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Received: 6 January 2011 / Revised: 17 March 2011 / Accepted: 21 March 2011 / Published online: 16 April 2011
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Abstract
Background Juvenile neuronal ceroid lipofuscinosis (JNCL, NCL3, Batten disease) is usually caused by a 1.02-kb deletion in the *CLN3* gene. Mutations in the *CLN1* gene may be associated with a variant form of JNCL (vJNCL). We report the clinical course and molecular studies in 24 patients with JNCL collected from 1975 to 2010 with the aim of assessing the natural history of the disorder and phenotype/genotype correlations.
Patients and methods Patients were classified into the groups of vJNCL with mutations in the *CLN1* gene and/or granular osmiophilic deposit (GROD) inclusion bodies (n=11) and classic JNCL (cJNCL) with mutations in the *CLN3* gene and/or fingerprint (FP) profiles (n=13). Psychomotor impairment included regression of acquired skills, cognitive decline, and clinical manifestations of the disease. We used Kaplan-Meier analyses to estimate the age of onset of psychomotor impairment.
Results Patients with vJNCL showed learning delay at an earlier age (median 4 years, 95% confidence interval [CI]

Communicated by: Sedel Frederic

Competing interest: None declared.

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3.1–4.8) than those in the cJNCL group (median 8 years, 95% CI 6.2–9.7) (P=0.001) and regression of acquired skills at a younger age. Patients with vJNCL showed a more severe and progressive clinical course than those with cJNCL. There may be a Gypsy ancestry for V181L missense mutation in the *CLN1* gene.

Conclusions The rate of disease progression may be useful to diagnose vJNCL or cJNCL, which should be confirmed by molecular studies in *CLN1*/*CLN3* genes. Further studies of genotype/phenotype correlation will be helpful for understanding the pathogenesis of this disease.

Introduction

Neuronal ceroid lipofuscinosis (NCLs) is one of the most common groups of progressive neurodegenerative diseases in childhood (Goebel and Sharp 1998). The NCLs are autosomal recessive lysosomal-storage disorders characterized by a diffuse accumulation of lipofuscin and ceroid in neural and nonneural tissues. Electron microscopy studies remain essential to identify granular osmiophilic deposits (GROD), curvilinear profiles (CV), fingerprint profiles (FP) or mixed-type inclusions (Goebel 1996). Clinically, NCLs are characterized by seizures, progressive deterioration of cognition, motor function impairment (ataxia, spasticity), and vision loss.

Phenotypes have been classified considering age of onset, clinical course, and ultrastructural morphology into infantile, late-infantile, juvenile, and adult forms (Goebel et al. 1999; Santavouri et al. 2000; Haltia 2003) The current genetic classification encompasses clinical heterogeneity with more severe and more benign phenotypes, depending mostly on how profoundly the genetic defect affects the function of the encoded protein (Goebel and Wisniewski 2004). To date ten forms with nine disease genes causing human NCL have been identified: *CLN10/CTSD, CLN1/PPT, CLN2/TTP1, CLN3, CLN4, CLN5, CLN6, CLN7/MFSD8, CLN8, and CLCN6* (Siintola et al. 2006; Mole 2006).

Juvenile NCL (JNCL; NCL3, Batten's disease or Spielmeyer-Vogt-Sjögren's disease) is the most common form in the United States and Europe (Wisniewski et al. 1998b). The most frequent mutation causing JNCL is a 1.02-kb deletion in the *CLN3* gene on chromosome 16p12.1–11.2, accounting for about 80–85% of mutated alleles (The International Batten Disease Consortium 1995; Mole et al. 2005). Two different disease courses have been distinguished in JNCL patients. Most homozygous patients for the 1.02-kb deletion show classic JNCL (cJNCL). However, patients who are compound heterozygotes for this deletion may have atypical phenotypes, including a milder course of the disease (Lauronen et al. 1999). Mutations in the *CLN1* gene produce a variant form of JNCL (vJNCL) which is characterized by the presence of GROD and which is clinically similar to JNCL due to mutations in *CLN3* (Mitchison et al. 1998; Mazzei et al. 2002). The variation of distribution of different phenotypes of JNCL in various countries may be due to genetic differences and to date several disease point mutations have been identified (Hofmann et al. 1999) (http://www.ucl.ac.uk/ncl/mutation.shtml).

This study was conducted to describe the clinical course and the results of neuropathological and molecular studies carried out in 24 Spanish patients with JNCL with the aim of assessing the natural history of the disease and to establish genotype/phenotype correlations.

Subjects and methods

From 1975 to 2010, a total of 24 Spanish patients (11 males, 13 females) with a mean age 18.3 years (range 10–33) and from 18 unrelated families were diagnosed with JNCL. Eleven patients (median age 18 years, range 10–23) presented mutations in the *CLN1* gene and/or GROD inclusion bodies (vJNCL group) and 13 patients (median age 18 years, range 10–33) harbored mutations in the *CLN3* gene and/or FP profiles (cJNCL group). The study was approved by the Ethics Committee of Hospital Sant Joan de Déu in Barcelona, which was the reference hospital for the study. Written informed consent was obtained from all patients, parents, or legal guardians.

Data from each patient were collected from the patient's medical records and completed by information provided by the physicians in charge and the parents when necessary. The review of the medical records and the interviews were conducted by a single investigator (M.S.P-P.). A database with 50 items was developed to enter the data (Table 1), which included demographics; age at diagnosis; first symptom (delayed sitting /walking, seizures, visual failure, autistic behavior, attention deficit, delayed speech, learning delay, clumsiness, loss of speech); type of seizures (myoclonic, myoclonic-atonic, partial, generalized tonic-clonic, secondarily generalized, atypical absence); age at regression of acquired skills; cognitive decline; salient signs and symptoms; electroencephalographic findings (background abnormality, generalized and focal paroxysmal activity, atypical high voltage slow spikes and waves to low frequency photic stimulation in the posterior regions, vanishing EEG); ophthalmologic data (maculopathy, vessels attenuation, peripheral pigmentary clumping, optic nerve pallor/atrophy); electroretinographic findings (decreased amplitude, delayed latency, attenuation of cortical waves, extinguished); visual evoked potentials (VEP) (decreased amplitude, delayed latency, attenuation of cortical waves, abolished VEP, giant VEP); enzymatic assays (palmitoyl-protein thioesterase 1 [PPT1], tripeptidyl peptidase 1 [TTP1]); electron microscopy data; and molecular studies.

Neurophysiological (electroretinography, VEP), neuroimaging (brain MRI, CT scan), and ophthalmologic studies

Table 1 Items of the clinical database

Item#	Description	Item#	Description
1	Examination data	26	Learning delay
2	Name and surname	27	Mental retardation
3	Date and birth place	28	Bradypsychia
4	Name of attending physician	29	Autistic behavior
5	Age at examination	30	Behavior disturbances
6	Age at clinical diagnosis	31	Epilepsy
7	Age at structural diagnosis	32	Type of seizures
8	Age at molecular diagnosis	33	Anticonvulsants and response to treatment
9	Consanguinity	34	Visual failure
10	Other family members with NCL	35	Blindness
11	Pregnancy	36	Apraxic gait
12	Delivery	37	Ataxia
13	Weight at birth	38	Pyramidal signs
14	Early psychomotor development	39	Spasticity and retraction of limbs
15	Age at onset of symptoms	40	Electroencephalographic findings
16	First symptom	41	Brain/cerebellar atrophy (MRI/CT)
17	Age at loss of walking ability	42	Ophthalmologic findings
18	Age at wheelchair-bound	43	Electroretinographic data
19	Age at loss of sitting ability	44	Visual evoked potentials
20	Age at loss of purposeful hand use	45	Vacuolated lymphocytes
21	Age at loss of sentence	46	Enzymatic assays
22	Age at nonverbal communication	47	Electron microscopic features
23	Dysphagia	48	Molecular studies
24	Nasogastric tube/feeding button	49	Clinical form
25	Age at urinary incontinence	50	Age of death

were available for 22 of the 24 patients. Electroretinography was performed in 21 patients at least once during the course of the disease. All patients underwent EEG. Brain imaging studies were performed in 19 of 24 patients, and ophthalmologic evaluations in 18.

Electron microscope examinations of biopsied tissues were the main morphological diagnostic technique being obtained according to routine methods. The histological examination of biopsy samples was carried out in 16 patients, including biopsies of the skin (nine patients), conjunctive (two patients), appendix (three patients), and muscle (two patients). The samples were fixed in 2.5% glutaraldehyde in phosphate buffer, post-fixed in osmium tetroxide, dehydrated through graded acetone or alcohol and embedded in epoxy resins. Semi-thin sections were stained with toluidine blue and selected ultra-thin sections with uranyl acetate and lead citrate.

Palmitoyl-protein thioesterase (PPT-1) activity was measured in samples of leukocytes or fibroblasts of eight patients. The fluorimetric enzymatic analyses were performed using 4-methylumbelliferyl-6-thiopalmitoil β-D-glucoside (Moscerdam Substrates, Rotterdam, Holland) as substrate. Protein measurements were performed according to the method of Lowry et al. (1951).

In all patients, peripheral blood DNA samples were studied for the entire open reading frame and splice site (50 bp exon-intron boundaries) of the *CLN1* and *CLN3* genes. The only large deletion studied was 1.02-kb in the *CLN3* gene. Molecular studies were performed by single-strand conformation polymorphism analysis and sequencing of abnormal patterns in the *CLN1* and *CLN3* genes. Genetics studies were carried out on the most patients. DNA was not available in two patients. One patient died prior genetic studies were available (case 1) and the second family did not approve this study (case 8).

The primary endpoint of the study was the assessment of psychomotor impairment (time to reach regression of acquired skills, cognitive decline, and clinical symptoms/signs of the disease) in the groups of vJNCL and cJNCL. The age of onset of psychomotor impairment was taken as survival data (i.e., time interval from birth to the onset of psychomotor impairment). In the absence of this information, data were censored at the age at the last visit. Estimates were made using the Kaplan-Meier survival analysis and survival curves were compared using the log-rank test. Adjustment for multiple comparisons was made using Bonferroni's correction. Data were analyzed with the SPSS computer program (version 17.0).

Table 2 Clinical features, enzymatic, and ultrastructural studies in JNCL Spanish patients

Case number	Sex	Age years	Consanguinity	Presenting symptom	Age at first symptom/ age at diagnosis	Myoclonic/ generalized tonic-clonic seizures	Response to antiepileptic treatment	Ophthalmologic findings / extinguished ERG (age, years)	Cerebral/ cerebellar atrophy (age, yrs)	PPT1 activity nmol/h/mg (control range)	Electron microscopy (biopsied tissue)	Group
1[a]	M	18	Yes	Visual failure	6 / 11	+++++/++	Refractory	Papillary pallor / (16)	+/+ (11)	NA	GROD, RL (skin)	vJNCL
2a[a]	F	23	Yes	Delayed speech	3 / 8	++++/−	Refractory	NA	NA	NA	NA	vJNCL
2b[a]	F	17	Yes	Loss of speech	4 / 7	++++/−	Refractory	Optic nerve pallor / (7)	+/− (7)	NA	GROD, RL, CV (skin)	vJNCL
3[a]	F	20	No	Delayed speech	2 / 11	+++/++	Refractory	Papillary pallor / (11)	+/− (9)	NA	GROD (conjunctiva)	vJNCL
4a[a]	M	19	Yes	Delayed speech	2 / 9	++++/−	Controlled	Optic nerve atrophy/ (9)	+/+ (9)	NA	GROD (appendix)	vJNCL
4b[a]	M	18	Yes	Learning delay	4 / 6	++++/−	Controlled	Optic nerve atrophy/ (8)	NA	NA	GROD (skin)	vJNCL
4c	F	12	Yes	Learning delay	3 / 7	+++/−	Controlled	NA / (7)	+/+ (7)	NA	NA	vJNCL
4d	F	12	Yes	Clumsiness	4 / 2	+++++/−	Controlled	NA / (7)	NA	1.6 (6–38)	NA	vJNCL
4e	M	10	Yes	Delayed speech	2 / 1	+++++/−	Controlled	NA / (7)	NA	1.8 (6–38)	NA	vJNCL
5	F	23	Yes	Learning delay	4 / 8	+++++/++	Refractory	Optic nerve atrophy/ (8)	+/+ (8)	0.45 (6–38)	GROD, FP (appendix)	vJNCL
6[a]	F	17	Yes	Learning delay	6 / 8	+++/−	Controlled	Optic nerve atrophy/NA	+/+ (7)	2.4 (control 114)	NA	vJNCL
7a[a]	M	33	No	Visual failure	6 / 18	−/+++	Refractory	Optic nerve atrophy/ (6)	−/− (7)	NA	Intracytoplasmic inclusions consistent with NCL (muscle)	cJNCL
7b	M	29	No	Visual failure	6 / 6	−/+++	Refractory	Optic nerve atrophy/ (6)	−/+ (15)	NA	NA	cJNCL
8[a]	F	26	No	Seizures	4 / 18	++/+++	Refractory	NA	NA	NA	FP (skin)	cJNCL
9	M	25	No	Visual failure	5 / 15	−/++	Controlled	Papillary pallor/ (12)	+/− (15)	NA	NA	cJNCL
10	F	24	No	Visual failure	7 / 7	−/++	Controlled	Optic nerve atrophy/ (17)	−/− (9)	NA	Intracytoplasmic inclusions consistent with NCL (skin)	cJNCL
11[a]	M	18	Yes	Delayed speech	2 / 7	+++++/++	Refractory	Papillary pallor/ (7)	+/− (7)	NA	FP (conjunctiva)	cJNCL
12	F	19	No	Loss of speech	4 / 6	+++++/+++	Refractory	Normal / (7)	+/+ (5)	Normal	FP, CV (appendix)	cJNCL
13	M	17	No	Visual failure	5 / 13	−/++	Controlled	NA/ (9)	−/− (7)	NA	Intracytoplasmic inclusions consistent with NCL (skin)	cJNCL
14	F	14	Yes	Learning delay	7 / 10	−/++	Controlled	Peripheral pigmentary clumping / (10)	−/− (7)	NA	FP, CV (skin)	cJNCL
15	F	13	No	Delayed walking	2 / 9	++/−	Controlled	Papillary pallor/ (10)	−/− (9)	Normal	FP, GROD (skin)	cJNCL

16	M	12	Yes	Visual failure	8 / 10	++/–	Controlled	Papillary pallor / Low amplitude (9)	–/– (11)	Normal	NA	cJNCL
17	M	12	No	Learning delay	6 / 8	–/–	NA	Papillary pallor / Low amplitude (7)	–/– (8)	NA	Intracytoplasmic inclusions consistent with NCL (skin)	cJNCL
18	F	10	No	Visual failure	6 / 8	–/++	Controlled	Papillary pallor / Low amplitude (7)	–/– (7)	Normal	Intracytoplasmic inclusions consistent with NCL (muscle)	cJNCL

[a] Died; M: male; F: female; NA: not assessed; CV: curvilinear inclusions; RL: rectilinear complex.

Results

Enzymatic and molecular studies

In the group of 11 patients with vJNCL, a partial deficiency of PPT1 activity was found in four patients (cases #4d, #4e, #5, and #6) who also showed mutations in the *CLN1* gene. In the remaining seven patients, data on PPT1 activity were not assessed; five of these patients belonged to two unrelated families harboring mutations in the *CLN1* gene (cases #2a, #2b, #4a, #4b, and #4c) and the remaining two patients were included in the vJNCL group because of a typical clinical course compatible with vJNCL and ultrastructural studies that showed mixed profiles, predominantly GROD.

In the group of 13 patients with cJNCL, mutations in the *CLN3* gene were identified in 12 (92.3%). Five patients (41.7%) were found to have the 1.02-kb deletion on both chromosomes, whereas three patients were compound heterozygotes for the 1.02-kb deletion. The remaining four patients showed different mutations in the *CLN3* gene. One patient (case #8) was included in the cJNCL group due to typical clinical course compatible with JNCL and ultrastructural studies showing FP inclusions. Vacuolated lymphocytes in peripheral blood were present in three cJNCL patients (cases #9, #11, and #15). In the remaining cJNCL patients, this study was not performed.

Individualized clinical, enzymatic, and ultrastructural data as well as *CLN1* and *CLN3* allele mutations are shown in Tables 2 and 3. GROD inclusions in the blood vessel wall in a case of vJNCL are shown in Fig. 1, and FP inclusions in endothelial cells in two different *CLN3* cases are shown in Fig. 2.

Regression of acquired skills

As shown in Table 4, regression of acquired skills occurred at an earlier age in patients with vJNCL than in those with cJNCL, with statistically significant differences for all comparisons. On the other hand, the percentage of patients who did not show psychomotor impairment was higher in the cJNCL group than in the vJNCL group. At the time of the study, all patients with vJNCL had lost their walking and sitting abilities, as well as to speak in full sentences, and urinary sphincter control. Loss of sentences was observed at a median age of 8 years (95% CI 6.4–9.8) among vJNCL patients as compared with a median of 20 years (95% CI 10.4–29.5) among those with cJNCL ($P<0.001$); absence of language appeared at a median age of 10 years (95% CI 9–10.9) and 23 years (95% CI 7–38.9), respectively ($P=0.002$). Both vJNCL and cJNCL patients lost their walking ability and

Table 3 Mutations identified in JNCL Spanish patients

Case number	Nucleotide change	Mutation	Aminoacid change/ predicted consequence	Status	Location	Number of individuals with mutation in the family
Mutations in the CLN1 gene						
2	c.541 G>T	Missense	V181L	Homozygous	Exon 6	2
4	c.541 G>T	Missense	V181L	Homozygous	Exon 6	5
5	c.541 G>T	Missense	V181L	Homozygous	Exon 6	1
6	c.541 G>T	Missense	V181L	Homozygous	Exon 6	1
Mutations in the CLN3 gene						
7	c.462-677del (g.6060-7025del) c.374 G>A	1.02-kb deletion Missence/Splice site	Frameshift after C153 S125N	Compound heterozygous	Intron 6–8 Exon 5	2
9	c.462-677del (g.6060-7025del)	1.02-kb deletion	Frameshift after C153	Homozygous	Intron 6–8	1
10	c.462-677del (g.6060-7025del)	1.02-kb deletion	Frameshift after C153	Homozygous	Intron 6–8	1
11	c.622-623insT	1 bp insertion	Frameshift after L207	Homozygous	Exon 8	1
12	c.462-677del (g.6060-7025del) Not found	1.02-kb deletion	Frameshift after C153	Compound heterozygous	Intron 6–8	1
13	c.462-677del (g.6060-7025del)	1.02-kb deletion	Frameshift after C153	Homozygous	Intron 6–8	1
14	c.462-677del (g.6060-7025del)	1.02-kb deletion	Frameshift after C153	Homozygous	Intron 6–8	1
15	c.575 G>A c.622-623instT	Missense 1 bp insertion	G192E Frameshift after L207	Compound heterozygous	Exon 8 Exon 8	1
16	c.265 C>T	Nonsense	R89X	Homozygous	Exon 4	1
17	c.462-677del (g.6060-7025del)	1.02-kb deletion	Frameshift after C153	Homozygous	Intron 6–8	1
18	c.370insT c.1001 G>A	1 bp insertion Missense	Frameshift after C153 R334H	Compound heterozygous	Exon 5 Exon 13	1

References: M. Milà and J. Mallolas, personal communication (cases 2, 4, 5, and 6); The International Batten Disease Consortium, 1995 (cases 7, 9, 10, 12, 13, 14, and 17); M. Milà, personal communication (case 11); M. Milà, personal communication and Taschner et al., unpublished results (case 15); Sims, personal communication and Munroe et al. 1997 (case 18); the present study (cases 7 and 16).

purposeful hand use few years after loss of sentences. Sitting ability was lost during adolescence in the vJNCL group and in adulthood in cJNCL patients (12 *vs* 31 years, $P=0.001$). Kaplan-Meier estimates of the age of the patients at the time of regression of acquired skills are shown in Fig. 3A and B.

Cognitive decline

Except for behavior disturbances, all parameters of cognitive decline (learning delay, mental retardation, bradypsychia, and autistic behavior) appeared at an earlier age in the vJNCL group as compared with the cJNCL group, and all differences were statistically significant (Table 4). Learning delay and mental retardation were observed in all vJNCL patients and in 92% of cJNCL patients. Learning delay occurred at a median age of 4 years among vJNCL patients and mental retardation at a median age of 5 years. However, in the cJNCL group learning delay and mental retardation occurred later and at a more variable age. Kaplan-Meier estimates of the age of the patients at the time of regression of acquired skills are shown in Fig. 3 C and D.

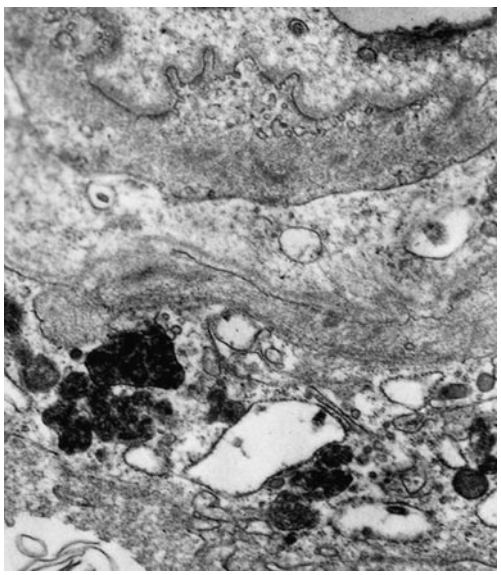

Fig. 1 GROD inclusions in the blood vessel wall in a case of vJNCL (ultrathin section stained with uranyl acetate and lead citrate x 12,000)

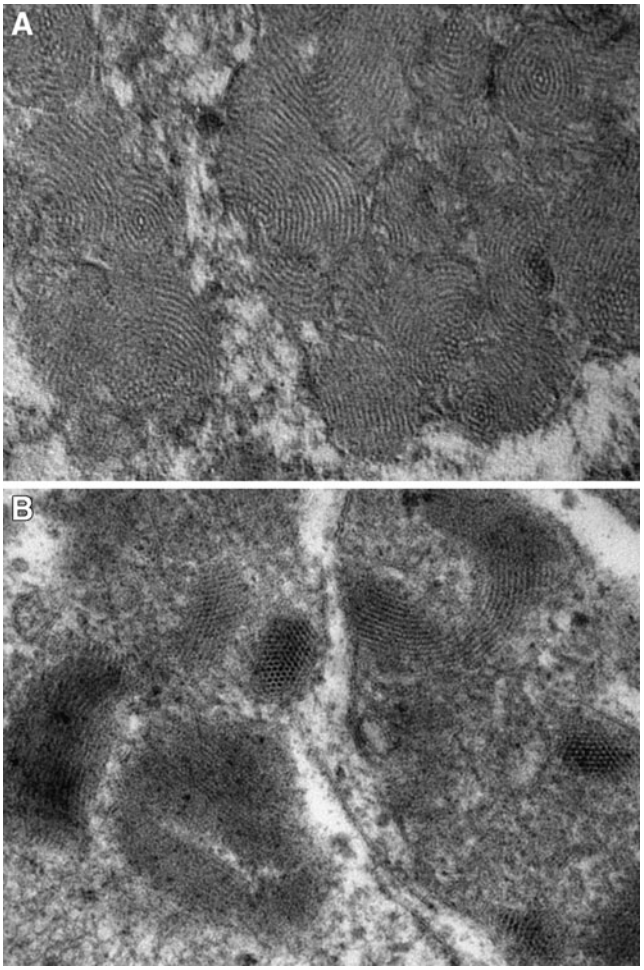

Fig. 2 A and B. Fingerprint inclusions in endothelial cells in two different cases of *CLN3* mutations (ultrathin section stained with uranyl acetate and lead citrate x 42,000)

Clinical signs and symptoms

Presenting symptoms of learning delay and delayed and loss of speech were observed predominantly in vJNCL (8 of 11 patients) compared to cJNCL (4 of 13 patients) group. Visual failure was more common as initial symptom in cJNCL than vJNCL patients (Table 2). The median age at which visual failure appeared in the two groups of patients was quite similar (P=0.088) (Table 4). Significant differences were found for the age at which vJNCL patients were blind (median 8 years, 95% CI, 6.8–9.1) compared to cJNCL (median 12 years, 95% CI 11.2–12.7) (P<0.001). All vJNCL patients developed apraxic gait and ataxia during their childhood whereas 69% of cJNCL patients both appeared during adolescence (P<0.001, P=0.001, respectively). The median age at which pyramidal signs and spasticity were observed was the later and it was significant earlier in vJNCL compared to cJNCL patients (P<0.001) (Table 4).

With respect to epilepsy, the median age was not significantly different between two groups (P=0.003) and occurred before than blindness both vJNCL (median 7 years, 95% CI 5.9–8) and cJNCL patients (median 10 years, 95% CI 7–12.9). The vJNCL patients had mainly myoclonic seizures whereas the cJNCL patients showed predominantly generalized tonic-clonic seizures. Pharmacological control of seizures with anticonvulsant drugs was more satisfactory in cJNCL patients than in vJNCL (Table 2). Optimal seizure control was found in patients on valproic acid monotherapy, combination of valproic acid and carbamazepine, or combination of valproic acid and clobazam.

Kaplan-Meier estimates of the age of the patients at the time of appearance of clinical signs and symptoms are shown in Fig. 3E and F.

Discussion

JNCL is the most prevalent type of NCL in North European countries (Uvebrant and Hagberg 1997). Several mutations in the *CLN1* and *CLN3* genes are associated with families from specific countries (http://www.ucl.ac.uk/ncl.genetics.shtml). The progression of the disease has been shown to be different and patients with a more benign or delayed course have been reported (Wisniewski et al. 1999). Most mutations in the JNCL genes are associated with a classic phenotype and a less severe disease is probably due to mutations that do not completely abolish the function of the encoded protein (Mitchison et al. 1998; Das et al. 1998; Lauronen et al. 1999; Mazzei et al 2002). At the time we undertook this study no comprehensive detailed studies of JNCL had been carried out in Spain. We aimed to describe the natural history of the disease between two groups JNCL patients (vJNCL and cJNCL). Our study tries to find an accurate genotype-phenotype correlation.

Most patients who did not show regression of acquired skills belonged to cJNCL group and vJNCL patients showed loss of acquired skills at an earlier age than did patients with cJNCL. We observed slower progression of the disease in cJNCL than in vJNCL patients. Careful clinical assessment regarding the regression of developmental skills in these two groups of JNCL patients allowed us to observe that it may be initiated by the onset of loss of sentences followed by loss of walking ability. During the second stage of the disease the patients lose the purposeful hand use. Finally, patients lost their sitting ability. According to other authors, the learning disabilities presented at the earliest age in vJNCL (Philippart et al. 1995) and most vJNCL patients presented mental retardation earlier than did cJNCL patients. Additionally, learning delay occurred after the onset of visual loss in cJNCL patients (Marshall et al. 2005). This finding suggests that the clinical course of the disease is more severe and progressive in vJNCL than in cJNCL patients. An initial diagnosis of vJNCL patients

Table 4 Age at the time of psychomotor impairment and clinical signs and symptoms

	vJNCL patients (n=11)		cJNCL patients (n=13)		P value
	Age, years median (95% CI)	No impairment % patients	Age, years median (95% CI)	No impairment % patients	
Regression acquired skills					
Loss of walking ability	9 (7.3–10.6)	0	21 (11.6–30.3)	38.5	< 0.001
Becoming wheelchair-bound	10 (6.7–13.2)	0	23 (12.6–33.3)	38.5	0.003
Loss of sitting ability	12 (8.4–15.5)	0	31	61.5	0.001
Loss of purposeful hand use	11 (8.3–13.6)	9.1	22 (13.6–30.3)	50	0.001
Loss sentences	8 (6.4–9.8)	0	20 (10.4–29.5)	38.5	< 0.001
No language	10 (9–10.9)	9.1	23 (7–38.9)	46.2	0.002
Dysphagia	12 (9.9–14)	9.1	22 (17.8–29.6)	53.8	< 0.001
Urinary incontinence	9 (7.5–10.4)	0	23 (18.2–27.7)	38.5	< 0.001
Cognitive decline					
Learning delay	4 (3.1–4.8)	0	8 (6.2–9.7)	7.7	0.001
Mental retardation	5 (4.9–5.6)	0	11 (9.4–12.5)	7.7	< 0.001
Bradypsychia	7 (5.3–8.6)	9.1	14 (7–20.9)	23.1	0.024
Autistic behavior	7 (5.3–8.6)	9.1	17 (1.6–32.3)	46.2	0.001
Behavior disturbances	8 (6.5–9.7)	9.1	10 (8.3–11.6)	15.4	0.150
Clinical signs and symptoms					
Visual failure	5.5 (4.8–6.1)	0	6.5 (5.6–7.3)	0	0.088
Blindness	8 (6.8–9.1)	0	12 (11.2–12.7)	15.4	< 0.001
Epilepsy	7 (5.9–8)	0	10 (7–12.9)	7.7	0.003
Apraxic gait	6.6 (5.8–7.4)	0	12 (9.9–14)	7.7	< 0.001
Ataxia	8 (6.9–9)	0	17 (7.7–26.2)	30.8	0.001
Pyramidal signs	8.5 (6.8–10)	0	20 (11.5–28.4)	38.5	< 0.001
Spasticity	10 (8.3–11.6)	0	18 (6.9–29)	38.5	< 0.001

may be suspected in the presence of learning delay and regression or delayed speech. Visual failure was the first symptom of the disease by 7 years of age as described in patients with mutations in the *CLN3* gene (Marshall et al. 2005; Moore et al 2008; Sarpong et al 2009), while other authors referred it between 10 to 14 years in vJNCL patients (Hofman and Taschner 1995; Philippart et al 1995). A phenotype characterized by visual failure and increased survival was observed in compound heterozygous *CLN3* (cases 7a, 7b). In these patients the evolution to blindness was more severe and the cognitive deterioration occurred slightly later and more slowly than in homozygous patients (Wisniewski et al. 1998a and 1998b; Lauronen et al. 1999; Munroe et al. 1997; Aberg et al. 2009).

Epilepsy was the third symptom of the disease after the onset of visual failure and learning delay in both vJNCL and cJNCL patients. The estimated median age at onset of seizures in our cJNCL patients was similar to what previously has been reported in patients with mutations in the *CLN3* gene (Aberg et al. 2009; Moore et al. 2008; Sarpong et al. 2009). In our series, epilepsy with later onset appeared only in compound heterozygous *CLN3* patients (cases 7a, 7b) (Wisniewski et al. 1998b; Aberg et al. 2009). Most cJNCL patients had generalized tonic-clonic seizures and were often reasonably well controlled as described in other series (Aberg et al. 1999; Aberg et al. 2000; Sarpong et al. 2009). The blindness occurred a few years later than epilepsy in our series. Motor decline with ataxia and spasticity was observed during advanced stages of the disease. Most cJNCL patients showed normal MRI brain imaging in the early stages of disease (Santavouri et al. 2001) and vJNCL patients showed brain atrophy before the age of 12. Cerebellar atrophy was the main radiological sign in both groups of patients, although it is not an unequivocal sign of the disease (D'Incerti 2000; Autti et al. 2008).

A relationship between the absence of PPT1 activity and GROD (Das et al. 1998) and mutations in *CLN1* gene and GROD has been reported (Mitchison et al. 1998; Mazzei et al. 2002; Mole et al. 2005). The present results confirm that mutations in *PPT1/CLN1* gene are associated with reduced PPT1 activity in vJNCL, as previously reported (Kalviainen et al. 2007). Moreover, GROD inclusions have been found in

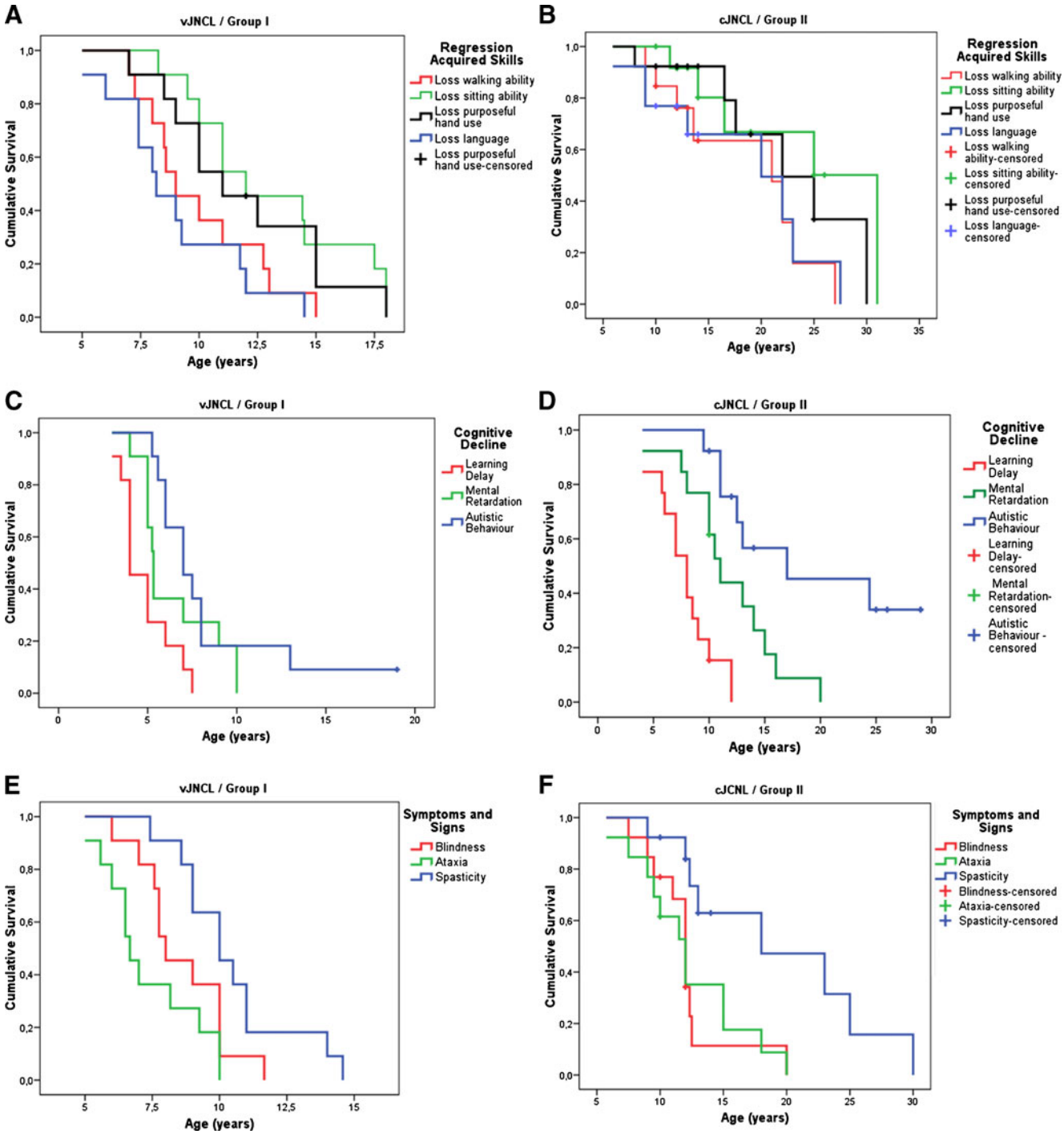

Fig. 3 Kaplan-Meier estimates of the age of the patients at the time of regression of acquired skills (**a** and **b**), cognitive decline (**c** and **d**), and main signs and symptoms of the disease (**e** and **f**) among 24 Spanish patients with JNCL

all six cases carrying mutations in *CLN1* gene. Interestingly, other inclusions including rectilinear, curvilinear and FP were also found in some individuals.

Regarding *CLN3* cases, intracytoplasmic inclusions with FP were found in five cases, whereas the characteristics of the deposit were not specified in the other five cases. However, in these cases, the pathologic study disclosed 'deposits consistent with NCL'. These biopsies were not available for further re-examination. Curvilinear bodies and GROD inclusions associated with FP were present in three cases, as described in other studies (Aberg et al 1998).

The present observations highlight the need for accurate combined methods in the diagnosis of juvenile NCL. Clinical

symptoms prompt the identification of abnormal deposits, enzymatic deficits and genetic studies. Although crucial some years ago, neuropathological findings with negative results in biopsy samples do not rule out NCL (Das et al. 1998), and a combination of variegated deposits may occur in some individuals. Regional variations should also be considered when using skin, appendix, rectal mucosa or conjunctive tissue, as there is no proof that different tissues are equally affected at the same time in the progression of the disease. Further genetic studies in this area are still needed.

The presence of V181L missense mutation in *CLN1* gene was found in homozygosis in nine patients belonging to four unrelated consanguineous Gypsy families. There may be a Gypsy ancestry for this mutation. Variability in the age at onset and presenting symptoms in families harboring other mutations (V181M) has been reported (Wisniewski et al. 2000). It may be possible that still unknown epigenetic factors may account for this interfamilial and interfamilial phenotypic variability.

The most common form of JNCL is caused by mutations in *CLN3* gene, being a 1.02-kb deletion the most prevalent mutation identified (The International Batten Disease Consortium 1995). On the basis of our results, the 1.02- kb deletion was present in 54% of the mutated alleles. This frequency is lower than previously reported (Munroe et al. 1997; Wisniewski et al. 2000; Mole et al. 2005).

In summary, early clinical diagnosis of JNCL may be suspected on the basis of the symptomatology and age at disease onset. The rate of disease progression may lead us to a clinical diagnosis of vJNCL or cJNCL which may be confirmed by molecular studies in *CLN1/CLN3* genes. Further studies of genotype/phenotype correlation will be helpful for understanding the pathogenesis of the disease. Early diagnosis is crucial to be able to apply therapeutic options.

Acknowledgments We are indebted to Drs Armstrong, Vernet, Vilaseca, Coll and Gort, and the families of the patients for the valuable support and indispensable cooperation, and Dr Marta Pulido for editing the manuscript and editorial assistance (medical editing services were provided on behalf of the authors).

Funding None

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