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LRSAM1 variants and founder effect in French families with ataxic form of Charcot-Marie-Tooth type 2

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Abstract

Currently only 25–30% of patients with axonal forms of Charcot-Marie-Tooth disease (CMT) receive a genetic diagnosis.
We aimed to identify the causative gene of CMT type 2 in 8 non-related French families with a distinct clinical phenotype.
We collected clinical, electrophysiological, and laboratory findings and performed genetic analyses in four different French
laboratories. Seventy-two patients with autosomal dominant inheritance were identified. The disease usually started in the
fourth decade and the clinical picture was dominated by sensory ataxia (80%), neuropathic pain (38%), and length-
dependent sensory loss to all modalities. Electrophysiological studies showed a primarily axonal neuropathy, with possible
isolated sensory involvement in milder phenotypes. Disease severity varied greatly but the clinical course was generally
mild. We identified 2 novel variants in *LRSAM1* gene: a deletion of 4 amino acids, p.(Gln698_Gln701del), was found in 7
families and a duplication of a neighboring region of 10 amino acids, p.(Pro702_Gln711dup), in the remaining family. A
common haplotype of ~450 kb suggesting a founder effect was noted around *LRSAM1* in 4 families carrying the first variant.
LRSAM1 gene encodes for an E3 ubiquitin ligase important for neural functioning. Our results confirm the localization of
variants in its catalytic C-terminal RING domain and broaden the phenotypic spectrum of *LRSAM1*-related neuropathies,
including painful and predominantly sensory ataxic forms.

Introduction

Charcot-Marie-Tooth disease (CMT) comprises a group of
clinically and genetically heterogeneous hereditary neuro-
pathies. With an estimated prevalence of 1 in 2500 indivi-
duals, CMT is the most common inherited neurological
disorder, affecting about 200,000 people in European
countries [1, 2].

The current classification of CMT is based on neuro-
physiological parameters, mode of inheritance, and the gene
involved [1]. A cut off of 38 m/s for the motor conduction
velocity (MCV) of the ulnar or median nerve is used to
distinguish demyelinating (MCV <38 m/s) from axonal
forms (MCV ≥38 m/s) [3]. The intermediate CMT forms
may display a median MCV between 25 and 45 m/s [4].
Mode of inheritance is most frequently autosomal dominant
but may also be autosomal recessive or X-linked.

So far, more than a thousand different mutations have been
discovered in over 90 genes encoding for proteins with
metabolic or structural functions that primarily affect the
myelin or the axon [5]. In demyelinating forms a genetic
diagnosis can be reached in about 80% of cases whereas in
axonal forms this occurs only in 25% [6]. There is, therefore, a
need to identify new genes involved in this subtype of CMT.

LRSAM1 gene mutations are a rare cause of axonal CMT,
named CMT2P (OMIM 614436) [7]. Both autosomal
dominant and recessive forms have been reported secondary
to 9 different pathogenic variants [7–14]. Clinical pheno-
type is characterized by onset in adulthood of slowly pro-
gressive length-dependent motor deficit, atrophy, sensory
loss to all modalities and foot deformities. Marked sensory
gait ataxia, sometimes in association with elevated serum

creatine kinase (CK), has also been reported [8, 11]. Clinical severity varies but the vast majority of patients maintain the ability to walk.

Here, we report 8 non-related French families presenting an adult-onset disease dominated by sensory ataxia with either electrophysiological features of CMT2 or sensory neuropathy and in which two novel variants of *LRSAM1* were identified.

Materials and Methods

Editorial policies and ethical considerations

The Institute of Myology obtained the approval of the French Ethics committee for these studies (Method of reference MR-003).

All participants gave their written informed consent prior to their inclusion in the study.

Patients and clinical investigations

Seventy-two patients from 8 unrelated families were recruited in several hospitals in France (G-H Pitié-Salpêtrière, CH La Rochelle, CHU Lyon, CHU Bordeaux, CHU Grenoble, CHU Besançon, CHU Limoges, CHU Montpellier, CHU Kremlin-Bicêtre).

Forty-one patients underwent a complete clinical assessment. Skeletal muscle strength was tested and scored according to the Medical Research Council (MRC) grading system, ranging from 0 (no movement) to 5 (normal strength).

Neurophysiological and laboratory investigations

Nerve conduction studies using standard techniques were performed in 33 of our patients. Compound muscle action potential amplitudes (CMAPs) and MCV were considered for the ulnar and peroneal nerves. Sensory nerve action potential (SNAPs) and sensory nerve conduction velocities (SNCVs) were recorded for the ulnar and sural nerves. Electromyography was performed in the tibialis anterior.

Biological analyses to exclude secondary causes of peripheral neuropathy comprised glucose level, renal, liver and thyroid function, autoimmune screening, vitamin B12 level, serum immunofixation, hepatitis serology, and salivary gland biopsy.

In the absence of a clear family history at onset, nerve and muscle biopsy specimens were obtained and analyzed in 7 patients according to standard procedures [15].

Genetic analysis

After exclusion of the CMT1A duplication, DNA sequencing using custom panels of genes was performed in 4 different laboratories [16]. In each laboratory, index cases were tested with panels for peripheral neuropathy including the following genes relevant for axonal CMT: *AARS, AIFM1, COX6A1, DNM2, DHTKD1, DYNC1H1, FAM134B, FBXO38, GAN, GARS, GDAP1, GJB1, HSPB1, HSPB8, IGHMBP2, KARS, KIF1B, LMNA, LRSAM1, MED25, MFN2, MPZ, MORC2, NEFH, NEFL, PLEKHG5, RAB7, TRIM2, TRPV4,* and *YARS.* Confirmation of variants and familial studies were done by Sanger sequencing. Whole exome sequencing (WES) was used as previously described to compare haplotypes of 9 patients from the following families: family 1 (patients V.14 and V.18), family 2 (patient IV.1), family 3 (patient III.1), family 4 (patient III.3), family 5 (patients I.2 and II.9), family 6 (patient III.7), and family 7 (patient III.2) [17]. Numbering of *LRSAM1* variants was done with reference transcript NM_138361.5.

We also screened genes involved in hereditary Parkinson diseases, since patient IV.1 (family 2) suffered from this disease. The following genes were examined *VPS13, DNAJC13, UCHL1, ADH1C* but no pathogenic variant was found.

Results

Molecular findings

An in-frame deletion, c.2093_2104del, p.(Gln698_Gln701del), was found for 7 families. An in-frame insertion, c.2104_2133dup, p.(Pro702_Gln711dup), was found in the remaining one (family 8). Neither of these variants was present in either the gnomAD or the dbSNP database. These variants are located in the essential RING finger domain of the protein (zinc binding and E3 enzymatic function). Variant c.2093_2104del is predicted to delete 4 amino acids and variant c.2104_2133dup is predicted to insert 10 amino acids (Fig. 1). All affected tested members were found to be heterozygous. Pedigrees were consistent with autosomal dominant inheritance (Fig. 2). Haplotyping with WES showed a very high likelihood for families 1, 2, 3, and 5 of a common haplotype of ~450 kb surrounding *LRSAM1* between single nucleotide polymorphisms (SNPs) rs7027153 (hg19 position 130 125 963) and rs10106 (130 576 075) (eTable 1, Supplement). Family 6 was partially compatible with this putative founder haplotype for ~100 kb. Haplotypes in families 4 and 7 showed several differences (eTable 1, Supplement).

Clinical findings

In our cohort of 72 unrelated patients, we identified two distinct *LRSAM1* variants: 7 families presented an in-frame deletion, c.2093_2104del, and the remaining family (family 8) presented a duplication of a neighboring region,
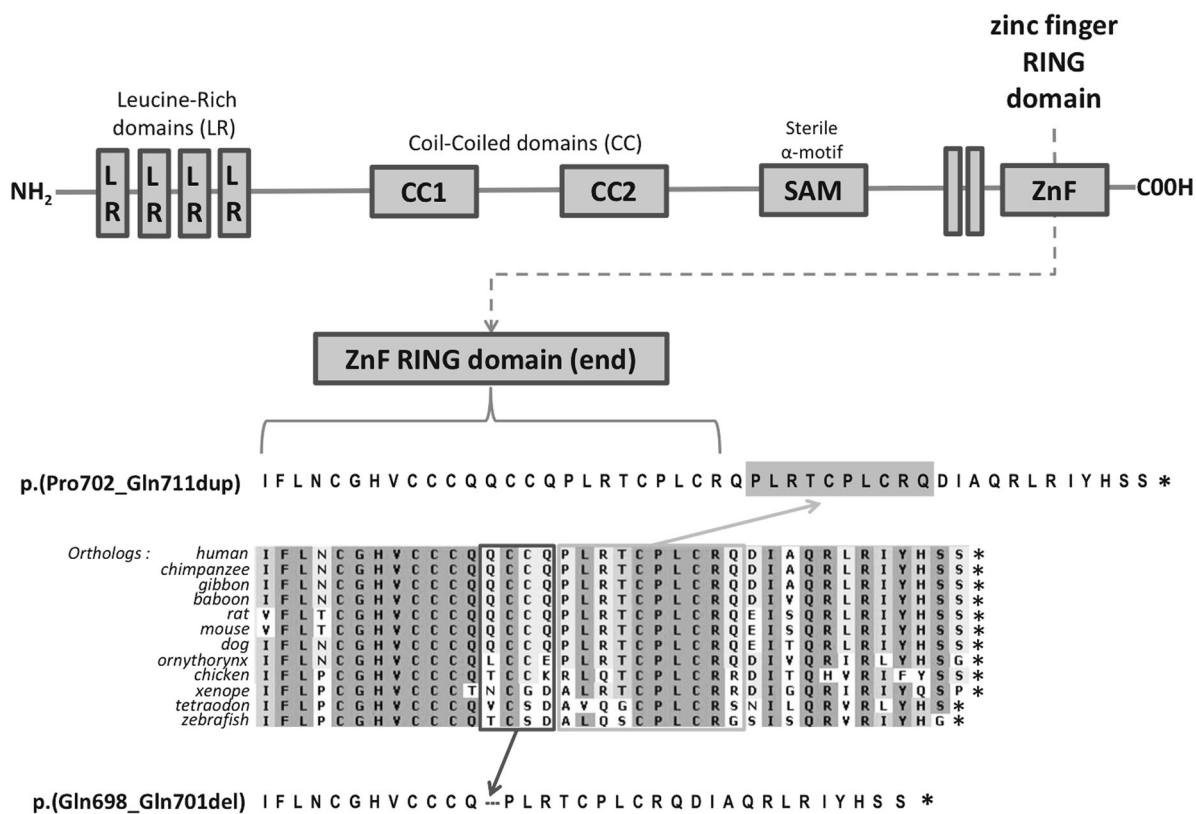
Fig. 1 Changes in C-terminus of LRSAM1. Schematic representation of LRSAM1 adapted from Bogdanik et al. 2013. Orthologs are shown for the C-terminus of LRSAM1 from amino acids 686 to 723. The stop codon is indicated by an asterisk. Protein changes observed in French families are indicated, respectively, above (family 8) and below orthologs (families 1–7)

c.2104_2133dup. The 7 families carrying the c.2093_2104del variant came from a large area of western and south-western France (including Loire Region, Brittany, Normandy and Poitou-Charentes). Family 8 was originally from the Center-East part of France (Jura). Clinical features were available for 41 patients and are summarized in Table 1. The mode of inheritance was autosomal dominant.

The mean age at onset was in the fourth decade (39 ± 12) but ranged from 15 to 70 years even within the same family.

Gait instability was the first symptom in most cases (71%): other possible symptoms at onset were feet numbness/paresthesia (20%), cramps (12%), neuropathic pain (12%), and distal motor deficit (7%), alone or in combination. Burning and shock-like pain in the lower limbs was frequent (38%) and sometimes intense and intractable, leading in patient II.3 (family 5) to suicide. Cramps were reported in 14 patients and were the only complaint in 3 of them. A genetically affected family member was still asymptomatic at the age of 36 (patient IV.3, family 4), although his neurological examination revealed subtle signs of neuropathy.

The clinical phenotype was dominated by sensory impairment with proprioceptive ataxia (80%) and marked reduction of sensory modalities in a length-dependent fashion, even in the asymptomatic patient. Motor deficit,

although present in 59% of patients, was generally mild and confined distally to the lower limbs (MRC 3–4 + /5). One subject presented proximal lower limb weakness (MRC 3/5) at about 70 years of age and became wheelchair-bound (IV.6, family 1). Slight calf atrophy was noted in 36% of patients and pes cavus was present in 39%. Deep tendon reflexes (DTR) were usually reduced or absent. One patient was also affected by early onset Parkinson disease from the age of 40 (IV.1, family 2), without any pathogenic or likely pathogenic variant in OMIM genes for Parkinson disease being identified.

Disease course was slowly progressive, with worsening of gait instability and numbness in 80% of the patients. After a median follow-up of 12 years (range 0–40), most patients (86%) were able to walk without aids. Three patients (7%) used a cane to move about and 3 (7%) were wheelchair-bound.

Description of clinical presentation in 2 patients belonging to families 1 and 2: LRSAM1 variant c.2093_2104del

Patient IV.6 (family 1) belongs to a large family showing autosomal dominant inheritance and comprising 33 family

Fig. 2 Pedigrees of the eight families with *LRSAM1* gene variants. c.2093_2104del, p.(Gln698_Gln701del) and c.2104_2133dup, p. (Pro702_Gln711dup) variants are found in families 1–7 and family 8, respectively. Affected patients are indicated with black (first 7 families) or dashed (family 8) squares and circles. Tested unaffected members are marked with an asterisk
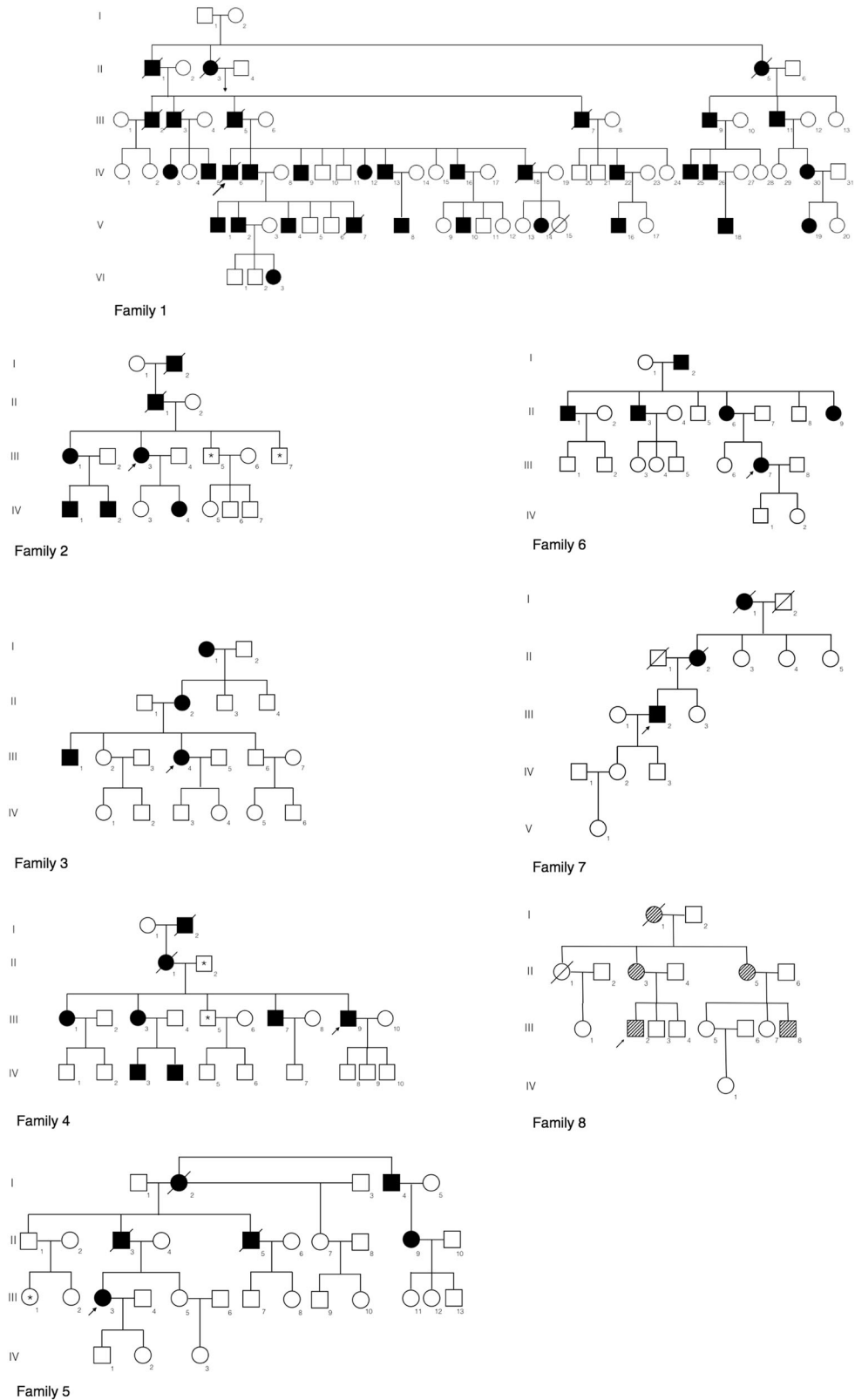
members. This family is originally from a region in western France called Poitou-Charentes. He was the most severely disabled. First symptoms appeared at the age of 33 with gait instability and difficulty in running. Ataxia was progressive and despite walking aids he reported many falls. Concurrently, lower limb proximal and distal weakness (MRC 3/

Table 1 Clinical Features of 41 Patients with either p.(Gln698_Gln701del) or p.(Pro702_Gln711dup) variant of *LRSAM1* gene

Patients	AAO	Symptoms at onset	ANE	Gait	Weakness (distal MRC)/atrophy LL	Weakness/atrophy UL	Pes cavus	DTR Ach/Knee	Vibration UL/LL	Pinprick UL/LL	Neuropathic pain	Additional features	DSLE (age)
FAMILY 1													
III.5	45	Gait instability	71	Ataxic, mild steppage	+(3)/+	-/-	?	-/-	+/-	+/↓	-		Ambulant (71)
IV.3	?	Gait instability	71	Ataxic	-/-	-/-	+	-/-	+/-	+/↓	-	Cramps	Use of a cane (71)
IV.5	55	Gait instability	65	Ataxic	+(4)/-	-/-	-	-/-	+/-	+/↓	-	I and II toes syndactyly	Ambulant (65)
IV.6[a]	33	Gait instability	67	Ataxic, LL weakness	+(3) and proximal (3)/+	-/-	-	-/-	+/-	+/↓	-	LL paresthesia, UL postural tremor, genu recurvatum	Wheelchair-bound (70)
IV.7	42	Gait instability and LL distal weakness	66	Ataxic	+(4)/-	-/-	-	-/-	+/-	+/↓	-	Cramps	Ambulant (66)
IV.12	35	Gait instability	40	Ataxic	+(4)/-	-/-	+	-/-	+/-	+/↓	-		Ambulant (40)
IV.16	47	Gait instability and LL distal weakness	59	Ataxic	+(4)/-	-/-	+	-/-	+/-	+/↓	-		Ambulant (59)
IV.22	48	Gait instability and LL distal weakness	64	Slightly ataxic	+(4)/+	-/-	+	-/-	↓/-	+/↓	-		Ambulant (64)
IV.25	70	Gait instability	72	Slightly ataxic	+(4)/+	-/-	-	-/-	+/-	+/↓	-		Ambulant (72)
IV.30	40	Gait instability	76	Ataxic	-/-	-/-	+	-/-	+/-	+/↓	-		Ambulant (76)
V.2	50	Gait instability	58	Ataxic	-/+	-/-	+	-/+	+/↓	+/↓	+		Ambulant (58)
V.4	20	Cramps	33	Normal	-/-	-/-	-	-/-	+/+	+/+	-		Ambulant (33)
V.14	35	Mild gait instability	43	Normal	-/+	-/-	+	-/+	+/↓	+/↓	-		Ambulant (43)
V.16	30	Cramps	39	Normal	-/-	-/-	+	-/-	+/↓	+/↓	-		Ambulant (39)
V.18	46	Gait instability	50	Ataxic	+(4)/-	-/-	-	-/-	+/-	+/↓	-		Ambulant (50)
FAMILY 2													
II.1	40	Gait instability	74	Slightly ataxic	?	?	?	?	?	?	?		?
III.1	35	Gait instability	51	Slightly ataxic	+(4)/-	-/-	-	-/-	↓/↓	↓/↓	+	Cramps	Ambulant (51)
III.3[a]	20	Asymmetric feet pain	56	Slightly ataxic	+(4)/-	-/-	-	-/-	↓/↓	↓/↓	+	Cramps	Ambulant (60)
IV.1	40	Feet numbness	40	Normal	-/-	-/-	-	-/-	+/↓	+/↓	-	UL rest tremor, bradykinesia	Ambulant (45)
IV.2	20	Fatigability	23	Normal	-/-	-/-	-	+/+	+/+	+/+	-	Cramps	Ambulant (25)
IV.4	20	Asymmetric feet pain	33	Normal	-/-	-/-	+	-/+	+/↓	+/↓	+	Cramps	Ambulant (40)

Table 1 (continued)

Patients	AAO	Symptoms at onset	ANE	Gait	Weakness (distal MRC)/atrophy LL	Weakness/atrophy UL	Pes cavus	DTR Ach/Knee	Vibration UL/LL	Pinprick UL/LL	Neuropathic pain	Additional features	DSLE (age)
FAMILY 3													
III.1	48	Feet paresthesia	49	Slightly ataxic	+(4)/+	−/−	+	−/−	+/↓	+/↓	+	Cramps	Use of a cane (60)
III.4[a]	46	Feet pain/gait instability	47	Slightly ataxic	+(4+)/+	−/−	+	−/−	+/↓	+/↓	+	Cramps, RLS	Ambulant (57)
FAMILY 4													
II.1	45	Gait instability	73	Slightly ataxic	−/−	−/−	−	−/±	+/−	+/↓	+		Ambulant (73)
III.1	44	Feet numbness/paresthesia	45	Ataxic, mild steppage	+(3)/+	−/−	−	−/+	+/−	+/↓	+	Cramps	Ambulant (45)
III.3	35	R foot numbness and pain	46	Ataxic, mild steppage	+(3)/-	−/−	−	±/±	↓/−	↓/↓	+	Cramps	Ambulant (69)
III.7	59	Gait instability	60	Slightly ataxic	+(4)/+	−/−	+	−/±	+/↓	+/↓	−		Ambulant (76)
III.9[a]	46	Feet numbness/gait instability	48	Ataxic	+(4)/+	−/−	+	−/+	+/↓	+/↓	+	Cramps, RLS	Use of a cane (57)
IV.3	?	Asymptomatic	36	Normal	−/−	−/−	+	+/+	+/+	+/↓	−		Ambulant (36)
IV.4	15	Mild gait instability	19	Normal	−/−	−/−	−	+/+	+/+	+/+	−		Ambulant (23)
FAMILY 5													
I.2	33	Gait instability	67	Wheelchair-bound	+(4)/+	−/−	−	−/−	↓/−	↓/−	+	Cramps, dysautonomia	Wheelchair-bound (58)
II.3	40	Pain and gait instability	60	Ataxic, burning feet	?	?	?	?	?	?	+	?	Ambulant (60)
II.5	34	Gait instability	42	Ataxic	+(4)/-	−/−	−	−/−	↓/−	↓/↓	−		Wheelchair-bound (50)
II.9	20	Running difficulties	54	Slightly ataxic	+(4)/-	−/−	−	−/−	+/−	↓/↓	+		Ambulant (60)
III.3[a]	15	Gait instability	39	Slightly ataxic	+(4+)/-	−/−	+	−/+	+/−	+/+	+	Cramps, RLS	Ambulant (49)
FAMILY 6													
II.3	58	LL Numbness	62	Slightly ataxic	−/−	−/−	−	−/−	+/−	↓/↓	−	Cramps, urinary urgency	Ambulant (62)
II.9	54	Feet numbness/gait instability	62	Ataxic	−/−	−/−	−	−/−	+/−	+/+	−	Cramps, urinary urgency	Ambulant (67)
III.7[a]	42	LL Numbness	46	Ataxic	−/−	−/−	−	−/+	+/↓	+/↓	−		Ambulant (46)
FAMILY 7													
III.2[a]	45	Gait instability	50	Ataxic	+/+	−/−	−	−/−	+/↓	+/↓	−		Ambulant (50)

Table 1 (continued)

Patients	AAO	Symptoms at onset	ANE	Gait	Weakness (distal MRC)/atrophy LL	Weakness/atrophy UL	Pes cavus	DTR Ach/Knee	Vibration UL/LL	Pinprick UL/LL	Neuropathic pain	Additional features	DSLE (age)
FAMILY 8													
III.2[a]	48	Gait instability	53	Ataxic	+(4)/+	−/−	−	−/−	+/↓	+/↓	+		Ambulant (52)
III.8	44	Gait instability	44	Slightly ataxic	−/−	−/−	−	−/−	+/↓	+/+	−		Ambulant (44)

+ present, − absent, ± weak, ↓ diminished, ? unknown, *MRC* medical research council scale for muscle strength, *AAO* age at onset, *Ach* Achilles tendon, *ANE* age at neurological examination, *DSLE* disability status at last examination, *DTR* deep tendon reflexes, *LL* lower limbs, *R* right, *RLS* restless leg syndrome, *UL* upper limbs

[a]indicated the proband for each family

5) appeared, leading to steppage gait and difficulty getting up from a chair. At the age of 55 he started using a cane to walk, at 65 he needed two crutches and at 70 he became wheelchair-bound. Neurological evaluation at the age of 67 showed absence of vibration perception and diminished thermoalgic sensitivity in the lower limbs. Sensory evaluation was normal in the upper limbs. Patellar and ankle reflexes were abolished. Among skeletal deformities genu recurvatum was noted. He had no foot deformities. Other family members were less affected: their clinical picture was dominated by sensory complaints, with onset occurring when they were in their 40s.

Patient III.3 (family 2) had disease onset at around the age of 20 with burning pain in the feet and a tendency to lose her slippers while walking. During the following years, she developed gait instability, which was accompanied by impaired manual dexterity and widespread cramps. At the age of 56, light touch, vibration, heat and pain sensation was markedly reduced in the knees and fingers and she had sensory gait ataxia with positive Romberg sign. DTR were abolished in the lower limbs. There was neither muscle atrophy nor pes cavus. Muscle strength was preserved except for symmetric weakness of ankle dorsiflexion (MRC 4/5). Electromyography was consistent with a length-dependent axonal sensory-motor neuropathy. Due to the suspicion of an underlying acquired cause, mainly because of the presence of severe neuropathic pain, extensive blood tests were performed, which proved normal except for a slight increase in CK level (213 U/L or 3.56 μkat/L). A salivary gland biopsy excluded Sjögren syndrome. As her older sister and their father developed the same complaints, they underwent genetic testing for hereditary neuropathies, which revealed a novel *LRSAM1* variant (c.2093_2104del). A total of 7 affected family members were then identified and they all presented a superimposable clinical picture. All of them were still ambulant without aid after an average of 14 years from disease onset.

Laboratory features

Neurophysiological, laboratory, and pathological findings are shown in Table 2.

Electrodiagnostic data were collected in 33 members of 7 families and were consistent with a length-dependent sensory-motor axonal neuropathy. In all patients, sural SNAPs were reduced or absent, whereas ulnar SNAPs were normal or only slightly impaired. Ulnar MCVs were faster than 38 m/s and amplitudes were preserved in all but 3 patients, in whom they were mildly reduced. Motor involvement in the lower limbs was confirmed by a reduction of peroneal nerve CMAPs and/or signs of distal chronic denervation on needle examination. Three patients presented only a reduced sural sensory amplitude: one was clinically asymptomatic and 2 were slightly ataxic.

Table 2 Neurophysiological, laboratory and pathological findings

	Age	Nerve conduction study (NCS)									EMG	CK (U/L)	Nerve biopsy	Muscle biopsy
		CMAP (mV)		MCV (m/s)		SNAP (µV)		SCV (m/s)						
		Ulnar (W)	Peroneal (A)	Ulnar	Peroneal	Ulnar	Sural	Ulnar	Sural	TA			
NV →		>6	>3	>48	>42	>8	>10	>45	>40		38–176		
FAMILY 1													
IV.3	71	4	–	53	–	1	1	43	39	Neurogenic	ND	ND	ND
IV.6[a]	67	4	–	53	–	1	–	43	–	Neurogenic	ND	ND	ND
IV.7	66	4	1	49	41	1	4	50	36	Neurogenic	ND	ND	ND
IV.12	40	7	1	70	47	4	6	48	39	Neurogenic	ND	ND	ND
IV.22	64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Prominent axon loss and occasional onion bulb-like formations	ND
IV.25	72	4	-	61	45	2	2	48	45	ND	ND	ND	ND
V.2	58	9	4	62	44	1	3	45	28	Neurogenic	ND	ND	ND
V.4	33	9	2	52	47	3	7	51	48	Neurogenic	ND	ND	ND
V.14	43	14	2	63	43	ND	17	ND	36	Neurogenic	ND	ND	ND
V.16	39	8	7	54	49	1	2	40	41	ND	ND	ND	ND
FAMILY 2													
II.1	73	ND	↓	ND	40	ND	–	ND	–	ND	ND	Prominent axon loss and occasional onion bulb-like formations	ND
III.1	51	8	6	53	42	5	6	45	42	Neurogenic	ND	ND	ND
III.3[a]	56	7	2	61	50	4	4	43	44	Neurogenic	213	ND	ND
IV.1	40	8	3	53	43	0	1	–	41	Neurogenic	141	ND	ND
IV.2	23	8	4	50	45	10	8	50	42	Neurogenic	ND	ND	ND
IV.4	33	10	5	62	47	8	5	53	46	Neurogenic	949	ND	ND
FAMILY 3													
III.1	49	7	5	62	47	8	5	53	47	Neurogenic	949–3000	Prominent axon loss and occasional onion bulb-like formations	Neurogenic changes
III.4[a]	47	9	3	56	39	5	3	49	33	Neurogenic	214–246	ND	ND
FAMILY 4													
II.1	73	N	2	N	38	N	↓	N	N	ND	ND	Prominent axon loss and occasional onion bulb-like formations	Normal

Table 2 (continued)

	Age	Nerve conduction study (NCS)								EMG	CK (U/L)	Nerve biopsy	Muscle biopsy
		CMAP (mV)		MCV (m/s)		SNAP (µV)		SCV (m/s)					
		Ulnar (W)	Peroneal (A)	Ulnar	Peroneal	Ulnar	Sural	Ulnar	Sural	TA			
NV →		>6	>3	>48	>42	>8	>10	>45	>40		38–176		
III.1	45	N	↓	N	N	N	↓	N	N	ND	ND	Prominent axon loss and occasional onion bulb-like formations	Neurogenic changes
III.3	46	7	1	52	47	7	1	44	36	Neurogenic	137	ND	ND
III.7	60	N	5	N	47	N	6	N	35	Neurogenic	ND	ND	ND
III.9[a]	48	8	2	55	37	3	3	40	30	Neurogenic	123	ND	ND
IV.3	36	8	5	52	47	12	10	47	46	N	ND	ND	ND
IV.4	19	10	3	52	44	10	9	48	44	N	ND	ND	ND
FAMILY 5													
I.2	67	6	1	55	44	1	0	46	–	Neurogenic	ND	Axonal features	Neurogenic changes
II.5	42	10	4	58	45	8	4	47	35	Neurogenic	ND	Prominent axon loss and occasional onion bulb-like formations	Neurogenic changes
II.9	54	9	1	65	47	4	0	52	–	Neurogenic	ND	ND	ND
III.3[a]	39	12	6	63	45	8	4	52	45	Neurogenic	141	ND	ND
FAMILY 6													
II.3	62	10	5	51	36	9	5	53	ND	Neurogenic	ND	ND	ND
II.9	62	N	↓	N	N	N	↓	N	N	N	359	ND	ND
III.7[a]	42	ND	5	ND	42	3	5	48	38	Neurogenic	ND	ND	ND
FAMILY 8													
III.2[a]	48	8	2	52	40	4	4	ND	37	Neurogenic	ND	ND	ND
III.8	44	5	4	50	40	5	5	ND	31	Neurogenic	ND	ND	ND

↓ diminished, − absent, A Ankle, CMAP compound muscle action potential, CK creatine kinase, EMG electromyography, MCV motor conduction velocity, N normal, ND not done, NCS nerve conduction studies, NV normal values, SCV sensory conduction velocity, SNAP sensory nerve action potential, TA tibialis anterior, W wrist
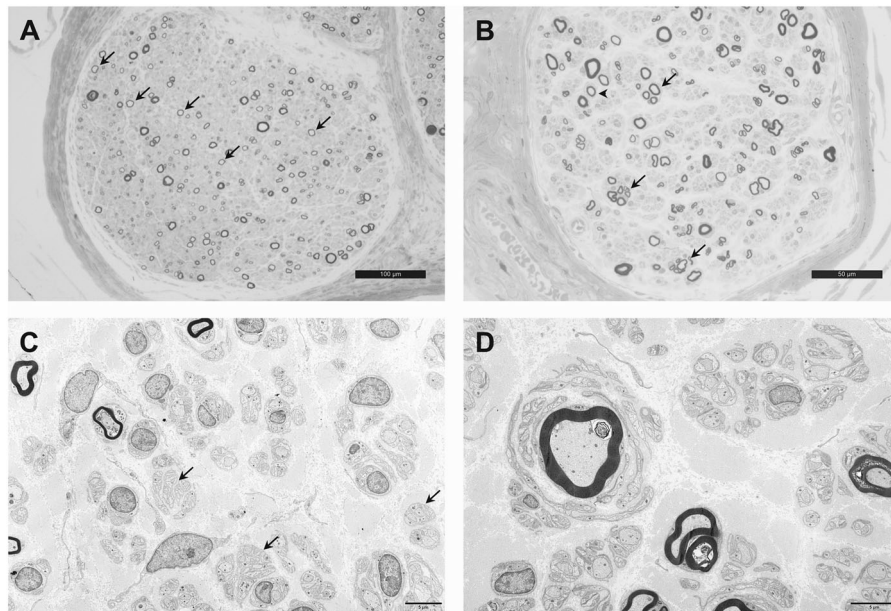
[a]indicated the proband for each family

Fig. 3 Nerve biopsy specimens from two patients. **a, b**: Resin-embedded sample of sural nerve. Transverse semifine section stained with toluidine blue. **a** (Patient III.1, family 4). There is an obvious moderate loss of large and small myelinated nerve fibers. Several of the remaining fibers have a myelin sheath too thin for their axon diameter (arrows). **b** (Patient II.1, family 4). Several clusters of regenerating fibers are observed (arrows). At higher magnification, a myelinated fiber is surrounded by an onion bulb-like formation (arrowhead). **c, d** Electron micrographs. **c** (Patient III.1, family 4). Note the loss of unmyelinated fibers and several stacks of non-myelinating Schwann cell membranes (arrows). **d** (Patient III.1, family 4). A myelinated nerve fiber is surrounded by an onion bulb-like formation composed of a few layers of Schwann cell membranes. Scale bars: **a** = 100 μm; **b** = 50 μm; **c, d** = 5 μm

Among the biological analyses, an elevated CK level was noted in 5 of 9 tested patients (range: 213–3000 U/L), the highest value being recorded in a patient complaining of frequent cramps. Blood tests excluded secondary etiologies of neuropathic pain.

A superficial peroneal nerve biopsy was performed in 7 affected members of 5 different families and showed predominant axonal loss. Reduction of large myelinated fibers, scattered onion bulbs and thin myelin sheaths were frequently encountered in 6 of them. Figure 3 shows nerve biopsy specimens from 2 patients.

All but one of the muscle biopsies showed features that were consistent with neurogenic atrophy.

Discussion

We report the clinical, electrophysiological, laboratory, and histopathological data of 8 unrelated CMT families, comprising 72 patients, with 2 different novel variants of the LRSAM1 gene (c.2093_2104del and c.2104_2133dup) inherited in an autosomal dominant fashion. In view of the presence of a common haplotype of ~450 kb around the LRSAM1 gene in carriers of the c.2093_2104del variant belonging to families 1, 2, 3 and 5, we postulate a common origin for these 4 families as a result of an ancestral founder effect; they likely came from a huge area of western and south-western France, since they are all concentrated there. However, for the other 3 families carrying the c.2093_2104del variant, several haplotype differences were noted in this region indicating that this variant might also occur independently.

The clinical and neurophysiological phenotype was very similar among patients, regardless of the type of variant. Disease onset was in adulthood (39 years on average) with a broad range extending from the second to the eighth decade. The clinical picture was dominated by sensory impairment with slowly progressive gait ataxia and neuropathic pain, respectively noted in 80% and 38% of patients. Mild to moderate motor weakness of the lower extremities was a frequent but marginal complaint except for one patient in whom proximal weakness appeared. The remaining patients complained of sensory disturbances, except for 3 who reported only cramps and one who was asymptomatic. Neurological examination revealed a length-dependent reduction of sensitivity, especially thermoalgic sensitivity, even in the asymptomatic patient. Among bone deformities, the most frequent was pes cavus (39%); genu recurvatum and toe syndactyly were separately noted in 2 patients.

Gait ataxia and sensory loss in the lower extremities were previously reported in 2 CMT2P families with different point mutations in the LRSAM1 gene, inherited in an autosomal recessive and dominant fashion [8, 13], (eTable 2, Supplement). On the other hand, the presence of

pauci- or asymptomatic patients is a possibility in association with point autosomal dominant mutations [10, 12]. However, neuropathic pain has never been described in *LRSAM1*-gene-mutated patients. In general, positive sensory symptoms are not common in CMT, which explains why many of our patients underwent an extensive diagnostic work-up to rule out secondary etiologies. Over the years, the appearance of other late onset familial cases has led clinicians to investigate hereditary causes of neuropathies.

Neurophysiology highlighted a length-dependent axonal sensory-motor neuropathy with a predominant involvement of lower extremity SNAPs. As already reported, in milder phenotypes the only electrophysiological finding could be an isolated reduction of sural nerve amplitude [12].

In line with the literature, the clinical course is generally mild, and most patients are able to walk without aid into later years [9–14]. In our cohort, only 3 patients needed a cane to move about and 3 others were wheelchair bound.

We found a significant rise in serum CK in 5 tested patients belonging to 2 families, who reported many cramps. An increase in CK level has been described in hereditary neuropathies and attributed to muscle denervation and reinnervation [8, 11, 18–20]. Indeed, muscle biopsies performed in our families did not show signs of a primary myopathic process.

Finally, one of our patients (patient IV1, family 2) was affected by early onset levodopa-responsive Parkinson disease. We have excluded other potential genetic causes of Parkinson disease through exome sequencing of patient DNA. Although this finding may remain merely incidental, we deem it worthy of mention considering the lines of evidence linking *LRSAM1* gene to neurodegeneration. In particular, an association between Parkinson disease and neuropathy has already been reported in 3 out of 11 affected family members carrying an *LRSAM1* mutation [9, 21]. LRSAM1 has a role in central nervous system homeostasis, as shown in murine models of Huntington's disease in which an over-expression of the gene was associated with a milder phenotype, probably due to its ability to regulate clearance of Htt protein [22]. On the other hand, the involvement of E3-ligase in Parkinson disease is well known: mutations in the *parkin* gene, which encodes a member of this enzyme family, cause the most common recessive form of genetic parkinsonism [23].

Most of the previously reported mutations truncate, disrupt, or abolish the catalytic RING zinc finger domain [12]. Two different missense mutations affecting Cystein 694 have also been reported [12, 13]. The 2 *LRSAM1* variants detected in our families also involved the RING domain. Both are in-frame changes affecting the final sequence of the RING domain (amino acids 675–710). The first variant (c.2093_2104del), which concerns 7 families, is expected to induce a deletion of 4 amino acids p.(Gln698_Gln701del), while the second one (c.2104_2133dup), in the remaining family, would result in a tandem duplication of a neighboring region of 10 amino acids p.(Pro702_Gln711dup). At protein level, a modification of the structure of the RING domain can be hypothesized, as previously described [7, 9–14].

The autosomal recessive inherited mutation described in the Canadian family leads to the complete loss of function of the protein, which remains undetectable in blood cells of affected members [8]. The remaining autosomal dominant mutations involve the C-terminal RING finger domain of LRSAM1, known to have both dimerization and ubiquitylation functions [7, 9–14]. These mutations to have been shown to abolish this latter property, preventing the essential interaction between LRSAM1 and E2 enzyme UBC13 [7]. In autosomal dominant cases, the mutations likely exert a dominant negative effect, since the clinical severity is comparable, irrespective of the mode of inheritance. A supposed mechanism is the formation of nonfunctional homodimers of wild type and mutated proteins [7]. However, an alternative hypothesis may be a toxic gain of function.

LRSAM1 is an E3-type ubiquitin-ligase highly expressed in adult spinal motor and sensory neurons, with a notable role in neural development and functioning, as demonstrated in zebrafish embryos [9, 24]. Equally, ubiquitination is a fundamental post-translational modification implied in several cellular functions, such as receptor signaling, intracellular trafficking, transcriptional regulation, and cell cycle progression [25]. Although the exact mechanism by which *LRSAM1* mutation induces neuropathy is not fully clarified, there are several lines of evidence linking *LRSAM1* to neurodegeneration [9, 12, 21, 22, 24, 26].

In conclusion, our work increases the number of *LRSAM1* variants and expands the phenotypic spectrum of CMT2P, extending from painful to predominantly sensory ataxic neuropathies. Furthermore, it confirms the importance of the RING domain in the correct functioning of LRSAM1 protein.

Data availability

Genetic data have been submitted to ClinVar (Submission ID: SUB4224652).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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