

Phenotypic variability in autosomal recessive axonal Charcot–Marie–Tooth disease due to the R298C mutation in lamin A/C

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Summary

Autosomal recessive forms of axonal Charcot–Marie–Tooth (ARCMT2) disease are frequent in some areas, such as North Africa and the Middle East, since consanguineous marriages are still common there. Recently, a unique homozygous mutation in *LMNA*, which encodes lamin A/C, a component of the nuclear envelope, was identified in members of three Algerian families with ARCMT2 linked to chromosome 1q21.2–q21.3. In the present study we describe a group of 21 ARCMT2 patients from seven unrelated Algerian families with the same R298C mutation in the lamin A/C gene and marked variability of the clinical phenotype. There is a wide range of age of onset, from 6 to 27 years, with a mean of 14.4 ± 4.6 years. The course of the disease varies considerably from one patient to another. Twelve patients with a disease duration of 10–15 years had a severe CMT phenotype with distal wasting and weakness of all four limbs and areflexia associated with involvement of the proximal lower limb muscles. In

contrast, nine patients had the classical CMT phenotype with mild functional disability without proximal lower limb involvement after a disease duration of 5–18 years. Electrophysiological studies showed a median motor nerve conduction velocity (MNCV) in the normal range in almost all the patients. MNCV and compound muscle action potential (CMAP) values were inversely correlated with the disease duration and the MNCV was strictly related to the CMAP, strongly supporting a pure axonal process without a demyelinating component. Six patients had a nerve biopsy, which revealed severe rarefaction of myelinated fibres in all cases and an increased density of unmyelinated fibres in the majority of cases. In conclusion, the ARCMT2 associated with the R298C mutation differs from other types of ARCMT2. The variability among patients in the age of onset and the course of the disease strongly suggests the action of modifying genes, which remain to be identified.

Keywords: autosomal recessive CMT; lamin A/C gene mutation; phenotypic variability; modifying genes

Abbreviations: ARCMT = autosomal recessive Charcot–Marie–Tooth; CMAP = compound muscle action potential; GDAP = ganglioside-induced differentiation-associated protein; MNCV = motor nerve conduction velocity

Introduction

Charcot–Marie–Tooth disease (CMT) or hereditary motor and sensory neuropathy (HMSN) represents a heterogeneous group of disorders which have been classified according to clinical, electrophysiological, morphological and genetic criteria (Dyck and Lambert, 1968; Harding and Thomas

1980a; De Jonghe *et al.*, 1998). Clinically, it is characterized by distal weakness and atrophy of the limb muscles, mild sensory loss and areflexia.

On the basis of motor nerve conduction velocity (MNCV) in the median nerve, the CMT disorders can be divided into

two main subtypes: demyelinating neuropathy, with MNVC <38 m/s and axonal neuropathy (CMT2), with MNCV >38 m/s (Dyck and Lambert, 1968).

Genetically, four loci for autosomal dominant CMT2 (ADCMT2) have been reported, at 1p (CMT2A), 3q (CMT2B), 8p (CMT2E) and 7q (CMT2F). Specific mutations in two genes have been identified: mutations in the neurofilament-light (*NF-L*) gene (CMT2E) (Mersiyanova *et al.*, 2000), which encodes a member of the intermediate filament (IF) family of proteins, and a mutation in *KIF1B* (CMT2A) (Zhao *et al.*, 2001), which encodes a microtubule motor protein that is a member of the kinesin superfamily of proteins. Mutations in myelin protein zero (*MPZ*), initially reported to cause CMT1B, Dejerine-Sottas disease and congenital hypomyelination, also cause axonal CMT (Chapon *et al.*, 1999; Misu *et al.*, 2000).

Autosomal recessive forms of axonal CMT (ARCMT2) are frequent in some areas, such as North Africa and the Middle East, as consanguineous marriages are still common there.

To date, three loci associated with an autosomal recessive axonal form of CMT (ARCMT) have been mapped: on chromosome 1q21.2 (ARCMT2A) in a large consanguineous Moroccan family (Bouhouche *et al.*, 1999), on 19q13.3 for CMT2B2 (Leal *et al.*, 2001), and on 8q21.3 (Barhoumi *et al.*, 2001). Two genes have been identified. The ganglioside-induced differentiation-associated protein 1 (*GDAP1*) causes axonal ARCMT and is linked to 8q (Cuesta *et al.*, 2002) and, surprisingly, to demyelinating ARCMT (Baxter *et al.*, 2002). Recently, a unique homozygous mutation in LMNA, which encodes lamin A/C, a component of the nuclear envelope, was identified in members of three Algerian families with ARCMT2 linked to chromosome 1q21.2-q21.3 (De Sandre-Giovannoli *et al.*, 2002). The eight patients from these three families and from an additional family have already been described clinically (Chaouch *et al.*, 2003).

In the present study, we describe the clinical, electrophysiological and neuropathological features of a large series of 21 patients from seven new Algerian families with the R298C mutation in the lamin A/C gene in order to analyse the clinical variability of the phenotype.

Patients and methods

Clinical assessment

Twenty-one patients from six families, their parents and most of their healthy siblings were assessed. All patients and the 41 at-risk relatives were examined for the presence of motor and sensory loss, areflexia, foot deformities, scoliosis and other associated signs, such as nerve hypertrophy, tremor, ataxia, pyramidal signs and cranial nerve involvement. Disease severity was evaluated in terms of the ability to walk and run and to use the hands in daily tasks, according to the following scales. For the lower limbs, stage 0 = normal; 1 = normal walking and running but fatigability and cramps; 2 = normal walking, running and jumping impossible; 3 = abnormal

walking without help; 4 = abnormal walking only with simple canes; 5 = abnormal walking, only with crutches; 6 = abnormal walking, only with a walker; 7 = wheelchair-bound; and 8 = bedridden. For the upper limbs, stage 0 = normal; 1 = mild disability with no effect on daily life; 2 = severe disability affecting daily life; 3 = claw hand; and 4 = no movements of fingers.

Ophthalmological and auditory examination, cardiological ECG examination and echocardiography were performed on all the probands and most of the secondary patients.

All subjects gave informed consent to take part in the study which was approved by the Ministry of Health, Health Ethic Committee, Algeria.

Electrophysiological analysis

Nerve conduction studies were performed with surface stimulation and recording electrodes. MNCVs in the median and the peroneal nerves were recorded. Antidromic sensory compound action potential was recorded from the median and sural nerves. Electromyography of the tibialis anterior and the first dorsalis interosseous muscle was performed with a concentric needle electrode.

Pathological study

Six patients from six families underwent superficial peroneal nerve biopsy. For analysis of the nerve biopsy, fascicles of the superficial peroneal nerves were divided into several pieces. One was fixed in formaldehyde (10%) and embedded in paraffin; routine sections were stained using conventional methods. Other fascicles were fixed in buffered glutaraldehyde, processed and embedded in epon. Transverse semithin sections were stained with toluidine blue and ultrathin sections were stained with uranyl acetate and lead citrate and viewed in a Philips CM10 electron microscope. For technical reasons morphometric studies were performed only in five cases.

Genetic study

Blood samples from the 41 individuals were obtained after informed consent. Genomic DNA was extracted using standard procedures. The R298C mutation was detected in the seven families with axonal ARCMT using restriction digestion by *AciI* of a polymerase chain reaction (PCR) fragment containing exon 5 of the lamin A/C gene. The mutation was confirmed by sequencing the exon 5 of each index case after amplification by PCR using previously published primers (De Sandre-Giovannoli, 2002). The 5'3' and 3'5' strands of the PCR products were sequenced with BigDye™ dRhodamine Terminators® (PE Applied Biosystems) on an ABI 377 sequencer and sequence chromatograms were analysed using Autoassembler® software (v. 1.4.0; PE Applied Biosystems). Rapid characterization of the genotypes of relatives was performed by restriction

Table 1 Clinical data

Case	Sex	Age 1 ^a (years)	Age 2 ^b (years)	Duration (years)	Areflexia	Weakness	Amyotrophy	Sensory impairment	Stage of disability		Deformities
									UL	LL	
I-1	M	10	28	18	All limbs	Dist all L	Dist all L	Dist all L	1	4	PEV
I-2	F	12	26	14	All limbs	Dist all L	Dist all L	Dist LL	1	3	PC
I-3	M	9	23	14	LL	Dist all L	Dist all L	Dist LL	1	3	PC
I-4	F	6	12	6	LL	Dist all L	Dist LL	Dist LL	0	3	PC
II-1	F	10	22	12	All limbs	Dist all L/prox LL	Dist all L/prox LL	Dist LL	1	6	PCV
II-2	M	16	28	12	All limbs	Dist all L/prox LL	Dist LL	Dist all L	1	4	PCV
II-3	F	12	30	18	All limbs	Dist all L/prox LL	Dist all L	Dist all L	1	7	PC/scoliosis
II-4	M	8	15	7	LL	Dist all L	Dist all L	Dist LL	0	3	PC/scoliosis
III-1	M	15	33	18	All limbs	Dist all L/prox LL	Dist all L/prox LL	Dist all L	2	4	PC
III-2	M	17	35	18	LL	Dist all L	Dist all L	Dist LL	1	3	PC
III-3	M	13	23	10	LL	Dist all L	Dist all L	Dist LL	0	3	PC
IV-1	M	17	29	12	All limbs	Dist all L/prox LL	Dist all L/prox LL	Dist LL	1	4	PC/ hyperlordosis
IV-2	M	18	38	20	All limbs	Dist all L/prox LL	Dist all L/prox LL	Dist all L	2	6	PC
IV-3	M	18	45	27	All limbs	Dist all L/prox LL	Dist all L/prox LL	Dist all L	2	7	PC
V-1	F	13	18	5	LL	Dist LL	Dist all L	Dist LL	0	3	PC/scoliosis
VI-1	F	18	36	18	All limbs	Dist all L/prox LL	Dist all L/prox LL	Dist LL	1	5	PC
VI-2	M	17	44	27	All limbs	Dist all L/prox LL	Dist all L/prox LL	Dist LL	1	5	PC
VI-3	M	14	33	19	All limbs	Dist all L/prox LL	Dist all L/prox LL	Dist LL	1	5	PC
VI-4	F	15	32	17	LL	Dist all L/prox LL	Dist all L/prox LL	Dist LL	0	3	PC
VII-1	F	27	41	14	LL	Dist all L/prox LL	Dist all L/prox LL	Dist LL	1	4	PC
VII-2	M	17	35	18	LL	Dist all L/prox LL	Dist all L/prox LL	Dist LL	1	4	PC

^aAge at onset; ^bAge at last clinical examination. L = limbs; LL = lower limbs; Dist = distal; prox = proximal; PC = pes cavus; PCV = pes cavus varus; PEV = pes equinovarus.

endonuclease digestion of the exon 5 PCR fragment with *AclI*.

Statistical analysis

Means were compared using Student's *t* test. The difference was considered significant at $P < 0.05$. Correlation studies were performed using regression analyses following three models: linear, exponential and logarithmic. The model with the maximum coefficient of correlation was taken into account. An intra- and interclass correlation analysis of age at onset was performed to study sibship resemblance and analysis of variance.

Results

Clinical data

The overall clinical features are summarized in Table 1. Consanguinity was found in families II, IV, V and VII. In families I and III, the parents were not known to be related but were both born in the same village. As in many neurodegenerative diseases, the true onset of symptoms was difficult to estimate. The first symptom described by the patients was difficulty in running and walking properly, the foot drop appearing some months later. The mean age of onset was 14.4 ± 4.6 years, with a wide range, from 6 to 27 years. The mean age at onset within families varied from 9.5 ± 2.5 years (family I) to 22 ± 7 years (family VII). The data in Fig. 1 strongly suggest that the difference in age at onset was larger between families than within a family. Variance analysis demonstrated that the mean of age at onset

was statistically different between siblings ($P < 0.01$). The intraclass coefficient of correlation, which measures the resemblance between siblings, was 0.63 ($P < 0.01$).

Duration of the disease was defined as time between age of onset (Age 1 in Table 1) and age at last clinical examination (Age 2 in Table 1). The mean duration of the disease was 15.4 ± 5.8 years, with a range from 5 to 27 years. The sex ratio (male : female) of the patients was 0.61. There were no statistical differences between the sexes in age at onset ($P = 0.87$) or in other clinical features (data not shown) were found. All the patients had distal weakness and amyotrophy of the lower limbs. The vast majority of them (18/21) also had weakness and amyotrophy of the upper limbs. Eleven patients also had proximal deficit and wasting of the lower limbs, but not of the upper limbs. Areflexia of the four limbs was present in 12 patients. The presence of weakness and amyotrophy of the proximal lower limbs was statistically related to disease duration ($P = 0.015$ and $P = 0.011$, respectively). In the vast majority of patients (18/21), there was parallel topographic progression of the muscular deficit and amyotrophy, which could be divided into three stages. (i) In the first 5 years the patients presented weakness and wasting in the tibialis anterior and the fibularis longus with a steppage gait; in the upper limbs slight amyotrophy was present on the first interosseous and opponens pollicis, without deficit. (ii) After 6–10 years of disease progression, we noticed an extension of the deficit and amyotrophy to the posterior leg muscles and the proximal lower limb muscles, leading to difficulty in standing up and to a waddling gait associated with the

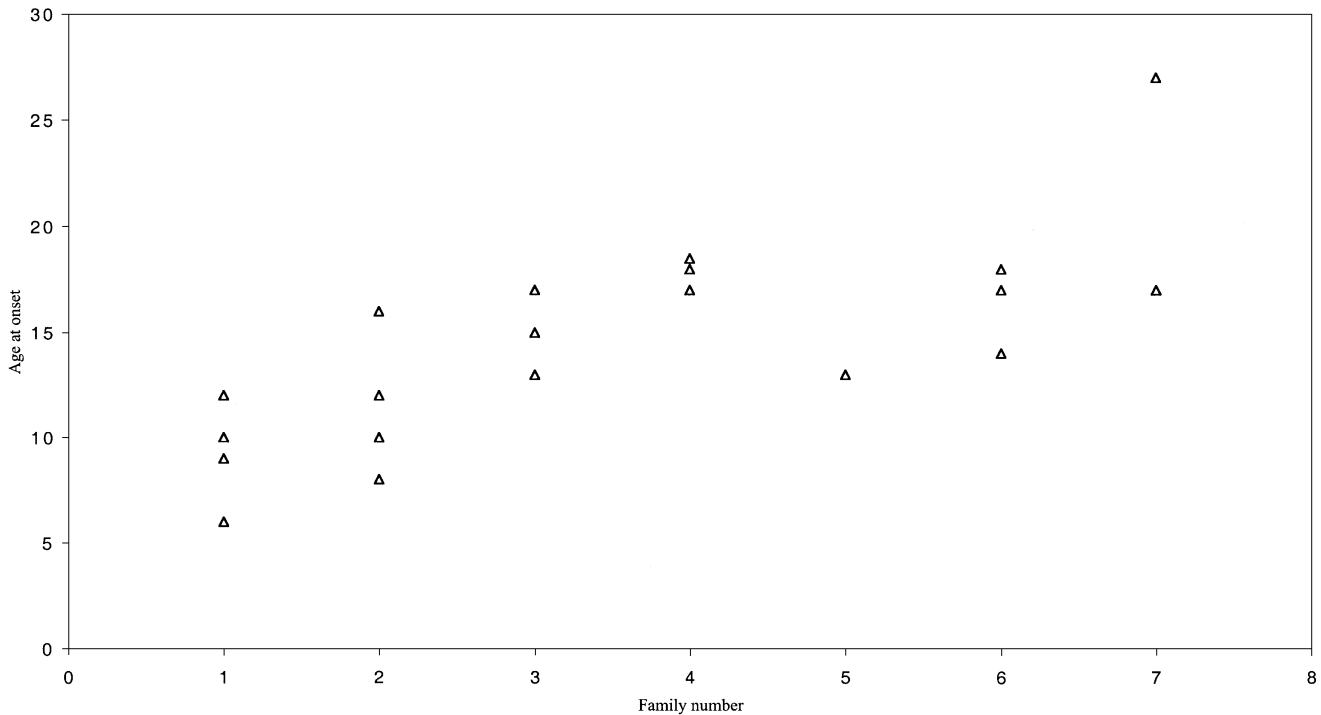


Fig. 1 Age at onset (years) in siblings with ARCMT associated with the R298C mutation in lamin A/C from the seven families. Each symbol represents a sibling.

steppage gait. In the upper limbs we noticed progression of wasting to the anterior and posterior forearm muscles. (iii) After 11–28 years of disease progression, the only proximal upper limb muscle to be affected was the triceps brachii. The muscles already affected were weaker but we noticed that the flexor and extensor muscles of the hands remained stronger than the other muscles of the hands. This allowed the patients to continue in their jobs (two of them were hairdressers), although they could not walk without aid. All patients presented sensory impairment of all modalities with a stocking distribution; only six showed a glove distribution. These latter had at least 12 years of disease duration, areflexia and a disability score ≥ 1 for the upper limbs. Deformities of the feet were present in all patients. Moderate scoliosis or hyperlordosis was observed in only four patients (19%), who did not have a particularly long disease duration or severe proximal deficit.

The functional disability was severe in seven cases (stages 5–7), moderate (stage 4 disability in the lower limbs, 1 or 2 in the upper limbs) in six cases, and mild (stage 3 disability in the lower limbs, 0 or 1 in the upper limbs) in eight cases (Table 1). No asymptomatic carriers (disability score = 0 for lower and upper limbs) were identified by molecular diagnosis. Functional disability in the lower limbs was statistically related to the disease duration ($P = 0.007$). All patients with the highest disability scores (>4) in the lower limbs also had proximal muscle involvement.

Despite a statistical link between functional disability and disease duration, the course of the disease was very hetero-

geneous at the individual level (Fig. 2). Indeed, after 18 years of disease duration, patient III-3 was still at stage 3, whereas patient II-3 had a disability score of 7. Moreover, among the seven patients with a disability score of 3 for the lower limbs, the affected individual V-1 had a disease duration of 5 years and III-3 had a disease duration of 18 years (Fig. 2). Only five patients with a mild disability (score 3 for the lower limbs) had a disease duration of more than a decade. However the majority of adult patients had a nearly normal life in the first decade, as they were independent and continued in their job. At the end of the second decade disability was more severe. Most of them quitted their job but remained independent at home. There were two wheelchair-bound patients after 18 and 27 years of disease progression. There was also clinical heterogeneity within families in terms of severity of the disease. For instance, in family VI the four cases had an onset between 14 and 18 years. Patient VI-4 was still walking without aid after 17 years of disease duration (disability score = 3), whereas her brother, case VI-3, could not stand up alone, was walking with great difficulty, with aid, and had proximal lower limb weakness (disability score = 5) after 19 years of disease duration. The audiological, ophthalmological and cardiac systems were normal in all the patients assessed.

Electrophysiological findings

The electrophysiological results are summarized in Table 2. The MNCV in the median nerve was in the normal range in

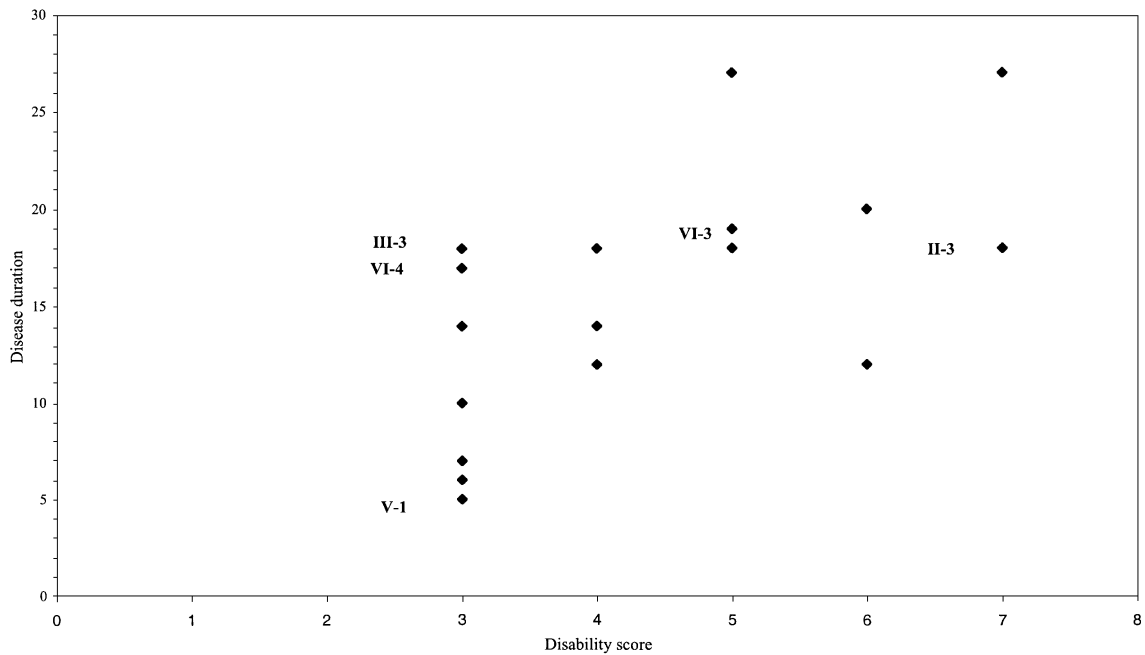


Fig. 2 Disease durations (years) for patients with the same disability score (from 0 to 8). Each symbol represents a patient. Cases III-2, V-1 and II-3 are discussed in the Results section.

Table 2 Electrophysiological data

Case	Median nerve					Peroneal nerve		
	MNCV (m/s)	DL (ms)	CMAP (mV)	SNCV (ms)	SNAP (µV)	MNCV (m/s)	DL (ms)	Amp (mV)
I-1	55	3.5	4	NR		44	6	0.68
I-2	50	4	7	50	10	35	10	4
I-3	57	4.4	5	NR		36	4	2
I-4	58	3.6	5	49		37	7	7
II-1	64	4	3	NR		NR		
II-2	55	3.5	2	NR		NR		
II-3	60	3	4	52	4	40	4.6	0.5
II-4	64	4	3	NR		47	5	0.7
III-1	52	4.5	3	NR		32	5	0.4
III-2	54	4	10.8	NR		40.5	6.6	0.32
III-3	60	3.5	9.6	NR		43	6	0.16
IV-1	44	4	0.9	NR		37.5	7	0.25
IV-2	40	4.5	0.7	NR		NR		
IV-3	40	4.7	0.6	NR		NR		
V-1	51	5	2	NR		30	5.7	0.5
VI-1	51	4.4	1.5	NR		NR		
VI-2	49	4	1	NR		NR		
VI-3	47	4.5	1.7	NR		NR		
VI-4	50	3.5	2	NR		NR		
VII-1	61	3	3.7	NR		34	5	0.9
VII-2	58	3.5	3	NR		32	5.8	0.5

Amp = amplitude; CMAP = compound motor action potential; DL = distal latency; MNCV = motor nerve conduction velocity; NR = not recorded; SNAP = sensory nerve action potential; SNCV = sensory nerve conduction velocity.

all patients except those in family IV, in which MNCV of the three affected members (IV-1, IV-2 and IV-3) was below the lower limit used in our laboratory, but above the threshold value of 38 m/s separating demyelinating from axonal CMT. MNCV values were inversely correlated with disease

duration, as shown in Fig. 3A ($r = 0.53, P < 0.02$). The distal latencies of the median nerve were normal or subnormal (3.96 ± 0.55 ms). The CMAP of the median nerve was recorded in all patients and had a large range (from 0.6 to 10.8 mV). CMAPs were also inversely correlated with

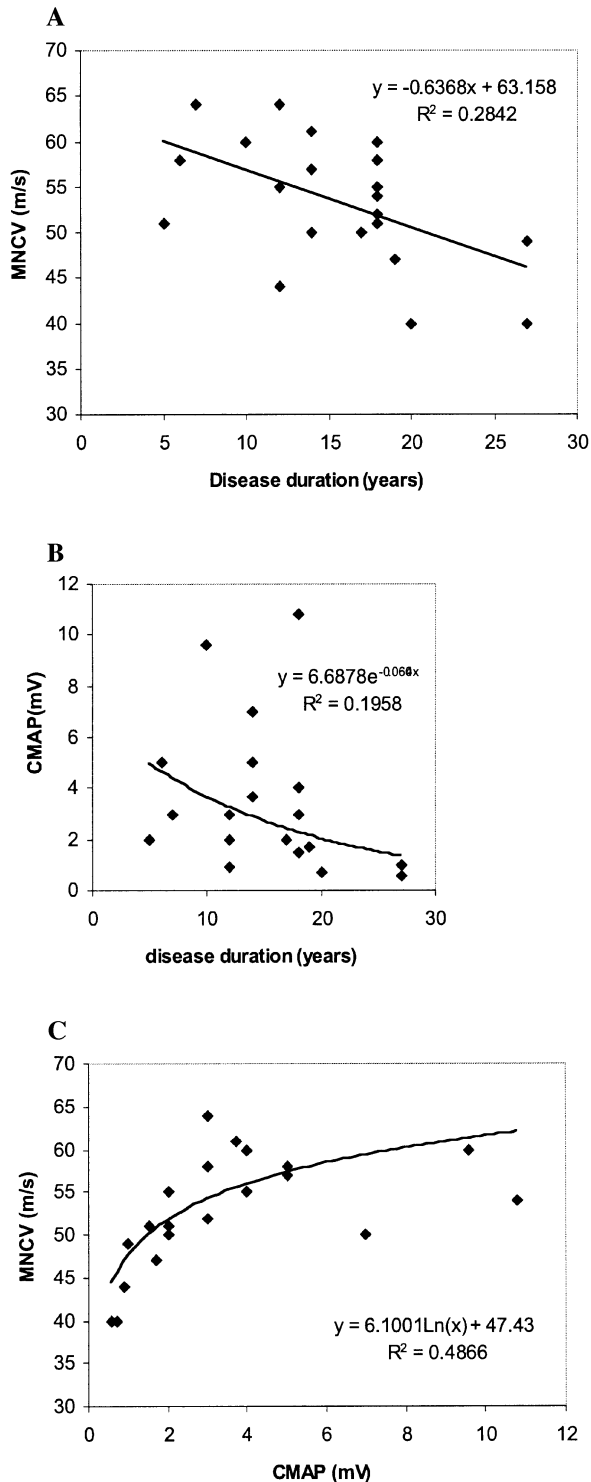


Fig. 3 Scattergrams with regression analysis between median nerve MNCV (m/s) and disease duration (years) (A), CMAP (mV) and disease duration (years) (B) and median MNCV and CMAP (C).

the disease duration (Fig. 3B; $r = 0.44$, $P < 0.05$), with the lowest values for patients IV-2 (0.7 mV) and IV-3 (0.6 mV) after 20 and 27 years of disease duration respectively. The

MNCVs were strictly related to the CMAP (Fig. 3C; $r = 0.69$, $P < 0.069$), strongly supporting a pure axonal process with no demyelinating component. The peroneal motor nerve potential was not recordable in eight cases. Peroneal MNCVs were decreased in 10 cases (normal values, 41–49 m/s). Amplitude was low in 11 cases (< 2.5 mV). In three cases MNCVs were in the normal range but amplitudes were decreased. A median nerve sensory action potential (SAP) was not obtained in 19 patients. Sural SAP was absent in all cases except patient II-4, the youngest patient of family II, in whom the sural nerve conduction velocity and amplitude were reduced (32 m/s and 6 μ V, respectively).

Electromyographic recording suggested a chronic neurogenic pattern in all cases. All these data are in favour of motor and sensory axonal neuropathy.

Pathological results

Light microscopy

The lesions were the same in all patients (I-3, II-3, III-3, IV-1, V-1 and VI-1). The number of myelinated fibres was severely reduced. Large-diameter fibres were almost totally lacking (Fig. 4). Active axonal degeneration was very exceptional. There was no evidence of demyelination or remyelination, or of onion bulb formation. Clusters of myelinated fibres were not present. Single teased fibres did not show any abnormality. No lesions were seen in the blood vessels or perineurium. Endoneurial connective tissue was increased.

Electron microscopy

These pathological aspects were confirmed by electron microscopy. The compaction of the myelin sheaths seemed normal in the remaining fibres. There were no demyelinated fibres. Few bands of Büngner were present. Most of the unmyelinated fibres seemed normal. There was no abnormality of the Schwann cell nuclei.

Morphometry

The density of myelinated fibres was statistically diminished in all six nerves. The density of unmyelinated fibres was significantly increased in four out of five cases (Fig. 5).

DNA diagnosis

Since the only defect in the LMNA/C gene reported so far in CMT patients is the R298C mutation, we searched for it systematically in the index cases from families with axonal ARCMT using PCR–restriction site analysis of exon 5 of LMNA/C with the *AciI* restriction enzyme, since this mutation creates or abolishes a restriction site. The mutation was detected in all seven families, and this was confirmed by direct sequencing in index cases. At-risk relatives were also tested. All tested patients were homo-

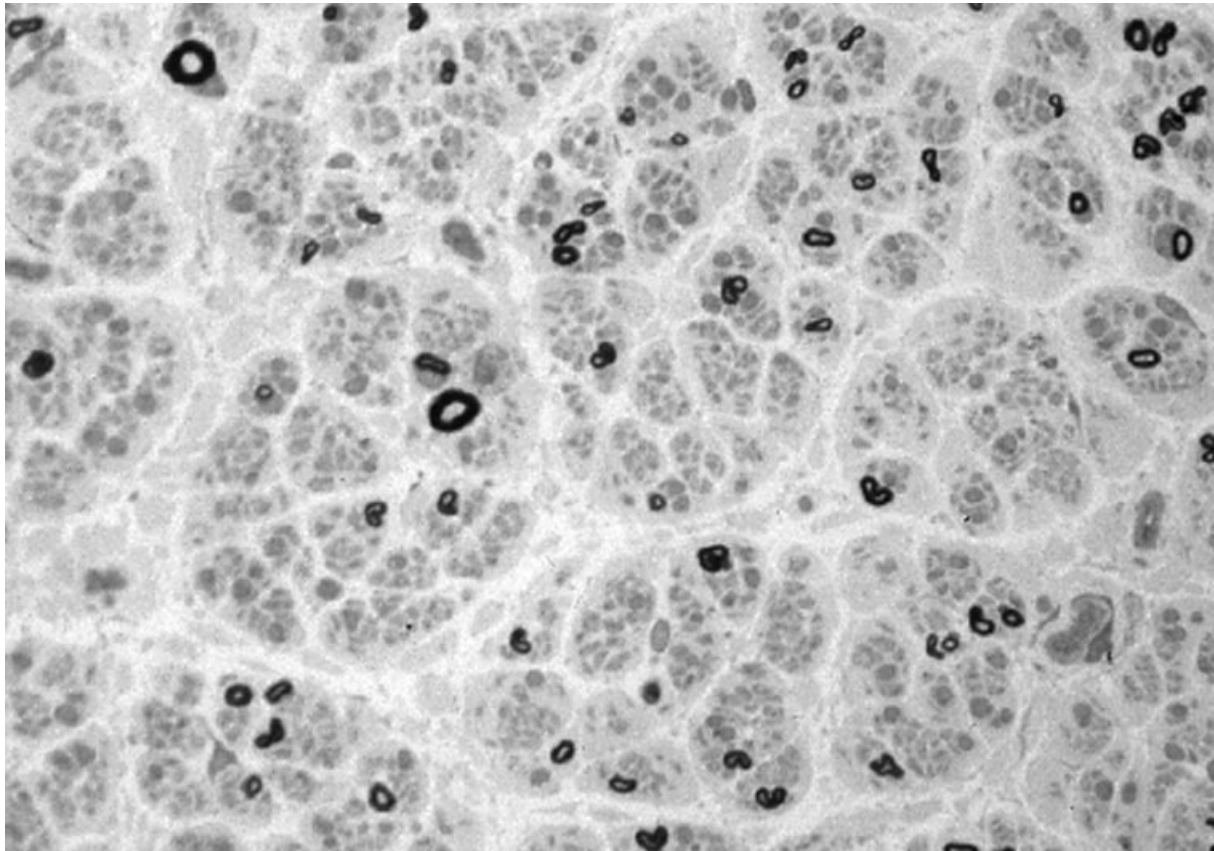


Fig. 4 Very severe rarefaction of large myelinated fibres. No clusters are seen. Semithin section. Original magnification $\times 20$.

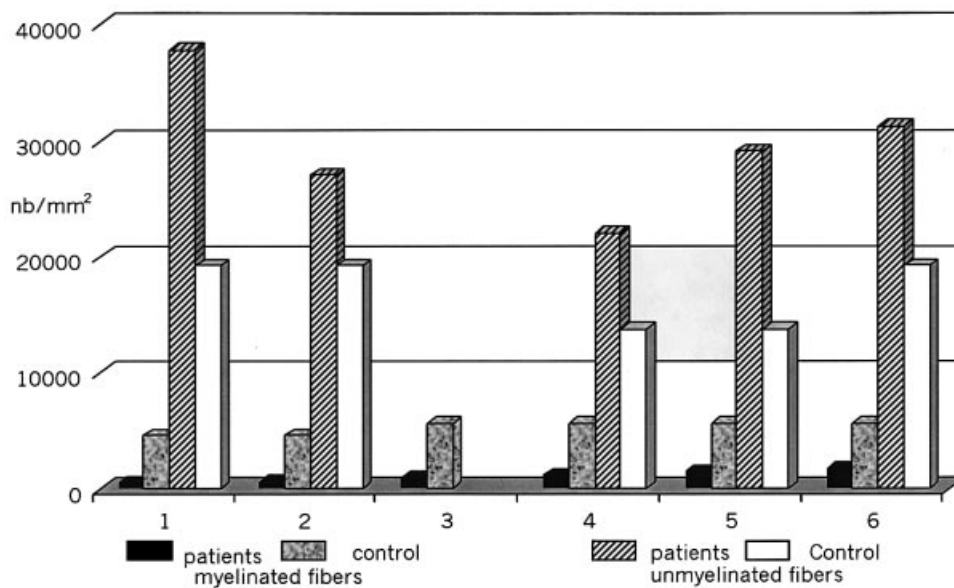


Fig. 5 Morphometry. The density of myelinated fibres was statistically diminished in all six nerves. As can be seen in the histogram, large fibre loss is prominent. The density of unmyelinated fibres is increased, statistically in five out of six cases.

zygous for this mutation and none of the 17 heterozygotes (including the eight available parents) developed any clinical

or electrophysiological signs of peripheral neuropathy or myopathy.

Discussion

There have been few clinical reports of axonal or demyelinating autosomal recessive CMT since the first description of six families by Harding and Thomas (1980b), two families by Ouvrier (1981) and 17 cases by Gabreels-Festen *et al.* (1991). These authors emphasized the severity of the clinical picture. The first locus associated with ARCMT2 was mapped to chromosome 1p21 in a large consanguineous Moroccan family with nine affected siblings, who presented a severe motor and sensory neuropathy with proximal muscle involvement in some cases (Bouhouche *et al.*, 1999). Recently, the unique R298C mutation in the *LMNA* gene encoding the lamin A/C nuclear envelope protein was identified in five patients from three Algerian ARCMT2 families with linkage to the 1q21–3 region (De Sandre-Giovannoli *et al.*, 2002). Their phenotype, as well as that of three additional patients, was also severe, with rapid evolution and involvement of the proximal lower limb, except in three cases with a disease duration of ≤ 7 years (Chaouch *et al.*, 2003). The present series, with a greater number of families and patients, a longer disease follow-up and a complete nerve biopsy study, makes it possible to define the phenotype of this form of ARCMT and to show its great variability in terms of the involvement of proximal muscles, evolution and severity. Variability of the age at onset within families has been demonstrated. The disease appeared earlier in the youngest siblings in most families. One of the reasons is that parents and children became more experienced with the disease and noticed the trouble earlier in the youngest siblings.

The involvement of the proximal muscles of the lower limbs, which determines the loss of walking, was very frequent (13/21) in the ARCMT2 associated with mutation of lamin A/C. This feature is very rare in CMT, having been considered as an exclusion criterion by some authors (De Jonghe *et al.*, 1998). However, some cases with autosomal dominant CMT2, ARCMT2 or ARCMT1 were reported with involvement of the proximal muscles of the lower limb (Gabreels-Festen *et al.*, 1991; Quattrone *et al.*, 1996; Kessali *et al.*, 1997; Salih *et al.*, 2000). The other types of axonal ARCMT associated with known loci or genes have different presentations. Leal *et al.* (2001) described a second locus for axonal ARCMT on 19q13.3 in a large inbred Costa-Rican family with a late age of onset (in the third decade) and a mild phenotype without proximal involvement. In contrast, early onset (before the age of 3 years) seems to be a constant for ARCMT caused by mutations in *GDAP1* (Baxter *et al.*, 2002; Cuesta *et al.*, 2002; Nelis *et al.*, 2002; Azzedine *et al.*, 2003; Birouk *et al.*, 2003; Boerkoel *et al.*, 2003; De Sandre-Giovannoli *et al.*, 2003; Senderek *et al.*, 2003). The patients with the axonal form, described in Spanish families, had a hoarse voice. Barhoumi *et al.* (2001) and Cuesta *et al.* (2002) reported patients with axonal ARCMT and pyramidal signs in a Tunisian family with linkage to 8q21, which contains the *GDAP1* gene. These data are in favour of a phenotype–genotype correlation in axonal ARCMT.

There are only a few studies of nerve biopsies in autosomal recessive CMT2 (Ouvrier *et al.*, 1981; Gabreels-Festen *et al.*, 1991; Thomas *et al.*, 1999; Barhoumi *et al.*, 2001; Cuesta *et al.*, 2002). As in our cases, these authors report that myelinated fibres are severely reduced in number and large-diameter fibres are affected to a greater extent than small-diameter fibres. There is no evidence of demyelination and remyelination, or of active axonal degeneration. Clusters of axons indicative of regeneration following axonal degeneration were seen in a few nerves of the patients (Ouvrier *et al.*, 1981). In contrast, as in our cases, the other authors found no cluster. As suggested in a previous report (Gabreels-Festen *et al.*, 1991), with respect to the pathogenetic mechanisms, the extensive lack of large-diameter fibres without appreciable signs of fibre degeneration and no cluster formation could be linked to a prenatal disturbance in maturation of peripheral motor and sensory neurons. The significant increase in unmyelinated fibres could be explained by the fact that some of them should have become myelinated fibres.

The main fundamental question concerning the mutations of the lamin A/C is the wide variability of their expression at the clinical level. First, in addition to ARCMT2, the mutations of lamin A/C can also result in limb girdle muscular dystrophy type 1B (Muchir *et al.*, 2000), dominant or recessive Emery–Dreifuss muscular dystrophy (Bonne *et al.*, 1999; Raffaele Di Barletta *et al.*, 2000), dilated cardiomyopathy (Brodsky *et al.*, 2000), familial partial lipodystrophy (Cao *et al.*, 2000) and, more recently, mandibulo-acral dysplasia (Novelli *et al.*, 2002). This suggests the existence of distinct functional domains in lamin A/C that are essential for the maintenance and integrity of different cell lineages. Until now, the R298C mutation was restricted to the CMT phenotype. However, a recent report of patients with lamin A/C mutations presenting with lipodystrophy in combination with cardiac and/or skeletal muscle abnormalities (Van der Kooi *et al.*, 2002) and other reports of patients with CMT associated with cardiomyopathy (Dyck *et al.*, 1987; Battistella *et al.*, 1988; Sevillano Fernandez *et al.*, 1994) or association of CMT with adipose tissue abnormalities (Enzi *et al.*, 1985) could suggest a possible common pathway. None of the patients in our series had cardiac or fat tissue abnormalities. However, careful cardiac evaluation with Holter monitoring is needed in all cases of ARCMT2 with the lamin A/C mutation because of the risk of cardiomyopathy concomitant with conduction disturbances resulting in heart failure and sudden death (Becane *et al.*, 2000). Thus, a complete investigation of skeletal muscle, cardiac function and adipose tissue in patients with these mutations, associated with experimental studies, will be helpful in elucidating the mechanisms which cause these disorders.

In this study, we had the opportunity, which is rare in the field of human genetics, to study the variability of the phenotype in a group of 21 ARCMT2 patients with the same R298C mutation in the lamin A/C gene. We found a wide range of age at onset, which was statistically lower within

than between families (Fig. 1). Moreover, the course of the disease was quite variable from one patient to another. Twelve patients with a disease evolution from 10 to 27 years had a severe CMT phenotype with distal wasting and weakness of the four limbs associated with involvement of the proximal lower limb muscles and with a high disability score in the lower limbs (≥ 4). In contrast, nine patients had the classical CMT phenotype with mild functional disability and without proximal lower limb involvement after 5 to 18 years of disease evolution. All these data are in favour of the interaction of the major gene LMNA/C with regulator genes or modifying genes, for which few examples are available among human inherited diseases (Nadeau, 2001). These modifying genes can affect penetrance, dominance, expressivity (as in the case of ARCM2) and pleiotropy. Two alternative approaches can be applied to identify these genes: (i) a genome scan or a candidate gene strategy in a large number of families with ARCM2 associated with the R289C mutation in lamin A/C gene, in which patients are carefully examined; and (ii) the construction of a R298C knock-in mouse model on various genetic backgrounds. This latter strategy was first applied for cystic fibrosis and led to the localization of a modifying gene for the occurrence of meconium ileus (obstruction of intestine by mucus in the fetus) (Rozmahel *et al.*, 1996). The syntenic locus was incriminated secondarily in humans (Zielenski *et al.*, 1999).

In conclusion, the type of ARCM2 associated with the R298C mutation differs from other types of ARCM2. This disorder is an axonal peripheral neuropathy clinically characterized by an age at onset in the second decade, involvement of proximal muscles after the first decade of evolution, and a severe course. The age at onset and the course of the disease are different among patients, strongly suggesting the action of modifying genes, which remain to be identified.

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