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REVIEW ARTICLE

Molecular Genetics of Autosomal-Recessive Axonal Charcot-Marie-Tooth Neuropathies

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Abstract

Autosomal-recessive forms of Charcot-Marie-Tooth (ARCMT) account for less than 10% of the families with CMT. On the other hand, in countries with a high prevalence of consanguinity this mode of inheritance accounts, likely, for the vast majority of CMT phenotypes. Like dominant forms, autosomal-recessive forms are generally subdivided into demyelinating forms (autosomal-recessive CMT1: ARCMT1 or CMT4) and axonal forms (ARCMT2). Until now, demyelinating ARCMT were more extensively studied at the genetic level than the axonal forms. Although the latter are undoubtedly the rarest forms among the heterogeneous group of CMT, three distinct forms have been genetically mapped and recent studies in the past 4 yr provided evidence that their respective causing genes have been characterized. Indeed, gene defects in encoding A-type lamins *(LMNA)*, encoding Ganglioside-induced Differentiation-Associated Protein-1 *(GDAP1)* and encoding the mediator of RNA polymerase II transcription, subunit 25 homolog *(MED25)* have been identified in ARCMT2 subtypes. Given the clinical, electrophysiological and histological heterogeneity of CMT2, it is likely that unreported forms of ARCMT2, related to novel genes, remain to be discovered, leading to an even more complex classification. However, our goal in this review is to provide the reader with a clear view on the known genes and mechanisms involved in ARCMT2 and their associated phenotypes.

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Index Entries: Axonal; Charcot-Marie-Tooth; CMT; *GDAP1*; Lamin; *LMNA*; recessive.

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Introduction

Charcot-Marie-Tooth disease (CMT) constitutes a large group of hereditary motor and sensory peripheral neuropathies, mainly characterized by its phenotypic and, above all, genetic heterogeneity. Dyck and Lambert (1968a,b) first noticed this heterogeneity, suggesting a distinction, on an electromyographic and pathological basis, between two main subtypes of hereditary motor and sensory neuropathies (HMSN) types I and II. This distinction between the demyelinating CMT1 type (HMSN type I) and the axonal CMT2 type (HMSN type II) is still used in the CMT nosological context. A third CMT type, called intermediate CMT that includes pathological features intermediate between the first two CMT types, with moderate reduction of motor nerve conduction velocities (MNCVs), has been added more recently. However, this classification has evolved and integrates, as an essential component, the molecular basis of each CMT subtype known to date. All the mendelian modes of inheritance have been described in CMT, including autosomal-dominant (AD) and -recessive (AR) forms of CMT1 and CMT2 and X-linked forms (for review *see* Shy, 2004; and other articles in this issue). Clinical and molecular features of CMT forms, as well as a classification, will be addressed in other articles of this issue and will not be detailed here. Nonetheless, three important points have to be raised:

1. In all series of patients reported to date, a very large number of sporadic cases makes it impossible to classify *a priori* these patients as being affected with AD, AR, or X-linked forms of CMT.
2. Although in recent years our knowledge of the molecular pathophysiology of CMT2 has dramatically increased, most of the explored and identified molecular defects involved in CMT are related to demyelinating forms.
3. Finally, while ARCMT represent only 10% among the whole CMT population, consanguineous unions are a clear basis underlying ARCMT, thus accounting for the vast majority of families in countries where culture and traditions make these unions frequent.

In this article, we specifically focus on the AR axonal forms of CMT (ARCMT2). Although this category overlaps somewhat with intermediate and demyelinating CMT, our goal is to provide the readers with a clear view of specific axonal neuropathies caused by mutations corresponding to defined loci and/or known genes.

ARCMT2 is reported as a rare and severe condition (Gemignani and Marbini, 2001; Vallat et al., 2004, 2005) and was first reported from a clinical and genealogical point of view in 1968 (Dyck and Lambert 1968a,b). Until recently, this condition was reported in isolated families (Harding and Thomas, 1980; Ouvrier et al., 1981; Gabrëels-Festen, 1991; Bouhouche et al., 1999; Bahroumi et al., 2001; Leal et al., 2001). Although different genes, whose mutations are mainly responsible for other severe neurological disorders, have been occasionally involved in AR CMT2-like forms such as spinal muscular atrophy type (SMN) 1 (Bouhouche et al., 2003; Rudnig-Schoneborn et al., 2003) or giant axonal neuropathy (GAN) (Nafe et al., 2003), genes for three distinct and specific ARCMT2 loci have been identified on chromosomes 8q (Ben Othmane et al., 1993; Barhoumi et al., 2001), 1q (Bouhouche et al., 1999), and 19q (Leal et al., 2001). For these three specific chromosomal localizations, *LMNA* (De Sandre-Giovannoli et al., 2002), *GDAP1* (Baxter et al., 2002; Custa et al., 2002), and the very recently reported *MED25* (Rautenstrauss et al, 2005a) genes, have been demonstrated to harbor disease-causing mutations (Table 1).

The identification of *LMNA* and *GDAP1* mutations has, however, changed our view of ARCMT2, leading us to better understand not only this specific group by itself, but also the possible pathophysiological links between previously distinct groups of CMT and between these forms and related phenotypes. In this respect, both *GDAP1*- and *LMNA*-related CMT forms constitute models, enabling to increase understanding of the causes of clinical and genetic heterogeneity in CMT.

Lamin A/C-Related Autosomal-Recessive CMT (ARCMT2A/CMT2B1)

In 1999, Bouhouche and colleagues (1999) reported a large consanguineous Moroccan family with CMT2, including nine affected sibs, showing linkage to 1q21.2-q21.3 in this family. Later in 2002, using a homozygosity mapping strategy in inbred Algerian families with ARCMT2, De Sandre-Giovannoli and colleagues (2002) established a linkage to chromosome 1q21.2-q21.3 in two additional and

Table 1
Classification of ARCMT2

Reference	Chromosomal localization	Gene	Denomination	OMIM symbol	OMIM no.
Bouhouche et al. (1999)	1q21.2				
De Sandre-Giovannoli et al. (2002)		*LMNA*	ARCMT2A	CMT2B1	605588
Leal et al. (2001)	19q13.3				
Rautenstrauss et al. (2005)		*MED25*	ARCMT2B	CMT2B2	605589
Ben Othmane et al. (1993, 1998)	8q21.1				
Baxter et al. (2002)		*GDAP1*		CMT4A	214400
Cuesta et al. (2002)		*GDAP1*		CMT2 + VCP, AR	607706
Nelis et al. (2002); Senderek et al. (2003)		*GDAP1*		CMTRIA	608340
Birouk et al. (2003)		*GDAP1*		CMT2K (AR)	607831
Bahroumi et al. (2001)	8q21.3 (overlap with *GDAP1* locus)		CMT4C2	CMT2H	607731

unrelated families. All patients shared a common homozygous ancestral haplotype, and the marker *D1S2721* detected a rare allelic variant that was in linkage disequilibrium. These data were suggestive of a founder mutation as the cause of the phenotype. Interestingly, both critical linkage intervals from the Moroccan and Algerian families were found to partially overlap, and, owing to common clinical features in all linked families, it was suggested that a unique gene was involved in the ARCMT2 disease segregating in the families. Moreover, owing to linkage disequilibrium, we hypothesized that the disease-causing gene mapped near or at *D1S2721* (De Sandre-Giovannoli et al., 2002).

Candidate genes within the critical interval were sequenced entirely. *LMNA* was regarded as a strong candidate in ARCMT2 linked to chromosome 1q21, owing to its location within the linkage-disequilibrium region, its expression pattern during neuronal development (Pierce et al., 1999), and the fact that it encodes Lamins A/C, major components of the nuclear lamina (Fig. 1). As well, Lamins A/C belong to the intermediate filaments multigenic family, also including the neurofilament light chain, in which mutations cause CMT2E (Mersiyanova et al., 2000; De Jonghe et al., 2001; Jordanova et al., 2003; Züchner et al., 2004a). The wide spectrum of phenotypes associated with *LMNA* mutations (Bonne et al., 1999; Fatkin et al., 1999; Muchir et al., 2000; Shackleton et al., 2000) and the clinical phenotype of mice bearing homozygous *Lmna* inactivation (Sullivan et al., 1999) constituted additional arguments to search for mutations in this gene. A unique homozygous mutation in *LMNA* was identified in all affected members from the Algerian families linked to the ARCMT2A locus, as well as in additional patients with ARCMT2 from a third, unrelated family (De Sandre-Giovannoli, 2002).

This mutation (c.892C > T) lies in exon 5 of the *LMNA* gene, and cosegregates with the disease in all ARCMT2 families linked to 1q21 (De Sandre-Giovannoli et al., 2002; Tazir et al., 2004). This missense variation leads to an amino acid substitution (p.R298C) lying in the Lamin A/C central rod domain, essential for protein–protein interactions (Fig. 2). This domain is highly conserved throughout evolution from *Caenorhabditis elegans* to *Homosapiens*, and the arginine at position 298 is also conserved in lamin B1, a close homolog of Lamin A/C.

The Founder R298C Variation in Lamin A/C Specifies ARCMT2B1

The identification of a common ancestral haplotype (at markers *D1S303*, *D1S2777*, and *D1S2721*)

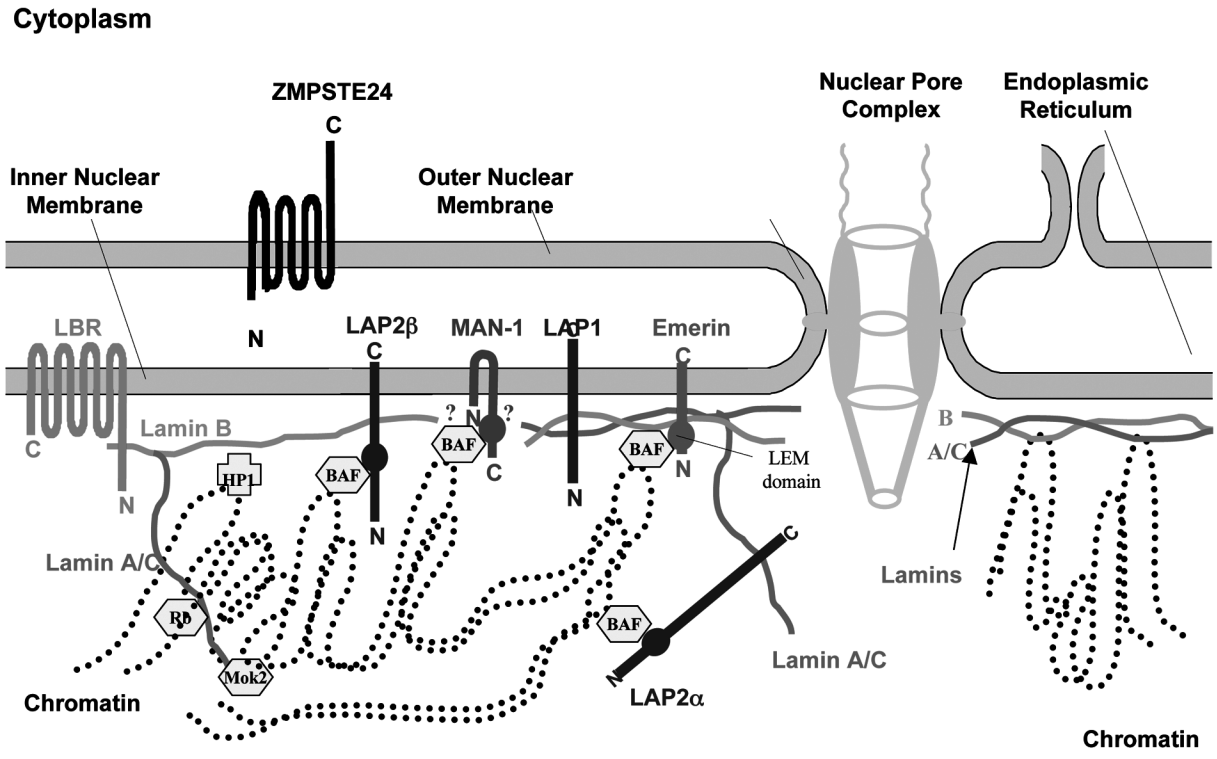

Fig. 1. Schematic representation of the nuclear envelope structure and composition (adapted from Gisèle Bonne). Inner and outer nuclear membranes, in continuity with the endoplasmic reticulum and nuclear pore complexes are shown. Lamins A/C and B localization at the nuclear lamina, underlying the inner nuclear membrane, is shown, with Lamins A/C further extending into the nucleoplasm. The interaction of Lamins with different molecular partners, including chromatin, are shown. Inner nuclear membrane integral proteins are represented with the respective transmembrane and LEM domains (LEM domain = a ~40 residue motif contained in LAP2-emerin-MAN1 and necessary for the interaction with the chromatin protein BAF [barrier to autointegration factor]). HP1 (heterochromatin protein 1, a component of condensed chromatin), and the transcription factors Rb and MOK2 are indicated. Chromatin is represented by the dashed line. ZMPSTE24, the metalloprotease involved in Prelamin A maturation, is a seven transmembrane-domain protein localizing in the endoplasmic reticulum.

suggested a founder mechanism underlying the Algerian families' disease. To date, the homozygous p.R298C mutation in Lamin A/C causing an axonal neuropathy, has only been identified in patients from Algeria and Morocco. This is the first mutation causing a neurodegenerative disorder, limited to a restricted region in the northwest of Algeria and east of Morocco. Eleven reported families, as well as 10 additional unpublished families, originated from this region and were found to carry the common ancestral haplotype on which the p.R298C mutation segregates (DeSandre-Giovannoli et al., 2002; Tazir et al., 2004). It is not yet clear why this specific *LMNA* mutation has not spread among neighboring populations or even elsewhere. One possible explanation would be that it occurred recently in the genome of a common ancestor living in the region. Meanwhile, this interpretation contradicts the genetic data that define a common haplotype limited to a 1.6 cM or 3 Mb

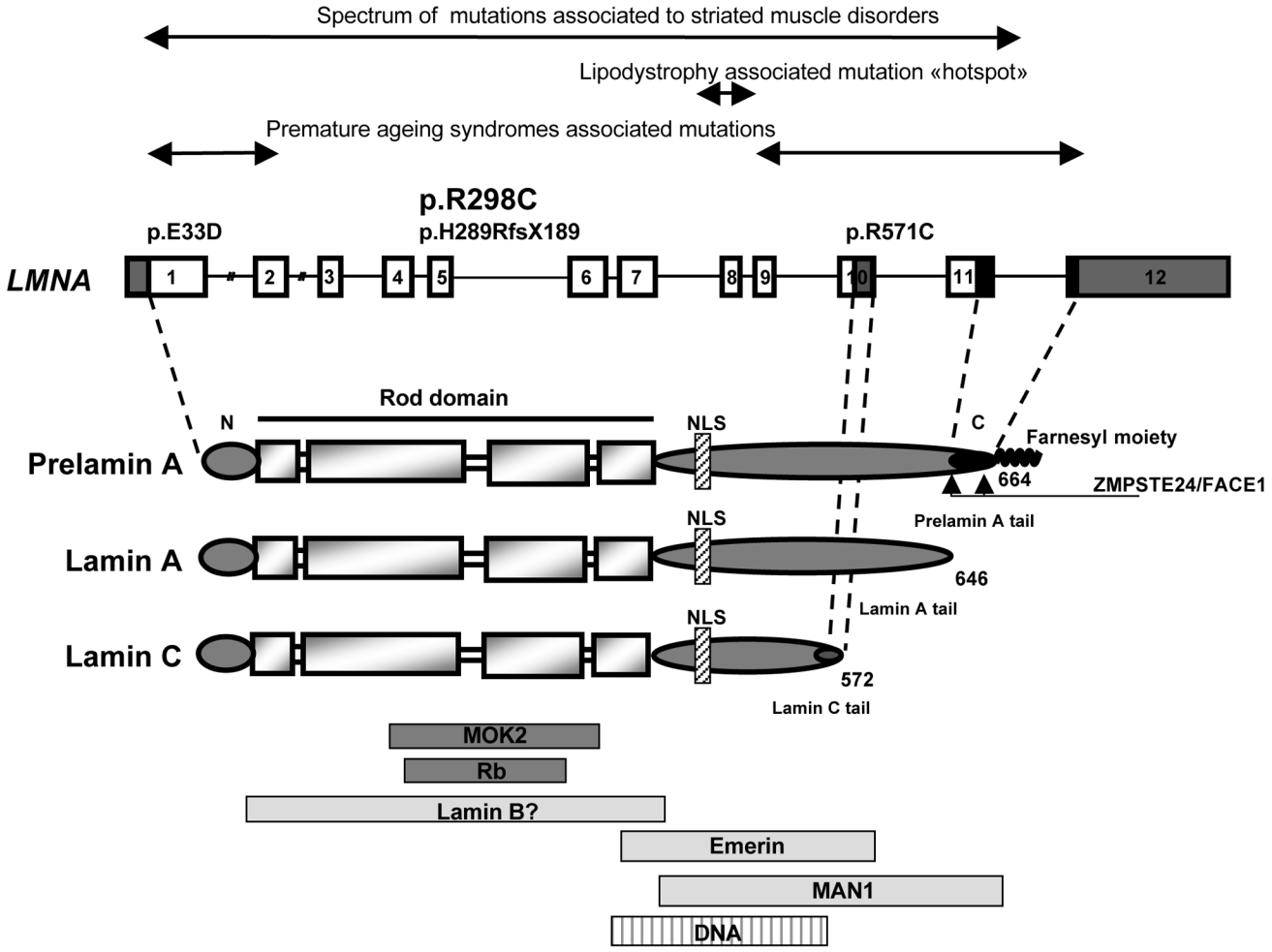

Fig. 2. *LMNA* and A-type lamins. Schematic representation of the *LMNA* gene, Lamins A/C structural domains (N,N-terminal globular head; C,C-terminal globular tail, NLS, nuclear localization signal), known or putative molecular interactors, the mutational spectrum of main groups of laminopathies and mutations causing specific peripheral neuropathic phenotypes. Prelamin A, mature Lamin A and Lamin C differ at their C-terminal tails and are composed of different numbers of amino acids as indicated; the parts of the exons encoding specific Prelamin A and Lamin C tails are filled in with corresponding colors (black for Prelamin A and dark grey for Lamin C) and are indicated by dashed black lines. The farnesyl moiety that is added to Prelamin A during its first posttranslational processing step is shown as a zigzag at Prelamin A tail. The two sites of ZMPSTE24 proteolytic cleavage on Prelamin A tail are shown as well by two arrowheads. The mutational spectrum of main groups of laminopathies is indicated by double arrows in the upper part of the figure. Autosomal-recessive *LMNA* p.R298C mutation linked to CMT2B1 is indicated in bold, whereas autosomal-dominant *LMNA* mutations linked to neuropathic phenotypes associated with other features are also indicated in clear (Goizet et al., 2004; Benedetti et al., 2005). The filled boxes located in the lower part of the figure indicate the regions of Lamins A/C required for each named molecular partner to be bound. Lamin A/C partners that are possibly related to CMT2B1 pathogenesis, owing to the region of interaction with Lamin A/C, are filled in dark grey. Other molecular interactors are filled with light grey; the region responsible of interaction with DNA is shown by the vertically hatched box; interactors with yet undefined region of interaction are followed by a question mark.

interval thus rather suggestive of an older mutational event. Further molecular and population analysis will be necessary to resolve this particular point of interest, and dating this particular mutation will be of great help to understand this apparent paradox.

Clinical, Electrophysiological, and Histopathological Phenotypes

Detailed phenotypic analyses have been reported (Chaouch et al., 2003; Tazir et al., 2004). In summary, the age of onset ranges from 6 to 27 yr, with a mean at around 15 yr. The course of the disease is also highly heterogeneous among patients. After 10 ± 15 yr of disease duration, most patients present with a severe CMT phenotype including distal wasting and weakness of all four limbs and areflexia. It is noteworthy that these common features coexist with the involvement of the proximal lower and sometimes upper limb muscles. However, some patients have been reported as presenting a more classical phenotype with milder disability without proximal limb deficit, even after disease of a prolonged duration. Electrophysiological studies showed that median MNCV were either preserved or slightly reduced in almost all the patients supporting the axonal process without demyelination participating in the disease process.

Histopathological exploration revealed a severe rarefaction of myelinated and nonmyelinated fibers in all homozygous patients and, comparatively, a relative abundance of unmyelinated fibres in most of them. Large myelinated fibers were almost totally lacking. No active axonal degeneration was usually observed. There is also no evidence of de- or remyelination, or of onion bulb formation; in particular, neither proliferations of Schwann cells (onion bulbs) nor regenerating "clusters" can be observed. Thus, from a clinical and pathological point of view, ARCMT2A presents with differences when compared with other forms of CMT2. The wide variability among patients for the age of onset, the disease course and the involvement of proximal limbs is somehow unusual within the same subtype. Nonetheless, such clinical variability should be correlated with the extreme clinical heterogeneity observed in the large groups of *LMNA*-related disorders, also called "laminopathies" (later in this review; Somech et al., 2005; Gruenbaum et al., 2005). In particular, mutations in *LMNA* lead to severe muscular phenotypes such as Emery Dreifuss muscular dystrophy (EDMD) and limb girdle muscular dystrophy (LGMD1A) (Bonne et al., 1999; Muchir et al., 2000). Moreover, the proximal involvement of the upper limbs in ARCMT2A could be regarded as a hallmark of muscle- and nerve-specific laminopathies. Indeed, in EDMD patients, proximal involvement of the upper limbs usually coexists with distal amyotrophy at the lower limbs, associated with severe joint retractions, whereas in LGMD1B, a more proximal deficit of the four limbs dominates the clinical picture (Wehnert et al., 2002).

Mouse Models for LMNA-Associated ARCMT2

De Sandre-Giovannoli et al. (2002) reported that *Lmna*-null mice (Sullivan et al., 1999) presented with an axonal clinical and pathological phenotype that is highly similar to patients with autosomal-recessive CMT2. Homozygous *Lmna* knockout mice (Sullivan et al., 1999) indeed display an abnormal gait with a stiff walking posture, characterized by splayed hind legs and the inability to hang on to structures with their forepaws. During development, they exhibit distinct scoliosis/kyphosis and become progressively hunched. These aspects are not observed in heterozygous littermates and are extremely evocative of a severe peripheral neuropathy, because they are also reported in animal models of other peripheral neuropathies (Sereda et al., 1996; Huxley et al., 1998; Norreel et al., 2001). When sciatic nerves of *Lmna* knockout mice were further analyzed at the ultrastructural level and compared with age-matched controls' nerves, the responsibility of *LMNA* homozygous defects for the injury of peripheral axons is even more evident. Whereas heterozygous ($Lmna^{+/-}$) knockout mice display a preserved peripheral nerve histology, null mice lacking all *Lmna* transcripts exhibit a strong reduction of axon density, an increase in axon diameter, as well as the presence of nonmyelinated axons, making these histological aspects reminiscent of those observed in ARCMT2A patients.

However, other mouse models carrying specific mutations in *Lmna* have been reported (Mounkes

et al., 2003; Arimura et al., 2005; Kozlov et al., 2005; Mounkes et al., 2005) and, as in humans, they do not present any of the typical symptoms associated with ARCMT2A. This is not surprising because most *LMNA* mutations reported in humans are associated with tissue-specific phenotypes. Thus, the deep clinical, behavioral, molecular, and morphological explorations of the *Lmna* R298C$^{-/-}$ knock-in mice, that are in process, should provide essential information related to the pathophysiological mechanism leading to ARCMT2A.

Altered Lamin A/C Function and Diseases: The "Laminopathies"

The *LMNA* gene encodes four Lamin A/C isoforms through alternative splicing, also known as A type Lamins, of which Lamins A and C constitute the two major ones (Fig. 2). They are expressed in nuclei of all vertebrate differentiated cells (Rober et al., 1989; for review, *see* Gruenbaum 2005), localizing both at the nuclear periphery, in which they constitute the nuclear "lamina" underlying the inner nuclear membrane, and in the nucleoplasm. Lamins A/C have been shown to participate in many diverse nuclear functions, notably involving the global regulation of gene expression patterns, through their interactions with a great number of molecular partners, and the maintenance of genome stability (Zastrow et al., 2004; Gruenbaum et al., 2005; Liu et al., 2005) (Figs. 1 and 2).

Mature Lamin A is obtained from a precursor (Prelamin A) through a series of posttranslational processing steps taking place at Prelamin A's C-terminal tail, including farnesylation by a farnesyl transferase, cleavage by a metalloprotease ZMPSTE24 (FACE-1), methylation by α-methyl transferase (ICMT) and as a last step, a second cleavage by ZMPSTE24 (Corrigan et al., 2005). Either primary Lamin A/C defects, or secondary ones, owing to the alteration of enzymes involved in prelamin A processing, as well as defects in molecular partners of Lamins A/C, gives rise to a group of diseases called "laminopathies" (Gruenbaum et al., 2005). These are made up of heterogeneous disorders, from both a clinical and a genetic point of view, to which CMT2B1 has been shown to belong. In a general way, laminopathies cause a nonsatisfactory quality of life, demand expensive medical care and, in many cases, lead to premature death. This group of disorders is characterized by a very large phenotypic spectrum, which has continued evolving, because their first implication in the pathogenesis of Emery–Dreifuss muscular dystrophy, in 1999, to the more recent implication in progeria and restrictive dermopathy (Table 2).

Laminopathies affect different tissues in separate or combined fashions, with variable severity, from mild-to-lethal in the perinatal period. Although different laminopathies present with overlapping features, they can somehow be separated into distinct subgroups and clinical entities.

Tissue-specific laminopathies include several disorders. Some affect skeletal and cardiac muscles, autosomal-dominant and -recessive Emery–Dreifuss muscular dystrophy (respectively EDMD2 and EDMD3) (Bonne et al., 1999; Raffaele di Barletta et al., 2000); Limb–Girdle muscular dystrophy type 1B with cardiac conduction defects (LGMD1B) (Muchir et al., 2000); autosomal-dominant dilated cardiomyopathy with conduction defects type 1A (DCM 1A) (Fatkin et al., 1999). Other disorders affect specifically adipose tissue, such as familial partial lipodystrophy, Dunnigan type (Shackleton et al., 2000). Additionally, laminopathies affecting peripheral nerves include the autosomal-recessive axonal CMT type 2B1 (CMT2B1) (De Sandre-Giovannoli et al., 2002). Notably, axonal CMT disease has recently been shown to be due, as well, to dominant *LMNA* mutations, usually in association to other clinical features. These include namely skeletal muscle dystrophy with or without cardiomyopathy and leuconychia (Goizet et al., 2004; Benedetti et al., 2005).

Combined laminopathies, affecting several tissues (skeletal, cardiac, cutaneous, and adipose) include mandibuloacral dysplasia (MAD) types A and B, respectively, owing to *LMNA* and *ZMPSTE24* mutations, and clinically characterized by: hypoplastic clavicles and mandible, acro-osteolyses, retarded closure of fontanels, joint contractures, lipodystrophy, insulin-resistance, alopecia, hepatomegaly, and alteration of cutaneous pigmentation (*Acanthosis nigricans*), (Novelli et al., 2002; Agarwal et al., 2003). Beside MAD, another *LMNA*-associated syndrome was reported, combining lipodystrophy, insulin-resistant diabetes, leucomelanodermic papules, liver steatosis, and cardiomyopathy (LDHCP) (Caux et al., 2003). Later after this report, the so-called atypical Werner syndrome was shown to be associated to

Table 2
Clinical Spectrum of Laminopathies Caused by Mutations in *LMNA*, *ZMPSTE24*, and *STA* (*Emerin*)

Abbreviation	Disease name	Genes involved	OMIM no.	References
AWS	Atypical Werner Syndrome	*LMNA*	150330	Chen (2003)
ARCMT2	Axonal ARCMT (CMT2B1)	*LMNA*	605588	De Sandre-Giovannoli (2002)
CMT2	Axonal AD CMT	*LMNA*		Goizet (2004); Benedetti (2005)
CMD1A	Cardiomyopathy, dilated 1A	*LMNA*	115200	Fatkin (1999)
EDMD1	X-linked Emery Dreifuss dystrophy type 1	*STA (Emerin)*	310300	Bione (1994)
EDMD2/3	Emery–Dreifuss muscular dystrophy types 2 (AD) and 3 (AR)	*LMNA*	181350	Bonne (1999)
FPLD	Dunnigan-type familial partial lipodystrophy	*LMNA*	151600	Cao and Hegele (2000) Shackleton (2000)
HPGS	Hutchinson-Gilford Progeria syndrome	*LMNA*	176670	De Sandre-Giovannoli (2003) Eriksson (2003)
LDHCP	Lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, leukomelanodermic papules	*LMNA*	608056	Caux (2003)
LGMD1B	Limb-girdle muscular dystrophy type 1B	*LMNA*	159001	Muchir (2000)
MADA	Mandibuloacral dysplasia type A	*LMNA*	248370	Nowelli (2002)
MADB	Mandibuloacral dysplasia type B	*ZMPSTE24*	608612	Agarwal (2003)
RD	Restrictive dermopathy	*LMNA*	275210	Navarro (2004)
RD	Restrictive dermopathy	*ZMPSTE24*	275210	Navarro (2004); Navarro (2005)

Several syndromes associating symptoms that overlap with many of the earlier mentioned disorders have been reported and are not mentionned here. Mutations in functionally related genes *LBR* encoding the Lamin B receptor protein, and *MAN1* encoding the LEMD3 protein (Fig. 1) also cause genetics disorders that are not reported here.

different or identical *LMNA* mutations (Chen et al., 2003). Unfortunately, this clinical entity lacks several of the phenotypic criteria that usually satisfy the diagnosis of Werner syndrome (Vigouroux et al., 2003; Bonne and Lévy, 2003).

Finally, the more recently reported syndromes associated to A-type lamins or related proteins defects fit the criteria of premature aging syndromes and are considered as systemic laminopathies. The paradigmatic syndrome is Hutchinson–Gilford Progeria syndrome (HGPS-Progeria) that was shown to be associated, in most cases, with a *de novo* splicing mutation in the specific domain encoding Lamin A (De Sandre-Giovannoli et al., 2003a; Eriksson et al., 2003). In addition, restrictive dermopathy represents a neonatally lethal laminopathy caused either by *LMNA* or *ZMPSTE24* mutations (Navarro et al., 2004, 2005).

Laminopathies are thus a model of clinical heterogeneity, their clinical spectrum being extremely large, both in the wide range of tissues involved, and in the severity of the phenotype. For some laminopathies (namely affecting nerve, skeletal, and cardiac muscles), a high degree of intrafamilial clinical variability has been observed (Vytopil et al., 2002; Mercuri et al., 2004, 2005; Tazir et al., 2004;

Benedetti et al., 2005). The clinical heterogeneity observed in the case of *LMNA* mutations is thus highly contrasted with our previous view of clinical heterogeneity. CMT disorders are often considered as examples for both clinical and genetic heterogeneity. Nonetheless, mutations within the same CMT gene usually lead to a more or less severe neuropathic phenotype and, only in rare occasions, to non-neurologically associated symptoms. Thus, as far as we know, phenotypic heterogeneity of a group of related diseases (including CMT) mainly depends on the associated disease-causing gene. In this context, *LMNA*-related ARCMT2 is of particular interest because different mutations in this gene cause distinct clinical entities, ranging from mild to extremely severe conditions including progeria and the neonatal lethal restrictive dermopathy. Whereas the pathophysiological mechanism in the latter disorders is at least partially elucidated and corresponds to the accumulation of a toxic protein within the nucleoplasm (Fong et al., 2004; Goldman et al., 2004; Navarro et al., 2005), molecular mechanism for CMT2B1 and relations with other laminopathies remains enigmatic. At least two other pathophysiological mechanisms have been postulated and partially proven as being involved in laminopathies. One corresponds to a reduced resistance to mechanical strain in tissues subjected to continuous physical stress, such as muscle fibers (Broers et al., 2004; Lammerding et al., 2004; Nikolova et al., 2004), whereas another corresponds to the impaired interaction with tissue-specific or systemic factors involved in the regulation of gene expression (Lloyd et al., 2002; Favreau et al., 2004). Among these, the latter "gene expression pathophysiological hypothesis" has to be further explored. Lamins A/C interact directly or indirectly with many nuclear proteins located both at the inner nuclear membrane and within the nucleoplasm (Figs. 1 and 2). Among them, several transcription factors, including the human orthologue of the mouse Kruppel-like (MOK2) and the retinoblastoma (RB) proteins, are exclusively or predominantly expressed from nervous tissues. Since the R298C mutation lies in the Lamin A/C rod domain in the specific region of interactions with MOK and RB, a possibility exists that a specific missense mutation in this Lamin A/C interacting domain disrupts the proper assembly of these partners, thus being the key pathomechanism involved in ARCMT2 (Ozaki et al., 1994; Dreuillet et al., 2002).

GDAP1 Related Autosomal-Recessive CMT

Linkage to chromosome 8q (8q13-q21.1) was initially evidenced in families affected with demyelinating CMT (CMT4A) (Ben Othmane et al., 1993, 1998). Subsequently, a linkage region was found to overlap with the CMT4A locus in a large consanguineous Tunisian family, in which the affected members presented with an axonal form of the disease, associated with a mild pyramidal syndrome (Barhoumi et al., 2001). Although the clinical phenotypes were distinct, the question was raised of the possibility of a common disease-causing gene in these two CMT entities. Subsequently, the *GDAP1* gene located in the chromosomal overlapping interval, was identified as the disease-causing gene (Baxter et al., 2002; Cuesta et al., 2002).

Phenotypic Aspects of ARCMT Caused by Mutations in the GDAP1 Gene

Clinical Presentations

The simultaneous publication of the two initial reports of CMT patients with *GDAP1* mutations directly demonstrated the complexity of the phenotypic spectrum of the *GDAP1* mutations because the gene was involved in both autosomal-recessive demyelinating CMT (CMT4A) (Baxter et al., 2002) and axonal CMT (ARCMT2) diseases (Cuesta et al., 2002). Further reports have then confirmed the intertwining of demyelinating and axonal processes, and raised the notion of intermediate forms of CMT (Nelis et al., 2002; Senderek et al., 2003; De Sandre-Giovannoli et al., 2003a,b; Birouk et al., 2003).

Cuesta and colleagues reported three Spanish families in which the disease, segregating as an autosomal-recessive trait, was characterized by an onset in childhood with foot and hand weakness and wasting, bone deformations (pes cavus, claw hand, kyphoscoliosis), and a severe course leading to disability at the end of the first decade. Wheelchair dependence is almost constant in the second or third decade, except in rare patients. Remarkable associated features included hoarse voice, appearing in the second decade, and vocal cord paresis (Cuesta et al. 2002; Sevilla et al., 2003).

The Moroccan family reported by Azzedine et al. (2003) presents with a very similar clinical phenotype, in which is underlined the progressive and major distal to proximal muscle involvement, leading to inability to walk in the second decade in one of the two affected sibs. Hoarse voice is present, and diaphragmatic paralysis responsible for restrictive respiratory insufficiency in the third decade is reported as a new added feature. Intrafamilial variability of the severity is noticed in this family (Azzedine et al., 2003). A severe clinical presentation also characterizes the Moroccan and French families reported by Birouk et al. (2003) and Stojkovic et al. (2004) respectively, including hypotonia at birth or during early childhood and delayed age of walking (Birouk et al., 2003; Stojkovic et al., 2004). More generally, patients affected with ARCMT2 owing to *GDAP1* mutations present a severe early onset phenotype variably associated with additional clinical features (e.g., hoarse voice owing to vocal cord paresis).

Electrophysiological and Neuropathological Phenotypes

A typical axonal pattern of the electromyographic exploration was reported in different studies such as severe reduction or absence of compound motor action potential (CMAP) and sensory nerve action potential amplitudes, moderately delayed distal motor latencies, and normal or slightly reduced MNCVs. Also, the axonal degenerative process was shown as the prominent histological aspect of these forms of CMT (Azzedine et al., 2003; Birouk et al., 2003; Sevilla et al., 2003; Stojkovic et al., 2004; Claramunt et al., 2005).

Loss of myelinated fibers and axonal degeneration, with no or few signs of demyelination and remyelination, is reported in the initial publication of ARCMT2 with mutation in *GDAP1* (Cuesta et al., 2002). Nevertheless, diverse observations for CMT patients carrying *GDAP1* mutations have been reported and should be mentioned because they reflect the clinical and, overall electrophysiological and neuropathological heterogenity in *GDAP1*-related ARCMT (Baxter et al., 2002; Nelis et al., 2002; Ammar et al., 2003; Boerkoel et al., 2003; De Sandre-Giovannoli et al., 2003; Senderek et al., 2003; Di Maria et al., 2004; Parman et al., 2004).

Spectrum of Mutations in GDAP1

To date, 23 mutations have been reported in the *GDAP1* gene (http://www.molgen.ua.ac.be/CMT-Mutations/) and all of them are listed in Table 3 in relation to phenotypic features and the ethnic origin of the families when data were available. In details, 11 missense, 4 nonsense, 6 frame-shift, and 2 splicing mutations have been identified, as well as 1 recurrent polymorphism. The mutations span the entire coding sequence of the gene, with the exception of exon 2, and several mutations have been repeatedly reported. A founder effect has been demonstrated for p.Gln163X, p.Ser194X, p.Thr288fsX290 (Claramunt et al., 2005), and p.Met116Arg (Di Maria et al., 2004).

Most *GDAP1* mutations co-segregate with the disease in a recessive mode of inheritance. However, dominant inheritance of two mutations (c.358C > T; p.Arg120Trp and c.469A > C; p.Thr157Pro) has recently been reported, respectively, in association with a mild phenotype and a severe phenotype with optical nerve atrophy (Claramunt et al., 2005). It is noteworthy that the mutation p.Arg120Trp has been reported elsewhere (Ammar et al., 2003) in association with another mutation and causing a severe recessive form of the CMT disease.

Pathophysiology of GDAP1-Associated ARCMT

GDAP1 encodes a 358 aa protein, containing two predicted transmembrane helices (aa 292–311 and 319–343). As it had been isolated from human sural nerve and mouse sciatic nerve (Cuesta et al., 2002), it was supposed to be expressed in neurons and Schwann cells, and to play a fundamental part during the development and myelination of the peripheral nervous system. It has been recently demonstrated that GDAP1 is predominantly expressed in neuronal cells (Pedrola et al., 2005). Phylogenetic and secondary structural analysis has shown that GDAP1 belongs to a new subfamily of glutathione-*S*-transferases (Marco et al., 2004). In parallel, its primary structure predicts a likely localization at the cellular membrane, in which GDAP1 might participate to the complex and permanent interactions existing between Schwann cells and neurons (Fields and Stevens, 2000; Fields and

Table 3
Reported Mutations in *GDAP1*

						Clinical aspects		Electromyography (available MNCV)		Histology				
Mut n	Gene mutations	Exon/ intron	Proteic predicted effect	References	Associated mutations	Particularities	CNP	MNCV (m/s)	CMAP	Loss of fibers	Hypo or demyelination	OB	Country of origin	Phenotype
1	c.92G > A	1	p.Trp31X	Baxter et al. (2002)	c.92G > A			31		Yes	Yes	xx	Tunisian	DM
2	c.311-1 G > A	intron 2	splice	Kabzinska et al. (2005)	c.389C > G								Poland?	A
3	c.342_345 delAAAG	3	p.Glu114fs X145	Claramunt et al. (2005)	c.487C>T		No	24-31					Spanish	A
				Ammar et al. (2003)	c.715C > T		No	41	Severely reduced	Yes	Yes	x	Germany/ South Italy	A
4	c.347T > G	3	p.Met116Arg	Di Maria et al. (2004)	c.347T > G		No	29-55	Reduced	Yes	Yes	x	Italy	A/DM
5	c.349_ 350insT	3	p.Tyr117fs	Senderek et al. (2003)	c.349_350insT		No	31	Reduced	Yes	Yes	x	Southern Turkey	A/DM
6	c.358C > T	3	p.Arg120Trp	Claramunt et al. (2005)	none	Mild (AD)	No	45	Mildly reduced				Spanish Belgium	A DM
				Ammar et al. (2003)	c.811G > A		No	19	Severely reduced					
7	c.359G > A	3	p.Arg120Gln	Boerkoel et al. (2003)	c.359G > A	DSS	Yes (VCP, FW)			Yes		xx	Japan	DM
8	c.389C > G	3	p.Ser130Cys	Kabzinska et al. (2005)	c.311-1 G > A								Poland	A
9	c.445G > T	3	p.Asp149Tyr	Parman et al. (2004)	c.445G > T	Proximal involvement		UR		Yes	Yes	xx	?	DM/I
10	c.469A > C	3	p.Thr157Pro	Claramunt et al. (2005)	none	Severe + optic atrophy (AD)	Yes	45					Spain	A
11	c.482G > A	3	p.Arg161His	Baxter et al. (2002)	c.482G > A			31		Yes	Yes	xx	Tunisia	DM
				Ammar et al. (2003)			No	37-51		Yes	Yes	x to xx	Tunisia	DM/I
12	c.485-2A > G	intron 3	p.Ser162 fsX166	De Sandre-Giovannoli et al. (2003)	c.485-2A>G	Hands retractions ++		20	Reduced	Yes	Yes	xx	Algeria	A/DM
13	c.487C > T	4	p.Gln163X	Cuesta et al. (2002)	c.487C > T	Slower evolutivity	Yes (VCP)	42					Spain	A
				Boerkoel et al. (2003)	c.487C > T		Unconstant			Yes	Yes	xx	Spain	DM

(Continued)

Table 3 *(Continued)*

Mut n	Gene mutations	Exon/ intron	Proteic predicted effect	References	Associated mutations	Clinical aspects		Electromyography (available MNCV)		Histology			Country of origin	Phenotype
						Particularities	CNP	MNCV (m/s)	CMAP	Loss of fibers	Hypo or demyelination	OB		
				Claramunt et al. (2005)	c.487C > T		Yes (H)	26-normal		Yes		No	Spain	
				Cuesta et al. (2002)	c.581C > G		Yes (VCP)	UR	Absent	Yes	Yes	x	Spain	A
				Cuesta et al. (2002)	c.863insA		Yes (VCP)	UR	Absent	Yes	Yes	Few	Spain	A
				Claramunt et al. (2005)	c.342_345 delAAAG	See mutation 3								
	c.507G >T	4	p.Ser169Ser	Senderek et al. (2003)		Polmorphism								Absent
14	c.579 + 1G > A	intron 4	5′-splice site	Senderek et al. (2003)	c.579 + 1G > A		No	26,4		Yes	Yes	x	Germany	A/DM
15	c.558delT	4	p.Ile186fs	Stojkovic et al. (2004)	c.558delT	Proximal involvement	Yes (VCP, DP)		Absent or reduced				France	A
16	c.581C > G	5	p.Ser194X	Baxter et al. (2002)	c.581C > G		No	31		Yes	Yes	xx	Tunisia	DM
				Nelis et al. (2002)			No	42-50	Reduced	Yes	Yes	Few	Morocco	A
				Birouk et al. (2003)		Proximal involvement	No	40-50	Reduced	Yes		No	Morocco	A
				Cuesta et al. (2002)	c.487C > T	See mutation 13								
				Claramunt et al. (2005)	c.863insA		Yes (H)	UR					Spain	
				Azzedine et al. (2003)	c.929G > A	Intrafamilial variability	Yes (VCP, DP)		Abolished / reduced				Morocco	A
17	c.668T > A	5	p.Leu223X	De Sandre-Giovannoli et al. (2003)	c.668T > A	Hands retractions		45	Severely reduced	Yes	Yes	xx	Lebanon	DM/I
18	c.715C > T	6	p.lys239Phe	Ammar et al. (2003)	c.342_345del AAAG	See mutation 3								
19	c.786delG	8	p.Gly262fs	Nelis et al. (2002)	c.786delG		No	21		Yes	Yes	xx	Turkey	DM/I
20	c.811G > A	6	p.Gly271Arg	Ammar et al. (2003)	c.358C > T	See mutation 6								

21	c.844C > T	6	p.Arg282Cys	Nelis et al. (2002)	c.844C > T		No	43-61	Severely reduced			Turkey	A/DM
				Ammar et al. (2003)			No	42	Severely reduced	Yes	xx	Croatia	A/DM
				Senderek et al. (2003)	none		No					Germany	A/DM
22	c.863insA	6	p.Thr288fs X290	Cuesta et al. (2002)	c.487C>T	See mutation 13							
23	c.929G > A	6	p.Arg310Gln	Azzedine et al. (2003)	c.581C > G	See mutation 16							

Initial source: IPNMD: http://www.molgen.ua.ac.be/CMTMutations/.

In order that all the mutations reported till date appear in column 2 (gene mutations) some redundancies are present in this table and correspond to the grey boxes.

Empty boxes: Unavailable or unclear data. Phenotypic data reported in the last column reflect either the authors statements or a possible interpretation of the reported data from each mentionned publication.

Abbreviations: CNP, cranial nerve participation; VCP, vocal cord paresis; H, hoarseness; FW, facial weakness; DP, diaphragmatic paralysis; MNCV, motor nerve coduction velocities; CMAP, compound muscle action potential; UR, unrecordable; OBs, onion bulbs; A, axonal; DM, demyelinating; I, intermediate; AD, autosomal-dominant; DSS, Dejerine-Sottas syndrome.

Stevens-Graham, 2002). However, recent functional studies have shown that GDAP1 is localized in mitochondria (Pedrola et al., 2005).

Although the pathophysiological mechanism linking *GDAP1* mutations to motor and sensory disabilities remains to be elucidated, the hypothesis has been raised that, because terminal peripheral nerves are enriched in mitochondria (Fawcett et al., 1982; Morris et al., 1993), reducing the transport of mitochondria and thus depriving axons of a major source of energy might lead to peripheral neuropathies (Sue et al., 2000; Pedrola et al., 2005). Also, several mitochondria-associated diseases include peripheral neuropathy among their clinical features (Finsterer, 2005).

Recently, mutations in the nuclear-encoded mitochondrial GTPase Mitofusin 2 *(MFN2)* gene have been demonstrated to cause autosomal-dominant CMT2A, the most prevalent cause of inherited axonal neuropathies (Züchner et al., 2004b). MFN2 is necessary for mitochondrial fusion and its proper transport along the cytoskeleton (Bach et al., 2003; Züchner et al., 2004b). Although such functions are altered when *MFN2* is dysregulated (Bach et al., 2003), such mitochondrial defects have not been clearly demonstrated yet in the case of *GDAP1* mutations. However, morphologically abnormal mitochondria have been observed after GDAP1 transient overexpression and the neuropathic phenotype could thus be related to a similar mechanism (Pedrola et al., 2005). GDAP1 might thus have diverse possible functions in the development of the peripheral nervous system, although many of them probably remain to be elucidated. Animal models, along with more detailed functional studies in humans, will certainly help to further understand the role of *GDAP1* and its molecular partners in the complex molecular interactions underlying the physiological development as well as the pathophysiology of the peripheral nervous system in general and *GDAP1*-associated neuropathies in particular.

Genotype–Phenotype Correlations and Nosology of GDAP1-Related Neuropathies

The phenotypic data from patients carrying *GDAP1* mutations are thus clearly heterogeneous. Although the axonal process seems to be predominant, demyelination as the primary defect has also been reported by several authors (Baxter et al., 2002; Desandre-Giovannoli et al., 2003a,b). Additionally, identical mutations, when identified in distinct unrelated families, have been associated to types I, II, or intermediate forms of CMT, although the number of patients with identical *GDAP1* mutations remains limited. Regarding both the clinical severity and histopathological lesions, intrafamilial variability has been observed (Ammar et al., 2003; Azzedine et al., 2003). The recent observation of possible dominant mutations associated to variable phenotypes is an additional argument to conclude that to date, no reliable phenotype–genotype correlations can be established (Table 3).

Moreover, the various and complex phenotypic spectrum linked to *GDAP1* mutations has led to some confusion in the nosological classification of the CMT phenotypes associated to this gene (Table 1). Although CMT4A usually refers to demyelinating ARCMT forms, this appellation has also been used on several occasions in which the primary phenotype was found to be axonal. Moreover, according to different authors as well as to OMIM database references, autosomal-recessive *GDAP1*-linked CMT might be referred to as autosomal-recessive CMT2+VCP (Cuesta et al., 2002), autosomal-recessive intermediate CMT type A (CMTRIA) (Nelis et al., 2002; Senderek et al., 2003) and autosomal-recessive CMT2K (Birouk et al., 2003) (Table 1). In our opinion, the classification of the several entities related to *GDAP1* mutations should be revisited, taking into account the fact they are associated to different electrophysiological and histopathological phenotypes, representing a continuum in the ARCMT context. The present classification contains an additional point of confusion because the first ARCMT2 family ever reported to be linked to 8q21 (Barhoumi et al., 2001) was termed CMT4C2 by the authors, although it is referred to as CMT2H in public databases (OMIM no. 607731). Together in this family it is likely that affected patients carry *GDAP1* mutations, such a molecular analysis has not been reported to date. Altogether, these classification's discrepancies underlie the difficulties in the designation of the clinical and molecular entities in the field of CMT. Thus, because of the expanding knowledge in this field, with the growing number of genes and mutations identified as being associated to clinically specific or

overlapping phenotypes, it becomes indispensable for the scientific community to reach a classification consensus.

ARCMT2 Linked to Chromosome 19q13.3 (CMT2B2)

Leal and colleagues (2001) reported a large consanguineous Costa Rican family with autosomal-recessive axonal CMT. In this family, the clinical presentation was typical of a classical form of CMT. Mean age at onset was 33.8 yr (range, 28–42 yr). MNCVs were normal or slightly reduced, and potential amplitudes were decreased. Linkage to 19q13.3 was found in three branches of the family, and subsequent homozygosity mapping defined shared haplotypes in a 5.5-cM interval between markers *D19S902* and *D19S907*. Although CMT4F had previously been mapped to 19q13.1-q13.3 (Delague et al., 2000), and the causative gene, Periaxin (*PRX*) identified (Boerkoel et al., 2001; Guilbot et al., 2001), the latter was unlikely the cause for CMT2B2. Indeed, not only the respective phenotypes are different, but more convincingly, in terms of genetics analysis, Leal et al. (2001) ruled out the inclusion of *PRX* in the CMT2B2 linkage interval.

Berghoff and colleagues (2004) provided a clinical follow-up of eight affected members of the family reported by Leal et al. (2001). Distal symmetrical weakness, atrophy and hypo- or areflexia were present and more pronounced in the lower limbs than in upper extremities. Sensory deficit was observed and had a symmetrical "stocking-glove" pattern. The authors emphasized the onset at adulthood within this unique family, and the mildness of the clinical presentation when compared with the axonal-recessive forms of CMT caused by mutations in *LMNA* or *GDAP1*. No sign of cranial nerve or upper neuron involvement or other clinical features were associated. Skeletal deformities were mild when present, possibly occurring late in the course of the disease. Although nerve biopsies were unavailable, preventing histopathological studies, electrophysiological studies were indicative of a primary axonal degenerative process including some patterns of secondary demyelination (Berghoff et al., 2004).

In an attempt to identify the disease causing gene in ARCMT2B, Rautenstrauss and colleagues have conducted the molecular screening of 53 genes located in the critical interval defined from the initially reported Costa Rican family. In affected individuals, they consistently identified a homozygous missense mutation (A335V) in *MED25*, a gene encoding a subunit of the mediator complex associated with RNA polymerase II (Rautenstrauss et al., 2005a). MED25, also known as ARC92 or ACID1, is a subunit of the human activator-recruited cofactor (ARC), a family of large transcriptional coactivator complexes related to the yeast mediator. Its exact physiological function in transcriptional regulation remains obscure. Owing to moderate demyelination in CMT2B2 patients, Med25 expression was investigated in rodent models over- and underexpressing *Pmp22* (Rautenstrauss et al., 2005b). A positive correlation was observed between *Med25* and *Pmp22* expression levels. Globally, these results suggest a molecular etiology of CMT2B2 and a potential more general role of MED25 in the pathophysiology of different inherited peripheral neuropathies.

Conclusions

Autosomal-recessive CMT2 is a rare and severe condition. The spectrum of severity makes the genetic counseling challenging since the age of onset, ranges from primary infancy to the third decade. The course of the disease and the degree of functional impairment may dramatically vary. Although no strict phenotype–genotype correlations can be established, the phenotypic heterogeneity mainly depends on the associated disease-causing gene.

In this context, *LMNA*-related ARCMT2 is of particular interest. First, pure forms of CMT2B1 have been reported exclusively in families originating from a limited geographical area in Algeria and Morocco and is owing to a founder mutation in *LMNA*. Second, different mutations in this gene cause distinct clinical entities ranging from mild to extremely severe conditions, including Progeria and the neonatal and lethal restrictive dermopathy. Although the pathophysiological mechanism in these latter disorders is at least partially elucidated and corresponds to the accumulation of a toxic protein within the nucleoplasm, neither the mechanism of CMT2B1 nor its relations with other "laminopathies" has been established yet. Among

several, the gene expression hypothesis seems to be pertinent. Indeed, a loss of interactions between Lamins A/C and some of its molecular partners exclusively or predominantly expressed from nerve tissues should thus be further investigated.

By contrast, *GDAP1* mutations have been identified in various ethnical groups and, although founder mutations have been reported, its molecular defects are likely the cause of autosomal-recessive peripheral neuropathies in diverse geographical regions. Additionally, although various phenotypic features are associated with *GDAP1* mutations, they have been reported exclusively among ARCMT patients affected either with demyelinating or axonal or intermediate forms of CMT. In the CMT context, *GDAP1*-associated disorders thus represent a typical model for ruling out the classical and historical dichotomy between demyelinating and axonal CMT. Its particular function has been recently explored, making its mode of action partially understood. As for MFN2 whose mutations cause autosomal-dominant CMT2A, GDAP1 certainly constitutes a bridge between peripheral nerve defects and the mitochondrial pathway thus making CMT research expand into the field of mitochondriopathies.

ARCMT2B2 linked to chromosome 19q is the least severe clinical entity among ARCMT2. To date, its frequency remains to be determined because only one linked family has been reported in Costa Rica. Recently, a missense variation has been identified in *MED25*, encoding a subunit of the mediator complex associated with RNA polymerase II. Meanwhile, supplementary molecular studies will be necessary to elucidate the *a priori* complex pathophysiological mechanism of CMT2B2 and confirm the implication of MED25 defects in additional patients.

In all, ARCMT2 represents a quite complex subcategory in the molecular nosology of inherited peripheral neuropathies. For many sporadic patients affected with CMT2 for which none of the known genes may be involved, it is not possible to distinguish between *de novo* dominant or recessive mutations as the cause of their neuropathy. It is thus likely that other genes remain to be identified for ARCMT2. This will be one of the tasks in the near future, which will enable better understanding of the complex pathomechanisms involved in axonal-recessive neuropathies and their respective links one another.

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