NeuroMolecular Medicine

ISSN0895-8696/06/08:75–86/$30.00 (Online) 1559-1174
doi: 10.1385/NMM:8:1–2:75

REVIEW ARTICLE

Autosomal-Recessive Forms of Demyelinating Charcot-Marie-Tooth Disease

O. Dubourg,[1,2,3] H. Azzedine,[1] C. Verny,[1,4] G. Durosier,[1] N. Birouk,[5] R. Gouider,[6] M. Salih,[7] A. Bouhouche,[8] A. Thiam,[9] D. Grid,[10] M. Mayer,[11] M. Ruberg,[1] M. Tazir,[12] A. Brice,[1,13] and E. LeGuern*[,1,13]

[1]INSERM U679 (ex U289), la Pitié-Salpêtrière Hospital, Paris, France; [2]Centre de référence sur les Pathologies Neuromusculaires Paris-Est, Institute of Myology, la Pitié-Salpêtrière Hospital, AP-HP, Paris, France; [3]Laboratory of Neuropathology Escourolle, la Pitié-Salpêtrière Hospital, AP-HP, Paris, France; [4]Service of neurology, CHU of Angers, France; [5]Laboratory of Clinical Neurophysiology, Hospital of Specialties, Rabat, Morocco; [6]Service of Neurology, Razi Hospital, Tunis, Tunisia; [7]Division of Pediatric Neurology, Department of Pediatrics, College of Medicine, King Saud University, Riyadh, Saudi Arabia; [8]Laboratory of Neurogenetics, Hospital of specialties, Rabat, Morocco; [9]Service of neurology, CHU of Dakar, Senegal; [10]AFM-Généthon Paris, France; [11]Service of Pediatric Neurology, Trousseau Hospital, Paris, France; [12]Department of Neurology and Neurogenetics, CHU Mustapha, Algiers, Algeria; and [13]Laboratory of Neurogenetics, Department of Genetics, Cytogenetics and Embryology, la Pitié-Salpêtrière Hospital, AP-HP, Paris, France

Received August 5, 2005; Revised January 3, 2006; Accepted January 11, 2006

Abstract

Autosomal-recessive forms of Charcot-Marie-Tooth (ARCMT) account for less than 10% of the families in the European CMT population but are more frequent in the Mediterranean basin and the Middle East because of more widespread consanguinity. Until now, demyelinating ARCMT was more extensively studied at the genetic level than the axonal form. Since 1999, the number of localized or identified genes responsible for demyelinating ARCMT has greatly increased. Eight genes, *EGR2*, *GDAP1*, *KIAA1985*, *MTMR2*, *MTMR13*, *NDRG1*, *PRX*, and *CTDP1*, have been identified and two new loci mapped to chromosomes 10q23 and 12p11-q13. In this review, we will focus on the particular clinical and/or neuropathological features of the phenotype caused by mutations in each of these genes, which might guide molecular diagnosis.

doi: 10.1385/NMM:8:1–2:75

Index Entries: ARCMT; demyelinating CMT; axonal CMT; consanguinity.

*Author to whom all correspondence and reprint requests should be addressed. E-mail: leguern@ccr.jussieu.fr

Introduction

Pedigrees with a proven autosomal-recessive form of Charcot-Marie-Tooth (ARCMT) account for only 4% of the families in the European CMT population as in France (Dubourg et al., 2001). In contrast, in communities with a high percentage of consanguineous marriages, ARCMT is likely to account for 30–50% of all CMT cases (Martin et al., 1999). It should be noted that patients in Western countries who are affected by autosomal-recessive disease often appear as isolated cases because of the small size of the sibships. ARCMT forms are demyelinating or axonal, or even intermediate, as in dominant forms, but are generally distinguished by an earlier onset and a more severe disease course. Numerous genes have been identified, mostly in inbred families originating from North Africa or the Middle East. Table 1 summarizes the different loci and genes identified in demyelinating ARCMT (CMT4). All mutations (nonsense, frame-shift, deletions, etc.) cause a loss of function. In many patients, owing to the severity of the secondary axonal loss, electroneuromyographic examination is often incomplete or does not allow median nerve conduction velocities (MNCVs) to be measured. In such cases, a nerve biopsy may be needed to determine the nature of the neuropathy and is of great diagnostic value in the case of a demyelinating process. For this reason, nerve biopsy features will be mentioned for each of the CMT4 subtypes.

The Different CMT4 Subtypes

Most CMT4 subtypes are clinically characterized by an onset in early infancy, sometimes congenital or with delayed motor milestones. The atrophy and weakness initially involve distal limbs but may progress to proximal limbs, especially the lower limbs, resulting in the loss of the ability to walk. The involvement of peripheral nerves might even be more widespread in CMT4. Thus, vocal cord paresis, bulbar, facial, and diaphragmatic weakness and sensorineural deafness might sometimes be observed. Some of these associated signs are closely related to a given CMT4 subtype and must therefore be carefully looked for in order to guide the molecular diagnosis. Table 2 indicates the different CMT4 subtypes in terms of age at onset, disease course, associated signs, MNCV values, and histopathological features.

CMT4A

CMT4A was originally mapped to chromosome 8q21.3 in four Tunisian families with a severe phenotype (Ben Othmane et al., 1993), and was subsequently shown to be resulting from mutations in the gene encoding ganglioside-induced differentiation-associated protein 1 (*GDAP1*) gene (Baxter et al., 2002 and Cuesta et al., 2002). GDAP1 is expressed in Schwann cells and in the central nervous system and has sequence similarities to glutathione-*S*-transferase. The protein is localized in mitochondria and its function may be related to the maintenance of the mitochondrial network (Pedrola et al., 2005). CMT4A is characterized by a severe neuropathy beginning in early childhood (before the age of 3), rapidly progressive distal (and further proximal) weakness and atrophy of the limbs, leading to an inability to walk in adolescence or early adulthood. MNCVs range from 25 to 35 m/s. Histopathological features include loss of myelinated fibers, especially those of large diameter, hypomyelination, segmental demyelination, and onion bulb formations. The same gene is also responsible for autosomal-recessive axonal (Cuesta et al., 2002, Azzedine et al., 2003a; Birouk et al., 2003) as well as intermediate CMT (Senderek et al., 2003a). The locus is also close to that responsible for autosomal-recessive CMT with pyramidal signs (Barhoumi et al., 2001).

CMT4B1

CMT4B1 is caused by mutations in the gene encoding the myotubularin-related protein 2 (MTMR2) (Bolino et al., 2000). This protein is expressed at high levels in Schwann cells as well as in neurons and is a member of the dual-specificity phosphatase superfamily, characterized by a protein/tyrosine phosphatase domain and an SID (Suvar 3-9, Enhancer-of-zeste, Trithorax [SET]-interacting domain). The latter interacts with proteins containing an SET domain that associates with chromatin. Myotubularin 1 (*MTM1*), another member of the myotubularin family, is responsible for X-linked myotubular myopathy (Laporte et al., 1996). Berger et al. (2002) showed that mouse Mtmr2 dephosphorylates phosphatidylinositol 3-phosphate (PI3P) and phosphatidylinositol 3,5-bisphosphate (PI3,5P2) with

Table 1
Genes and Proteins Involved in Autosomal-Recessive Forms of Demyelinating CMT

Type of CMT	Localization of the gene	Gene	Protein	Function
CMT4A	8q13-q21	GDAP1	Ganglioside-induced differentiation-associated protein 1	This mitochondrial protein may be implicated in the maintenance of the mitochondrial network
CMT4B1	11q22	MTMR2	Myotubularin-related protein 2	MTMR2 dephosphorylates PI3P and PI35P2
CMT4B2	11p15	MTMR13/SBF2	Myotubularin-related protein 13/SET binding factor 2	MTMR13 (SBF2), which has an inactive phosphatase site, interacts with MTMR2
CMT4C	5q23-q33	KIAA1985	Unknown	Protein with TPR and SH3 motifs; function unknown
CMT4D or HMSNL	8q24	NDRG1	N-myc downregulated gene/ Protein regulated by oxygen 1-PROXY1	NDRG1 is essential for maintenance of the myelin sheaths in peripheral nerves
CMT4E	10q	EGR2	Early growth factor 2	Transcription factor; key factor in myelination
CMT4F	19q	PRX	Periaxin	Role in the maintenance of the myelin
CCFDN	18qter	CTDP1	FCP1 protein phosphatase	Essential component of the eukaryotic transcription machinery
HMNSR	10q23	Unknown	Unknown	Unknown
CMT4H	12q11.21-q13.11	Unknown	Unknown	Unknown

Table 2
Main Characteristics of Autosomal Recessive Demyelinating CMT or CMT4

Locus Gene	Reference	Origin	Age at onset	Clinical characteristics	Median MNCV	Histopathological features
CMT4A GDAP1	Ben Othmane et al. (1993); Baxter et al. (2002); Cuesta et al. (2002)	Tunisia, Libya, Algeria, Belgium	<3 yr	Distal + proximal limbs Diaphragm	27–35 m/s	Hypomyelination Onion bulbs Loss of myelinated fibers
CMT4B1 MTMR2	Bolino et al. (1996); Bolino et al. (2000)	Italy, Saudi Arabia, England, India	<4 yr	Distal + proximal limbs Facial, bulbar and diaphragmatic weakness	9–20 m/s	Myelin outfolding Loss of myelinated fibers
CMT4B2 MTMR13 /SBF2	Ben Othmane et al. (1999); Azzedine et al. (2003b); Senderek et al.(2003b)	Tunisia, Morocco, Turkey,	1st decade	Distal and sometimes proximal limbs; Congenital glaucoma in some patients	15–30 m/s	Myelin outfolding Loss of myelinated fibers
CMT4C KIAA1985	LeGuern et al. (1996); Senderek et al. (2003c)	Algeria, Germany, Greece, Iran, Italy, The Netherlands, Turkey	1st or 2nd decade	Distal and sometimes proximal limbs Severe and early scoliosis	4–37 m/s	Basal membrane onion bulbs Cytoplasmic expansions of Schwann cells Loss of myelinated fibers
CMT4D NDRG1	Angelicheva et al. (1999); Kalaydjieva et al. (2000)	Gypsies from Bulgaria, Slovenia, Spain, Italy	<10 yr	Distal + proximal limbs Sensorineural deafness Tongue atrophy	10–20 m/s	Hypomyelination Onion bulbs Myelin decompaction Axonal inclusions Loss of myelinated fibers
CMT4E EGR2	Warner et al. (1998)	United States	Birth	Congenital hypotonia	5–20 m/s	Congenital hypomyelination
CMT4F PRX	Delague et al. (2000); Guilbot et al. (2001); Boerkoel et al.(2001)	Libya, United States, Northern Europe	<7 yr	Distal and sometimes proximal limbs	<5 m/s	Onion bulbs Myelin outfolding Loss of myelinated fibers
CCFDN CTDP1	Angelicheva et al. (1999); Varon et al., (2003)	Bulgaria (Gypsies)	Congenital (cataract)	Congenital cataract and microcornea, facial dysmorphism, mental retardation and distal motor peripheral neuropathy	19–33 m/s	Diffuse hypomyelination
HMSN Russe 10q22-q23	Rogers et al. (2000)	Bulgaria	8–16 yr	Distal + proximal limbs	30–35 m/s	Hypomyelination Regenerating clusters
CMT4H	De Sandre-Giovanolli et al. (2005)	Lebanon, Algeria	1–2 yr	Distal limbs, lower>upper limbs	Decreased	Hypomyelination Small onion bulbs and sometimes myelin outfoldings

high efficiency, whereas MTM1 acts only on PI3P. Disease onset is in early infancy (before the age of 4). Early developmental milestones are normal. The atrophy and weakness initially involve the distal parts of the four limbs and progress toward proximal muscles, resulting in the need for a wheelchair from late childhood or adolescence. Facial, bulbar, and diaphragmatic involvement has been reported in some families (Quattrone et al., 1996; Houlden et al., 2001; Verny et al., 2004). Life span was shortened in the large Italian family reported by Quattrone et al. (1996), with death occurring in the fourth or fifth decade. MNCVs range from 9 to 20 m/s and are often undetectable in adult patients. Histopathological examination shows irregular folding and redundant loops of myelin, the so-called myelin outfoldings, and a secondary axonal loss.

CMT4B2

Genetic heterogeneity of CMT4B (i.e., CMT4 with focally folded myelin sheaths) was demonstrated by the absence of linkage to the 11q22.1 locus in Tunisian families, and was subsequently mapped to chromosome 11p15 (Othmane et al., 1999). This locus has been named *CMT4B2*. Two teams independently identified the responsible gene in consanguineous families with different phenotypes. In the Turkish family reported by Senderek et al. (2003b), the phenotype was less severe than in CMT4B1 patients. Disease onset was around 5 yr of age and the patients had severe distal motor and sensory neuropathies. The proximal muscles were spared, however, and two patients could still walk with aid in the third decade. The responsible gene was called *SBF2* (SET binding factor 2). In the two families from Tunisia and Morocco reported by Azzedine et al. (2003b), the demyelinating motor and sensory neuropathy segregated with a congenital glaucoma. They called the responsible gene myotubularin-related protein-13 (*MTMR13*), as it clearly belonged to the MTM1-related protein family. The same association has been reported in a Japanese family with an *MTMR13*/*SBF2* mutation (Hirano et al., 2004). Azzedine et al. (2003b) postulated that *MTMR13* could be involved in both the differentiation of Schwann cells of peripheral nerves during myelination and in the formation and development of the trabeculum meshwork, which permits the aqueous humor outflow, because both types of cells derived from the neural crest. Very recently, Robinson and Dixon (2005) have shown that *MTMR13*, in which the phosphatase domain is catalytically inactive, functions in association with *MTMR2*. They hypothesized that the loss of *MTMR13* function in CMT4B2 patients might lead to alterations in *MTMR2* function, and subsequent alterations in 3-phosphoinositide signaling.

CMT4C

CMT4C was mapped to chromosome 5q23-q33 (LeGuern et al., 1996) and was recently found to be associated with mutations in the *KIAA1985* gene (Senderek et al., 2003c). This gene encodes a protein of unknown function containing SH3 and TPR motifs that is expressed in neural tissues, including peripheral nerve. It could represent a relatively frequent cause of CMT4. Mutations in the *KIAA1985* gene have been found in families of diverse geographic origin: Algeria, France, Germany, Greece, Iran, Italy, the Netherlands, and Turkey (LeGuern et al., 1996; Gabreëls-Festen et al., 1999; Guilbot et al., 1999; Senderek et al., 2003c; Azzedine et al. [submitted]). A delay in walking may be observed. Disease onset is in the first decade or adolescence. Progression of the neuropathy is generally slow, but some patients become wheelchair-dependent as a result of involvement of the proximal lower limbs. The hallmark of CMT4C is the presence of early and severe scoliosis, which is reported to be the presenting sign in the majority of patients (Kessali et al., 1997; Gabreëls-Festen et al., 1999), and might require surgery. Median MNCVs range from 4 to 37 m/s, with a mean of 22 m/s. Nerve biopsies show a loss of myelinated fibers, relatively few and small classical onion bulbs, as observed in CMT1A, but also basal membrane onion bulbs, consisting of concentric Schwann cell lamellae intermingled with single or double basal membranes or concentric basal membranes alone, and multiple cytoplasmic processes of the Schwann cells of unmyelinated axons (Kessali et al., 1997; Gabreëls-Festen et al., 1999). Gabreëls-Festen et al. (1999) considered this combination of morphological features to be unique among the demyelinating forms of CMT.

HMSNL or CMT4D

CMT4D, which was originally designated HMSNL (hereditary motor and sensory neuropathy—Lom)

because it was first diagnosed in Bulgarian Gypsies from Lom, a small town on the Danube river, was mapped to chromosome 8q24 (Kalaydjieva et al., 1996). The responsible gene is *NDRG1* (N-myc downstream-regulated gene 1), which encodes a protein highly expressed in Schwann cells (Kalaydjieva et al., 2000). Okuda et al. (2004) showed that myelination in the sciatic nerve of Ndrg1 knockout mice was normal for 2 wk after birth, but the sciatic nerve degenerated with demyelination at about 5 wk of age, highly supporting that *NDRG1* plays an important role for maintenance of the myelin sheaths in peripheral nerves.

Disease onset is in the first decade with delayed walking in some patients. Difficulty in using the hands is noted between the ages of 5 and 15 yr. Distal lower limb involvement is usually severe and might compromise ability to walk. Tongue atrophy is a frequent finding. Sensorineural deafness generally develops during the second or third decade. Brainstem auditory evoked potentials (BAEPs) show an increase in the I–V interpeak latency. Nerve conduction studies give evidence of a profound and diffuse demyelinating neuropathy with median MNCVs around 14 m/s (Kalaydjieva et al., 1998). Nerve biopsies show a severe depletion of myelinated fibers, with a complete loss of large diameter fibers, hypomyelination, and multiple poorly developed onion bulbs, which tend to disappear in older individuals (Kalaydjieva et al., 1998). Myelin decompaction and axonal inclusions with curvilinear structures have also been described (Baethman et al., 1998; King et al., 1999).

CMT4E

CMT4E is caused by mutations in the early growth response 2 *(EGR2)* gene (Warner et al., 1998), a key factor in myelination. *EGR2* gene mutations are associated with a large spectrum of CMT phenotypes, ranging from congenital hypomyelination neuropathy (CHN) to late-onset demyelinating CMT1 (Boerkoel et al., 2001a; Yoshihara et al., 2001). They might be transmitted in an autosomal-dominant (*CMT1D*) or autosomal-recessive (*CMT4E*) fashion (Warner et al., 1998). Very few mutations have been reported to date, and *CMT1D/4E* appears to represent less than 1% of CMT cases in large cohorts (Boerkoel et al., 2002; Numakura et al., 2003). EGR2 is a transcription factor with zinc fingers, which binds DNA regulatory domains of target genes. It is expressed at a high level early in the myelination of the peripheral nervous system and is mainly implicated in the regulation of the expression of the myelin genes, such as *MPZ* (Hayazaka et al., 1993), *PMP22* (Lupski et al., 1991; Matsunami et al., 1992; Timmerman et al., 1992; Valentijn et al., 1992), and *CX32* (Bergoffen et al., 1993) and *PRX* (Boerkoel et al., 2001b; Guilbot et al., 2001).

CMT4F

CMT4F results from mutations within the periaxin (*PRX*) gene (Boerkoel et al., 2001b and Guilbot et al., 2001). It encodes two proteins with PDZ domains, L- and S-periaxin that are expressed in myelinating Schwann cells. During myelination, L-periaxin is predominantly located at the adaxonal membrane but, once myelination is finished, it is localized at the abaxonal membrane, Schmidt-Lantermann incisures and paranodal membranes (Gillespie et al., 1994). Periaxins interact through PDZ domains with the dystroglycan-dystrophin-related protein-2 complex linking the Schwann cell cytoskeleton to the extracellular matrix (Sherman et al., 2001). Periaxins are thought to be essential for the maintenance of peripheral nerve myelin. The phenotype has been reported as Dejerine–Sottas disease, because the onset is in early infancy and delayed motor milestones are frequently noted (Boerkoel et al., 2001b; Takashima et al., 2002). However, the disease course can be quite variable: severe in some patients with distal and proximal involvement of the limbs in the first decade or slow in others with predominantly distal sensory signs (Takashima et al., 2002; Kijima et al., 2004). In a cohort of 66 Japanese patients with demyelinating CMT who were negative for the gene mutation causing dominant or X-linked demyelinating CMT, Kijima et al. (2004) found three (4.5%) *PRX* gene mutations. MNCVs are unrecordable or very slow (<5 m/s), with a marked temporal dispersion of compound muscle action potentials (CMAPs). Nerve biopsies show a severe loss of axons of all diameters, tomacula, and onion bulbs. Paranodal abnormalities, including a reduced number of myelin loops and an absence of septate-like junctions between the paranodal myelin and axon, have been reported in a single patient (Takashima et al., 2002).

Congenital Cataract, Facial Dysmorphism, and Neuropathy Syndrome

Tournev et al. (1999) identified in Gypsies from Bulgaria a new autosomal-recessive disorder including congenital cataracts and microcornea, delayed motor and intellectual development, facial dysmorphism, and a progressive distal predominantly motor peripheral neuropathy in which the lower limbs are affected first. Motor and sensory conduction studies suggested neuropathy and nerve biopsies showed diffuse hypomyelination, associated with demyelination and axonal degeneration in older subjects. The central nervous system was also involved in some patients in whom a mild nonprogressive cognitive deficit was associated with extensor plantar responses, mild chorea, upper limb postural tremor, and mild ataxia. The gene responsible for this syndrome was mapped to chromosome 18qter (Angelicheva et al., 1999). A single nucleotide substitution in the antisense Alu element in intron 6 of the *CTDP1* gene encoding the FCP1 protein cosegregated with the phenotype. This mutation led to aberrant splicing. The FCP1 protein is a TFIIF-associating CTD phosphatase-1, which dephosphorylates the C-terminal domain of the largest RNA polymerase II subunit and regulates recruitment of proteins involved in transcription and mRNA processing (Varon et al., 2003).

HMSN-Russe

HMSNR has been identified in Bulgarian, Romanian, and Spanish Gypsies (Rogers et al., 2000; Thomas et al., 2001) and mapped to chromosome 10q23, close to the *EGR2* gene. Distal lower limb weakness begins between the ages of 8 and 16 yr and distal upper limb involvement between 10 and 43 yr. This progresses toward a severe distal weakness of the four limbs. MNCVs are moderately reduced in the upper limbs (32 m/s). Nerve biopsies show a loss of large myelinated fibers, abnormally thinly myelinated fibers, and profuse regeneration (Thomas et al., 2001).

CMT4H

Recently, De Sandre-Giovannoli et al. (2005) reported a new locus on chromosome 12p11.21-q13.11 in two consanguineous families, one from Lebanon and the other from Algeria. Onset was in the first or second year. In all cases, walking was delayed and patients presented with severe scoliosis and pes equinus with toe retraction. Some patients had thenar and hypothenar amyotrophy in the upper limbs. However, the progression of the disease was slow. Electrophysiological examination confirmed the demyelinating nature of the pathological process. Nerves biopsies show a severe loss of myelinated fibers generally surrounded by small onion bulbs or an abnormal proliferation of myelin sheaths with outfoldings (De Sandre-Giovannoli et al., 2005).

Conclusion

In the past 10 yr, molecular data on demyelinating ARCMT has accumulated, 10 loci have been mapped and 8 genes identified (Table 1). This "success story" was possible mainly because of a two-step strategy combining linkage analyses by homozygocity mapping in large consanguineous families and a candidate-gene approach in the incriminated genetic intervals. However, in some cases, studies in animal models suggested the identity of the causative gene, as in the case of *EGR2* (Warner et al., 1998) and *PRX* (Guilbot et al., 2001; Boerkoel et al., 2001b).

The identification of a new locus or gene makes it possible to perform molecular diagnoses in an increasing number of families. Because of the severity of demyelinating ARCMT, genetic counseling, and prenatal diagnosis are often crucial for at-risk family members.

The molecular diagnosis of CMT is very complex and time consuming owing to its very great genetic heterogeneity. As the systematic screening of all responsible genes is not technically feasible today, molecular strategies based on the phenotype, after a detailed clinical and electrophysiological examination, have been developed. In Western countries, patients with ARCMT often appear to be isolated cases. Genetic counseling is therefore problematical because several modes of inheritance are possible: (1) *de novo* mutations (as described for the 17p12 duplication and for *PMP22*, *MPZ*, *Cx32*, *EGR2* and *NEFL* gene mutations); (2) dominant transmission with incomplete penetrance, which is difficult to validate when the parents are not available for clinical and electrophysiological studies; or (3) autosomal-recessive transmission. Patients with isolated

demyelinating CMT should be systematically tested for the presence of the 17p12 duplication, as this accounts for 40% of cases. Mutations in the *MPZ*, *Cx32*, and *PMP22* genes account for an additional 10%. In other cases of demyelinating CMT without a familial history, molecular diagnosis might be orientated by particular clinical features and/or by nerve biopsy results. The presence of vocal cord paresis, facial and bulbar paralysis, early glaucoma, severe scoliosis, associated with either an early onset and/or a severe course, are suggestive of an autosomal recessive demyelinating CMT, implicating the *GDAP1*, *MTMR2*, *MTMR13*, or *KIAA1985* genes. Moreover, when a demyelinating ARCMT is suspected, a nerve biopsy can be extremely helpful: myelin outfoldings point to mutations in the *MTMR2*, *MTMR13*, or *PRX* genes, the presence of basal membrane onion bulbs are characteristic of a mutation in the *KIAA1985* gene and pseudo-onion bulbs are associated with mutations in the *GDAP1* gene. Neuropathological examinations also help to diagnose patients for whom motor nerve conduction velocities are not recordable, as is sometimes seen in CMT4. In the Mediterranean basin, especially in the Maghreb and the Middle East, it is often easy to identify an autosomal recessive mode of inheritance, as when two affected children of related parents are found in large sibships. There are some pitfalls, however. For example, transmission of the disease from one parent to his children is not sufficient to exclude autosomal-recessive mode of inheritance if the grandparents were also related and both carriers of the disease (pseudodominance). As for isolated cases, the molecular diagnosis has to be guided by the clinical and/or morphological phenotype. Molecular diagnosis is also complicated by the fact that we do not know precisely the relative frequency of each form of CMT4 in either Western or Mediterranean populations. Moreover, despite the large number of genes responsible for demyelinating ARCMT that have been identified in the past decade, other genes still remain to be identified.

The large spectrum of proteins responsible for demyelinating ARCMT and the great variety of their functions in Schwann cells (structural protein, such as PRX; zinc transcription factor, such as EGR2; Myotubularins, such as MTMR2 and MTMR13/SBF2 and so on) make it difficult to develop specific therapeutic approaches aimed at correcting each dysfunction. Such approaches are already being developed for the most frequent form of CMT, CMT1A. Sereda et al. (2003) reported that, in a rat model, treatment with onapristone, a progesterone antagonist, was able to downregulate PMP22 expression and decrease the motor deficit. Ascorbic acid treatment had similar effects in a CMT1A mouse model (Passage et al., 2004). In demyelinating ARCMT, a more global approach might be developed, aimed at preventing or decreasing axonal loss, which is common to all forms of CMT and responsible for the functional disability. Neuroprotective or neurotrophic factors might therefore be of use. However, myelination in humans begins in the prenatal period about 16 wk after amenorrhea, and at birth, the dysmyelination as caused by mutations in genes responsible for CMT4 is already in progress. Protective treatments would, therefore, have to begin very early in childhood and this would require an early molecular diagnosis.

Acknowledgments

This work was supported by Association Française contre les Myopathies (AFM), the Assistance Publique des Hôpitaux de Paris (AP-HP), the Institut National de la Santé et de la Recherche Médicale (INSERM) and the GIS-Maladies rares. All authors are members of the "French GIS-maladies rares research network on autosomal recessive forms of CMT."

References

Angelicheva D., Turnev I., Dye D., Chandler D., Thomas P. K., and Kalaydjieva L. (1999) Congenital cataracts facial dysmorphism neuropathy (CCFDN) syndrome: a novel developmental disorder in Gypsies maps to 18qter. *Eur. J. Hum. Genet.* **7,** 560–566.

Azzedine H., Ruberg M., Ente D., et al. (2003a) Variability of disease progression in a family with autosomal recessive CMT associated with a S194X and new R310Q mutation in the GDAP1 gene. *Neuromuscul. Disord.* **13,** 341–346.

Azzedine H., Bolino A., Taieb T., et al. (2003b) Mutations in MTMR13, a new pseudophosphatase homologue of MTMR2 and Sbf1, in two families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease associated with early onset glaucoma. *Am. J. Hum. Genet.* **72,** 1141–1153.

Baethmann M., Gohlich-Ratmann G., Schroder J. M., Kalaydjieva L., and Voit T. (1998) HMSNL in a 13-year-old Bulgarian girl. *Neuromuscul. Disord.* **8,** 90–94.

Barhoumi C., Amouri R., Ben Hamida C., et al. (2001) Linkage of a new locus for autosomal recessive axonal form of Charcot-Marie-Tooth disease to chromosome 8q21.3. *Neuromuscul. Disord.* **11,** 27–34.

Baxter R. V., Ben Othmane K., Rochelle J. M., et al. (2002) Ganglioside-induced differentiation-associated protein-1 is mutant in Charcot-Marie-Tooth disease type 4A/8q21. *Nat. Genet.* **30,** 21, 22.

Ben Othmane K., Hentati F., Lennon F., et al. (1993) Linkage of a locus (CMT4A) for autosomal recessive Charcot-Marie-Tooth disease to chromosome 8q. *Hum. Mol. Genet.* **2,** 1625–1628.

Ben Othmane K., Johnson E., Menold M., et al. (1999) Identification of a new locus for autosomal recessive Charcot-Marie-Tooth disease with focally folded myelin on chromosome 11p15. *Genomics* **62,** 344–349.

Berger P., Bonneick S., Willi S., Wymann M., and Suter U. (2002) Loss of phosphatase activity in myotubularin-related protein 2 is associated with Charcot-Marie-Tooth disease type 4B1. *Hum. Mol. Genet.* **11,** 1569–1579.

Bergoffen J., Scherer S. S., Wang S., et al. (1993) Connexin mutations in X-linked Charcot-Marie-Tooth disease. *Science* **262,** 2039–2042.

Birouk N., Azzedine H., Dubourg O., et al. (2003) Phenotypical features of a Moroccan family with autosomal recessive Charcot-Marie-Tooth disease associated with the S194X mutation in the GDAP1 gene. *Arch. Neurol.* **60,** 598–604.

Boerkoel C. F., Takashima H., Bacino C. A., Daentl D., and Lupski J. R. (2001a) EGR2 mutation R359W causes a spectrum of Dejerine-Sottas neuropathy. *Neurogenetics* **3,** 153–157.

Boerkoel C. F., Takashima H., Stankiewicz P., et al. (2001b) Periaxin mutations cause recessive Dejerine-Sottas neuropathy. *Am. J. Hum. Genet.* **68,** 325–333.

Boerkoel C. F., Takashima H., Garcia C. A., et al. (2002) Charcot-Marie-Tooth disease and related neuropathies: mutation distribution and genotype-phenotype correlation. *Ann. Neurol.* **51,** 190–201.

Bolino A., Brancolini V., Bono, F., et al. (1996) Localisation of a gene responsible for autosomal recessive demyelinating neuropathy with focally folded myelin sheaths to chromosome 11q23 by homozygosity mapping and haplotype sharing. *Hum. Mol. Genet.* **5,** 1051–1054.

Bolino A., Muglia M., Conforti F. L., et al. (2000) Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. *Nat. Genet.* **25,** 17–19.

Cuesta A., Pedrola L., Sevilla T., et al. (2002) The gene encoding ganglioside-induced differentiation-associated protein 1 is mutated in axonal Charcot-Marie-Tooth type 4A disease. *Nat. Genet.* **30,** 22–25.

Delague V., Bareil C., Tuffery S., et al. (2000) Mapping of a new locus for autosomal recessive demyelinating Charcot-Marie-Tooth disease to 19q13.1-13.3 in a large consanguineous Lebanese family: exclusion of MAG as a candidate gene. *Am. J. Hum. Genet.* **67,** 236–243.

De Sandre-Giovannoli A., Delague V., Hamadouche T., et al. (2005) Homozygosity mapping of autosomal recessive demyelinating Charcot-Marie-Tooth neuropathy (CMT4H) to a novel locus on chromosome 12p11.21-q13.11. *J. Med. Genet.* **42,** 260–265.

Dubourg O., Tardieu S., Birouk N., et al. (2001) The frequency of 17p12 duplication and Connexin 32 mutations in 282 CMT families in relation to the mode of inheritance and motor nerve conduction velocity. *Neuromuscul. Disord.* **11,** 458–463.

Gabreëls-Festen A., van Beersum S., Eshuis L., et al. (1999) Study on the gene and phenotypic characterisation of autosomal recessive demyelinating motor and sensory neuropathy (Charcot-Marie-Tooth disease) with a gene locus on chromosome 5q23-q33. *J. Neurol. Neurosurg. Psychiatry* **66,** 569–574.

Gillespie C. S., Sherman D. L., Blair G. E., and Brophy P. J. (1994) Periaxin, a novel protein of myelinating Schwann cells with a possible role in axonal ensheathment. *Neuron* **12,** 497–508.

Guilbot A., Kessali M., Ravise N., et al. (1999) The autosomal recessive form of CMT disease linked to 5q31-q33. *Ann. NY Acad. Sci.* **883,** 453–456.

Guilbot A., Williams A., Ravise N., et al. (2001) A mutation in periaxin is responsible for CMT4F, an autosomal recessive form of Charcot-Marie-Tooth disease. *Hum. Mol. Genet.* **10,** 415–421.

Hayasaka K., Himoro M., Sato W., et al. (1993) Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P(0) gene. *Nature Genet.* **5,** 31–34.

Hirano R., Takashima H., Umehara F., et al. (2004) SET binding factor 2 (SBF2) mutation causes CMT4B with juvenile-onset glaucoma. *Neurology* **63,** 577–580.

Houlden H., King R. H., Wood N. W., Thomas P. K., and Reilly M. M. (2001) Mutations in the 5′ region of the myotubularin-related protein 2 (MTMR2) gene in autosomal recessive hereditary neuropathy with focally folded myelin. *Brain* **124,** 907–915.

Kalaydjieva L., Hallmayer J., Chandler D., et al. (1996) Gene mapping in Gypsies identifies a novel demyelinating neuropathy on chromosome 8q24. *Nat. Genet*. **14,** 214–217.

Kalaydjieva L., Nikolova A., Turnev I., et al. (1998) Hereditary motor and sensory neuropathy-Lom, a novel demyelinating neuropathy associated with deafness in gypsies. Clinical, electrophysiological and nerve biopsy findings. *Brain* **121,** 399–408.

Kalaydjieva L., Gresham D., Gooding R., et al. (2000) N-myc downstream-regulated gene 1 is mutated in hereditary motor and sensory neuropathy-Lom. *Am. J. Hum. Genet.* **67,** 47–58.

Kessali M., Zemmouri R., Guilbot A., et al. (1997) A clinical, electrophysiologic, neuropathologic, and genetic study of two large Algerian families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease. *Neurology* **48,** 867–873.

Kijima K., Numakura C., Shirahata E., et al. (2004) Periaxin mutations causes early-onset but slow-progressive Charcot-Marie-Tooth disease. *J. Hum. Genet*. **49,** 376–379.

King R. H., Tournev I., Colomer J., Merlini L., Kalaydjieva L., and Thomas P. K. (1999) Ultrastructural changes in peripheral nerve in hereditary motor and sensory neuropathy-Lom. *Neuropathol. Appl. Neurobiol.* **25,** 306–312.

Laporte J., Hu L. J., Kretz C., et al. (1996) gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nature Genet.* **13,** 175–182.

LeGuern E., Guilbot A., Kessali M., et al. (1996) Homozygosity mapping of an autosomal recessive form of demyelinating Charcot-Marie-Tooth disease to chromosome 5q23-q33. *Hum. Mol. Genet*. **5,** 1685–1688.

Lupski J. R., Montes de Oca-Luna R., Slaugenhaupt S., et al. (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell* **66,** 219–232.

Martin J. J., Brice A., and Van Broeckhoven C. (1999) 4th Workshop of the European CMT-Consortium—62nd ENMC International Workshop. Rare forms of Charcot-Marie-Tooth disease and related disorders. 16–18 October 1998, Soestduinen, The Netherlands. *Neuromuscul. Disord.* **9,** 279–287.

Matsunami N., Smith B., Ballard L., et al. (1992) Peripheral myelin protein-22 gene maps in the duplication in chromosome 17p12 associated with Charcot-Marie-Tooth 1A. *Nat. Genet.* **1,** 176–179.

Numakura C., Shirahahta E., Yamashita S., et al. (2003) Screening of the early growth response 2 gene in Japanese patients with Charcot-Marie-Tooth disease type 1. *J. Neurol. Sci.* **15,** 61–64.

Okuda T., Higashi Y., Kokame K., Tanaka C., Kondoh H., and Miyata T. (2004) Ndrg1-deficient mice exhibit a progressive demyelinating disorder of peripheral nerves. *Mol. Cell Biol*. **24,** 3949–3956.

Othmane K. B., Johnson E., Menold M., et al. (1999) Identification of a new locus for autosomal recessive Charcot-Marie-Tooth disease with focally folded myelin on chromosome 11p15. *Genomics* **62,** 344–349.

Passage E., Norreel J. C., Noack-Fraissignes P., et al. (2004) Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. *Nat. Med.* **10,** 396–401.

Pedrola L., Espert A., Wu X., Claramunt R., Shy M. E., and Palau F. (2005) GDAP1, the protein causing Charcot-Marie-Tooth disease type 4A, is expressed in neurons and is associated with mitochondria. *Hum. Mol. Genet.* **14,** 1087–1094.

Quattrone A., Gambardella A., Bono F., et al. (1996) Autosomal recessive hereditary motor and sensory neuropathy with focally folded myelin sheaths: clinical, electrophysiologic, and genetic aspects of a large family. *Neurology* **46,** 1318–1324.

Robinson F. L. and Dixon J. E. (2005) The phosphoinositide 3-phosphatase MTMR2 associates with MTMR13, a novel membrane-associated pseudophosphatase also mutated in type 4B Charcot-Marie-Tooth disease. *J. Biol. Chem.* **36,** 31,699–31,707.

Rogers T., Chandler D., Angelicheva D., et al. (2000) A novel locus for autosomal recessive peripheral neuropathy in the EGR2 region on 10q23. *Am. J. Hum. Genet.* **67,** 664–667.

Senderek J., Bergmann C., Ramaekers V. T., et al. (2003a) Mutations in the ganglioside-induced differentiation-associated protein-1 (GDAP1) gene in intermediate type autosomal recessive Charcot-Marie-Tooth neuropathy. *Brain* **126,** 642–649.

Senderek J., Bergmann C., Weber S., et al. (2003b) Mutation of the SBF2 gene, encoding a novel member of the myotubularin family, in Charcot-Marie-Tooth neuropathy type 4B2/11p15. *Hum. Mol. Genet*. **12,** 349–356.

Senderek J., Bergmann C., Stendel C., et al. (2003c). Mutations in a gene encoding a novel SH3/TPR domain protein cause autosomal recessive Charcot-Marie-Tooth type 4C neuropathy. *Am. J. Hum. Genet*. **73,** 1106–1119.

Sereda M. W., Meyer zu Hörste G., Suter U., Uzma N., and Nave K. A. (2003) Therapeutic administration

of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A). *Nat. Med.* **9,** 1533–1537.

Sherman D. L., Fabrizi C., Gillespie C. S., and Brophy P. J. (2001) Specific disruption of a Schwann cell dystrophin-related protein complex in a demyelinating neuropathy. *Neuron* **30,** 677–687.

Takashima H., Boerkoel C. F., De Jonghe P., et al. (2002) Periaxin mutations cause a broad spectrum of demyelinating neuropathies. *Ann. Neurol.* **51,** 709–715.

Thomas P. K., Kalaydjieva L., Youl B., et al. (2001) Hereditary motor and sensory neuropathy-russe: new autosomal recessive neuropathy in Balkan Gypsies. *Ann. Neurol.* **50,** 452–457.

Timmerman V., Nelis, E., Van Hul W., et al. (1992) The peripheral myelin protein gene PMP-22 is contained within the Charcot-Marie-Tooth disease type 1A duplication. *Nat. Genet.* **1,** 171–175.

Tournev I., King R. H. M., Workman J., et al. (1999) Peripheral nerve abnormalities in the congenital cataracts facial dysmorphism neuropathy (CCFDN) syndrome. *Acta Neuropathol.* **98,** 165–170.

Valentijn L. J., Bolhuis P. A., Zorn I., et al. (1992) The peripheral myelin gene PMP-22/GAS-3 is duplicated in Charcot-Marie-Tooth disease type 1A. *Nat. Genet.* **1,** 166–170.

Varon R., Gooding R., Steglich C., et al. (2003) Partial deficiency of the C-terminal-domain phosphatase of RNA polymerase II is associated with congenital cataracts facial dysmorphism neuropathy syndrome. *Nat. Genet.* **35,** 185–189.

Verny C., Ravise N., Leutenegger A. L., et al. (2004) Coincidence of two genetic forms of Charcot-Marie-Tooth disease in a single family. *Neurology* **63,** 1527–1529.

Warner L. E., Mancias P., Butler I. J., et al. (1998) Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. *Nat. Genet.* **18,** 382–384.

Yoshihara T., Kanda F., Yamamoto M., et al. (2001) novel missense mutation in the early growth response 2 gene associated with late-onset Charcot-Marie-Tooth disease type 1. *J. Neurol. Sci.* **184,** 149–153.