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Myotubularin-Related (MTMR) Phospholipid Phosphatase Proteins in the Peripheral Nervous System

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Abstract Myotubularin-related proteins (MTMRs) constitute a broad family of ubiquitously expressed phosphatases with 14 members in humans, of which eight are catalytically active phosphatases, while six are catalytically inactive. Active MTMRs possess 3-phosphatase activity toward both PtdIns3*P* and PtdIns(3, 5)P_2 poliphosphoinositides (PPIn), suggesting an involvement in intracellular trafficking and membrane homeostasis. Among MTMRs, catalytically active MTMR2 and inactive MTMR13 have a nonredundant function in nerve. Loss of either MTMR2 or MTMR13 causes Charcot–Marie–Tooth type 4B1 and B2 neuropathy, respectively, characterized by demyelination and redundant loops of myelin known as myelin outfoldings. In *Mtmr2*-null mouse nerves, these aberrant foldings occur at 3–4 weeks after birth, a time when myelination is established, and Schwann cells are still elongating to reach the final internodal length. Moreover, *Mtmr2*-specific ablation in Schwann cells is both sufficient and necessary to provoke CMT4B1 with myelin outfoldings. MTMR2 phospholipid phosphatase might regulate intracellular trafficking events and membrane homeostasis in Schwann cells during postnatal nerve development. In this review, we will discuss recent findings on the MTMR family with a major focus on MTMR2 and MTMR13 and their putative role in Schwann cell biology.

Keywords Myelin · Schwann cells · Phosphatase

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Introduction

Impulse propagation by saltatory conduction in vertebrates is achieved by myelin, which wraps axons larger than 1 μm in the peripheral nervous system. Here, myelin is formed by compacted layers of Schwann cell membrane enrolling a single internode—the portion of the axon isolated by myelin—with numbers of "lamellae" that are proportionate to the axon diameter. In a myelinated fiber, both a radial as well as a longitudinal polarization are distinguished. The radial goes from the basal lamina, surrounding the outside of the Schwann cell, the compact myelin, to the periaxonal space. The longitudinal consists of few subdomains centered on the node of Ranvier, such as paranodal and juxtaparanodal regions (Fig. 1). This structure relies on the intimate relationship of Schwann cell with axon and Schwann cell with basal lamina [1, 2].

Charcot–Marie–Tooth diseases (CMTs) are the most frequent inherited disorders affecting myelinated fibers in the peripheral nervous system. CMT is usually characterized by progressive muscular atrophy and weakness affecting distal extremities, more often the lower limbs, with an age of onset around the first and the second decade. The clinical picture is very heterogenous, and variable expressivity is observed also among members of the same family. CMTs have been classically divided into demyelinating or CMT1, when the defect primarily occurs in myelin, characterized by nerve conduction velocity (NCV) below 38 m/s and axonal or CMT2, in which the pathogenesis initially involves the axon, characterized by normal or almost normal values of NCV [3]. CMTs are also genetically heterogenous with more than 40 loci mapped by linkage analysis and 23 genes identified thus far (http://www.molgen.ua.ac.be/CMTMutations/default.cfm). These genes encode a variety of proteins, which consist of structural proteins of compact myelin and

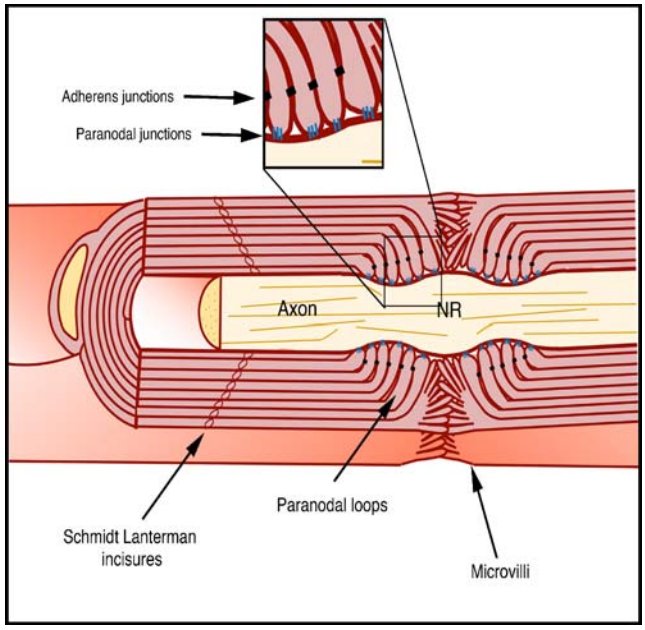

Fig. 1 Architecture of a myelinated fiber in the peripheral nervous system. Myelin is formed by layers of Schwann cell membrane, which wraps the axon along each internode. Apposition of these layers generates the so-called "compact-myelin" in contrast to the non-compact myelin, where Schwann cell cytoplasm is concentrated. The Schwann cell cytoplasm is restricted to a main region around the nucleus: to the layers adjacent to the axon (adaxonal) and outside of the sheath (abaxonal); the channels of communication between different layers of membrane—the Schmidt–Lanterman incisures; *microvilli*, the loops facing the node the Ranvier and to the *paranodal loops* flanking the node at the periphery of each internode

axons, molecules regulating protein synthesis or degradation and mitochondrial function, transcription factors, etc [3–5]. Surprisingly, also loss of the ubiquitously expressed phospholipid phosphatases MTMR2 and MTMR13—myotubularin-related 2 and 13—causes demyelinating CMT neuropathies [6–8]. Myotubularin-related proteins (MTMRs) belong to a newly identified family of phosphatases, so called because of the former member of the family, MTM1, myotubularin, mutated in the X-linked myotubular myopathy, XLMTM [9].

In this review, we will focus on MTMR2 and MTMR13, which are involved in the pathogenesis of demyelinating CMT neuropathies.

Myotubularin-Related Proteins

MTMRs are a broad family of proteins within the protein tyrosine phosphatase (PTP) superfamily. In humans, 14 members have been identified, of which eight are catalytically active proteins, while six are predicted to be catalytically inactive. Individual orthologues have been also demonstrated in Zebrafish, suggesting that most vertebrates have the same number of myotubularins in their genome [10]. Myotubularins all share a PH-GRAM (Pleckstrin Homology-Glucosyltransferase, Rab-like GTPase Activators and Myotubularins) domain at the N terminus, a PTP domain and a coiled-coil region at the C terminus (Fig. 2). The PH-GRAM domain binds to poliphosphoinositides, mainly PtdIns5*P* and PtdIns(3,5)P_2 [11, 12], and in MTMR2, it has been shown to mediate the targeting of the phosphatase to the membrane of vacuoles formed under hypoosmotic stress [13]. The PTP domain is responsible for the 3-phosphatase activity of MTM1, MTMR1, MTMR2, MTMR3, and MTMR6 toward both PtdIns3*P* and PtdIns(3,5)P_2 [11, 14–22]. Recently, the crystal structure of MTMR2 in complex with PtdIns3*P* and PtdIns(3,5)P_2 has provided the molecular basis for myotubularin substrate recognition [23]. Two catalytically essential residues that are present in the Cys-X_5-Arg conserved motif are mutated in six members of the family, which are predicted to be catalytically inactive. The coiled-coil domain mediates both hetero- and homodimerization between members of the family. Several interactions between active and inactive myotubularins have been demonstrated [20, 24, 25]. For example, the active MTMR2 phosphatase binds to MTMR5 and MTMR13, which are both inactive enzymes [26–28].

Specific subsets of myotubularins also contain other domains, such as the DENN (differentially expressed in normal versus neoplastic) domain, the FYVE (Fab1p/YOTB/Vac1p/EEA1), and PH domains, which bind poliphosphoinositides, and PDZ (PSD-95/Dlg/ZO-1) binding domains (PDZ-BD) at the C terminus (Fig. 2).

PtdIns3*P* and PtdIns(3,5)P_2 Are Substrates of Active MTMRs

Poliphosphoinositides (PPIn) play crucial roles in membrane trafficking, signal transduction, and cytoskeletal dynamics. PPIn differ in the phosphorylation status of their inositol ring, and their metabolism is strictly regulated [29, 30]. They are thought to function as spatially restricted lipid second messengers, which recruit and activate specific effectors at the cytosolic face of intracellular membranes. PPIn-effector proteins show conserved PPIn-binding domains, such as PH, PX (phox homology), FYVE, and ENTH (epsin N-terminal homology) domains [31, 32]. The subcellular distribution of poliphosphoinositides (PPIn) is controlled by organelle-specific PPIn kinases and PPIn phosphatases that allow interconversion between PPIn.

Myotubularins are 3-phosphatases that specifically act on PtdIns3*P* and PtdIns(3,5)P_2. PtdIns3*P* is produced by a class III PtdIns 3-kinase in mammals, which corresponds to the Vps34 protein (vacuolar protein sorting 34) in yeast. It is highly enriched on early endosomes and on the internal vesicles of multivesicular bodies. PtdIns3*P* is thought to function by the recruitment of effector proteins as the early

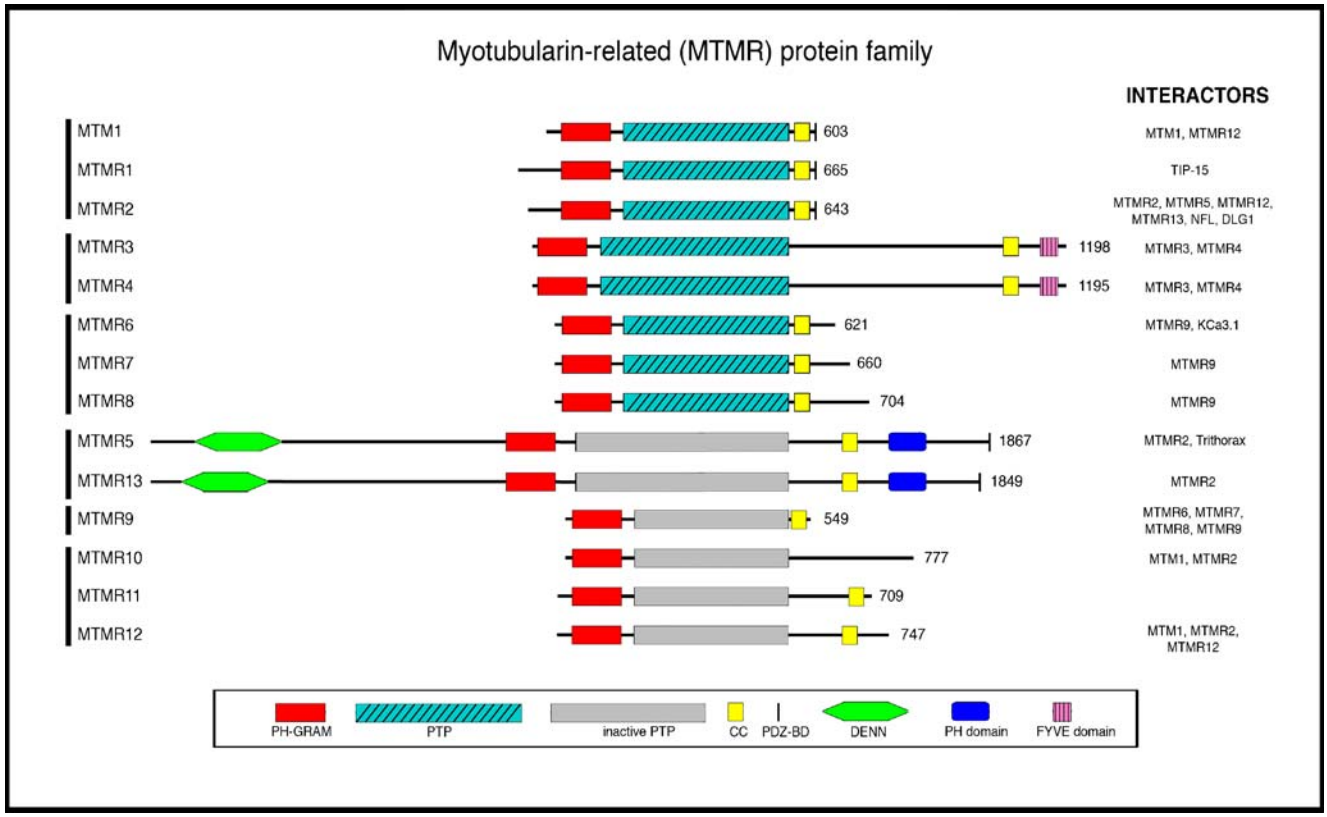

Fig. 2 Myotubularin-related (*MTMR*) protein family. *PH-GRAM*, pleckstrin homology-glucosyltransferase, Rab-like GTPase activators and myotubularins; *PTP*, protein tyrosine phosphatase; *CC*, coiled-coil; *PDZ-BD*, PSD-95/Dlg/ZO-1, binding domain; *DENN*, differentially expressed in normal versus neoplastic; *PH*, pleckstrin homology; *FYVE*, Fab1p/YOTB/Vac1p/EEA1

endosomal antigen 1 (EEA1) that cooperates with the activated Rab5 GTPase to regulate early endosomal fusion [33, 34].

PtdIns(3,5)P_2, the other myotubularin substrate, represents only the 0.1% of total cell poliphosphoinositides, and it is generated by the phosphorylation of PtdIns3*P* by PIKfyve in mammals and by Fab1p in *Saccharomyces cerevisiae* [35, 36]. Fab1p mutation induced grossly enlarged vacuoles because of defects in membrane and protein recycling at the level of multivesicular bodies [35, 37–39]. In mammalian cells, an overexpressed kinase-deficient mutant of PIKfyve leads to the accumulation of multiple swollen vacuoles of endosomal origin resembling the Fab1p mutant phenotype in yeast [36]. Although PtdIns(3,5)P_2 localization in mammalian cells is not well defined, PtdIns(3,5)P_2 is required for the sorting of membranes at the level of late endosomes and the limiting membranes of lysosomes.

MTMR phosphatase activity toward PtdIns3*P* and PtdIns(3,5)P_2 obviously points to an involvement in the regulation of membrane trafficking and homeostasis. However, MTMRs are mainly cytosolic or peripheral membrane-associated proteins [40], and they have not been reported to localize at early endosomes or late endosomes/lysosomes. Several studies have suggested a role of myotubularins in regulating endocytosis and the trafficking of membranes and proteins. In *Caenorhabditis elegans*, MTM-6 and MTM-9 (MTMR6-8 and MTMR9 in mammals) have been identified in a genetic screening as proteins required for endocytosis in coelomocytes: a dramatical alteration in PtdIns3*P* localization is observed in coelomocyte from mtm-6 and mtm-9 mutants [41]. MTM1 overexpression in COS7 cells extensively treated with EGF induces large endosomal vacuoles and inhibits EGFR trafficking from late endosome to lysosome, [12] while, in myotubes, MTM1 overexpression leads to the displacement of EEA1 from early endosomes and to a decreased insulin-induced glucose uptake [42].

Despite these results, the physiological role of myotubularins in mammalian cells is still not clear. For example, lack of endogenous MTM1 in XLMTM patient myoblasts does not significantly affect the pool of PtdIns3*P* or the localization of EEA1 [21]. Moreover, the finding that MTMR6 can inhibit the KCa3.1 channel in membranes of CD4 T cells by decreasing levels of PtdIns3*P* suggests that MTMRs might be involved in the regulation of specific cellular functions in different cell types [43].

Functional Redundancy and Specificity of MTMRs

Active MTMRs show similar 3-phosphatase activity, and almost all MTMRs are ubiquitously expressed, suggesting a broad functional redundancy. However, human disorders affecting only specific tissues such as skeletal muscle (loss of MTM1) or peripheral nerve (loss of either MTMR2 or MTMR13) demonstrated that the function of these proteins is not redundant at least in the tissues involved in the disease. X-linked myotubular myopathy (XLMTM) is a severe skeletal muscle disorder affecting newborn males, characterized by hypotonia and generalized muscle weakness. More than 200 mutations in *MTM1* have been identified thus far in XLMTM patients leading to loss of MTM1 protein [44–47]. Morphological studies showed that the muscle of these patients contains small fibers with centrally located nuclei, suggesting a defect in myogenesis. However, the generation of an animal model for the disease demonstrated that Mtm1 has a role in muscle maintenance rather than myogenesis [48]. Mtm1 deficient mice have, after birth, normal muscle fibers with nuclei located at the periphery. Starting at 4 weeks of age, these mice develop a progressive myopathy characterized by hypotrophy and an increasing number of fibers with centrally located nuclei. Hereafter, we will discuss more in detail MTMR2 and MTMR13 and their role in the pathogenesis of Charcot–Marie–Tooth type 4B neuropathy.

MTMR2 and MTMR13 in the Peripheral Nervous System

Charcot–Marie–Tooth Type 4B1 and B2 Demyelinating Neuropathies

MTMR2 is ubiquitously expressed in human and mouse tissues, as demonstrated mainly at the mRNA level. In mouse development, ubiquitous *Mtmr2* expression is observed starting at E9.5 [19, 49, 50]. In the adult rat peripheral nervous system, the Mtmr2 protein is detected in vivo in the cytoplasm of myelin-forming and nonmyelin-forming Schwann cells and in axons in peripheral nerves, in motor neurons, sensory neurons and satellite cells of dorsal root ganglia, and in both dorsal and ventral roots of spinal cord [51].

MTMR13 expression has been mainly investigated at the mRNA level on human tissues, and it was found to be almost ubiquitously expressed [7, 8].

Although ubiquitously expressed, loss of either MTMR2 or MTMR13 specifically causes Charcot–Marie–Tooth type 4B1 and B2 (CMT4B1 and CMT4B2) neuropathies, respectively. CMT4B is an autosomal recessive neuropathy with childhood onset usually characterized by decreased MNCV (mean 9–20 m/s), distal sensory impairment, demyelination with extensive loss of myelinated fibers, and redundant loops of myelin around the main myelinated axons or myelin outfoldings in the nerve [52]. We first demonstrated that putative loss of function mutations in *MTMR2* cause CMT4B1 [6]. Additional mutations have been identified mainly in familial CMT4B1 cases (Fig. 3). Most of the *MTMR2* identified mutations affect the broad PTP domain, resulting in loss of the enzymatic activity, as has been functionally demonstrated for some of them [19, 53]. Mutations in the PH-GRAM domain affect both binding to the membrane and the phosphatase activity, as demonstrated for the G103E mutation [13, 54, 55].

Putative loss of function mutations in *MTMR13*, encoding a catalytically inactive phosphatase, cause CMT4B2. The first reported mutation is an in frame deletion removing part of the DENN domain at the N terminus of the protein [8]. DENN/GEF/AEX-3 is a well-conserved motif in signaling molecules. Cytoplasmic proteins lacking part of this domain delocalize to the nucleus where they might activate signaling event leading to oncogenic transformation, such as MTMR5/SBF1 [56]. Whether MTMR13 lacking the DENN domain might also activate a transcriptional program leading to excessive myelination and myelin outfoldings remains to be assessed. Other *MTMR13* mutations are classical loss of function mutations that remove the PH-GRAM, PTP, and C-terminal PH domain [7, 57].

CMT4B1 Animal Models

To demonstrate that loss of MTMR2 phosphatase activity causes a demyelinating neuropathy with myelin outfoldings and to follow the natural onset and development of the pathology in the nerve, animal models of CMT4B1 have been generated. We generated the first mouse model, the *Mtmr2*-null mouse, using Cre/loxP technology. By removing exon 4, which encodes part of the PH-GRAM domain, a truncated product is produced lacking all the functional domains downstream such as PTP, coiled-coil, and PDZ-BD [58, 59] (Fig. 3). These mice are viable, and at external examination, they do not show any functional impairment. However, behavioral and electrophysiological tests suggested a neuromuscular defect. In both motor and sensory nerves of mutant mice, myelin outfoldings were observed starting at 3–4 weeks after birth, a time when myelinating Schwann cells are still elongating during postnatal development (Fig. 4). Myelin compaction and the ultrastructure of the myelinated fiber were normal, suggesting that the molecular architecture of the myelinated fiber with redundant myelin is not altered. The most peculiar observation came when semithin analysis was performed in longitudinal section of nerves. Myelin outfoldings predominantly arise at paranodal regions extending throughout the internode. At

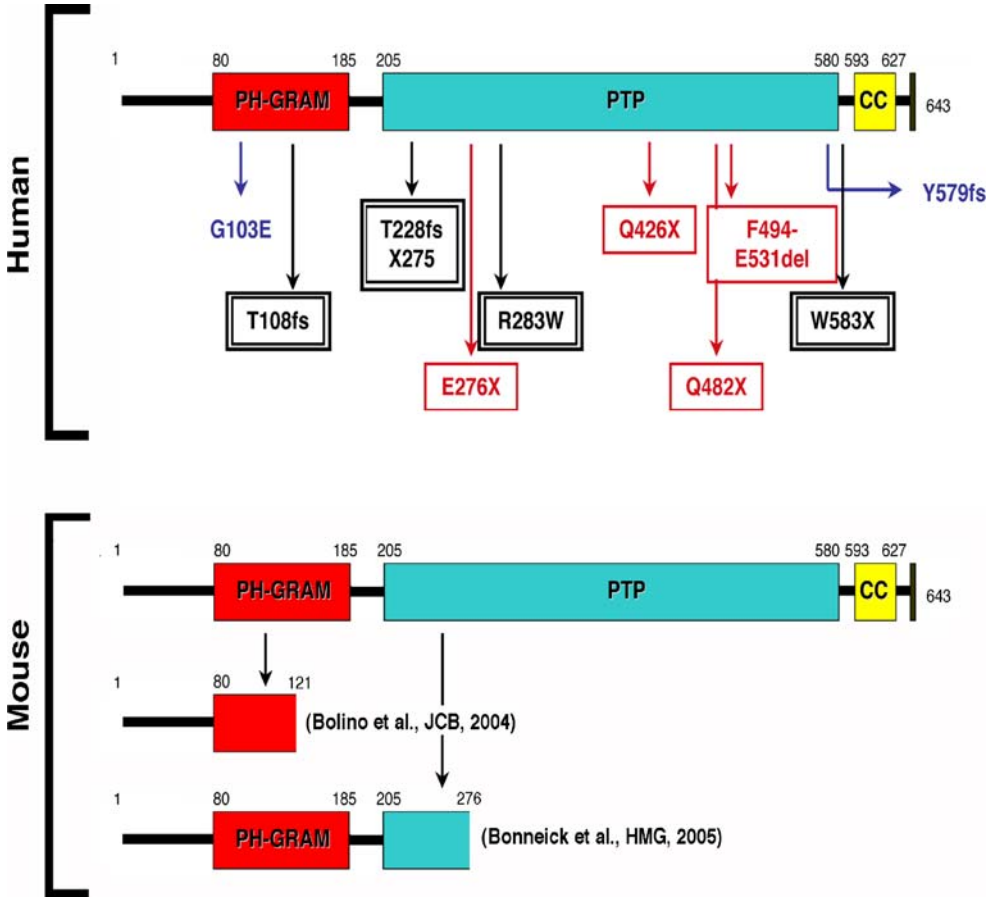

Fig. 3 MTMR2, myotubularin-related 2, mutations in human and mouse. The crystal structure of MTMR2 has recently demonstrated that this phosphatase has a PH-GRAM domain at the N terminus (aa 80–185), a broad PTP domain (aa 205–580), and a linker region (185–205) [65]. *Red* color (single square) labels mutations that abolish MTMR2 3-phosphatase activity toward both PtdIns3*P* and PtdIns(3,5)P_2 in vitro [19]. G103E and Y579fs, in *blue* (no square), affect binding to the membrane of vacuoles formed under hypo-osmotic stress and the dimerization of MTMR2 through its coiled-coil domain [13]. In *black* (double square) are depicted mutations, which have not been yet functionally tested

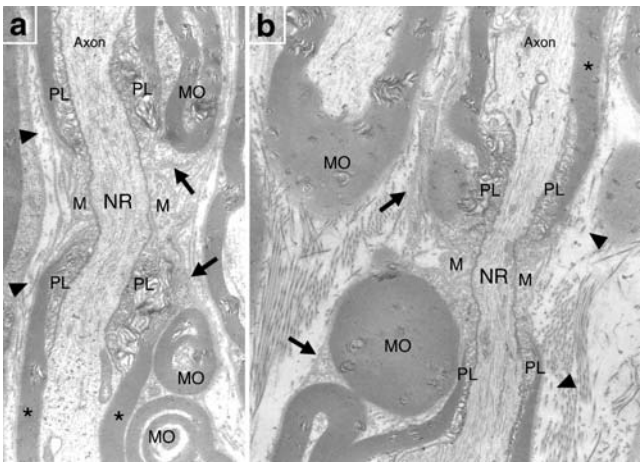

Fig. 4 Myelin outfoldings in *Mtmr2*-null mouse nerves. In **a** and **b** electronmicroscopy of two myelinated fibers from *Mtmr2*-null sciatic nerves in *longitudinal sections* is reported. Refer also to Fig. 1. *MO*, myelin outfoldings; *PL*, paranodal loops flanking the node of Ranvier, *NR*; *M*, microvilli. *Asterisks* indicate compact myelin; *arrows* indicate altered portions of the fiber where myelin outfoldings are evident. *Arrowheads* show normal segments of the same myelinated fiber. We are grateful to Dr. Angelo Quattrini, San Raffaele Scientific Institute, Milan, for these images

12–15 months of age, myelin outfoldings also arise at Schmidt–Lanterman incisures, other regions enriched in Schwann cell cytoplasm.

Mtmr2-null mice have also defects in spermatogenesis, again, starting at 3–4 weeks of age. Immature germ cells were found in the lumen of tubules of the seminiferous epithelium of these mice, suggesting that a loss of adhesion occurred between Sertoli cells and germ cells. Consistent with this, one familial CMT4B1 case has been reported with azoospermia [9]. Moreover, when the inactive Mtmr5 phosphatase, which interacts in vitro with Mtmr2, is ablated in mouse, a defect in spermatogenesis is also observed [60]. Thus, Mtmr2, perhaps through interaction with Mtmr5, has a relevant role in testis the absence of which is not compensated by other MTMRs.

Another animal model has been also produced by introducing the E276X mutation in exon 9 [61] (Fig. 3). This mutation has been found in one familial CMT4B1 case, in homozygosity [6], and it has been also demonstrated in vitro that the E276X–Mtmr2 protein lacks phosphatase activity toward PtdIns3*P* and PtdIns(3,5)P_2 [19]. The nerve

morphology in these mice was similar to that observed in *Mtmr2*-null mice, although a huge variability in the number of myelin outfoldings between different mice was noted. Moreover, no electrophysiological and behavioral alterations were reported and the testis was grossly normal.

In both mutants, *Mtmr2*-null and E276X, the dys-myelinating phenotype was less severe than that observed in human CMT4B1. Axonal loss was observed only in *Mtmr2*-null mice at later stages (around 15 months) in more distal nerves [59]. The variation in phenotype between mouse and humans might be due to a shorter mouse life-span and to shorter nerve lengths. Alternatively, a partial compensation by other MTMRs in mouse nerve might account for the less severe pathology. We investigated the expression of MTM1 and MTMR1 proteins in nerves, and we found that MTM1 but not MTMR1 is expressed by myelin-forming Schwann cells [58]. Using quantitative expression analysis, we also found that in mutant nerves—but not in muscles and brains—levels of *Mtm1* mRNA are significantly upregulated, suggesting a possible compensation played by Mtm1 in this tissue [59].

The nerve morphology of human and mouse CMT4B1 points to a defect originating in Schwann cells. The severity of human CMT4B1 with loss of large myelinated fibers starting at early stages suggests that MTMR2 might also play a cell-autonomous role in motor neurons [52]. We provided evidence that the neurofilament light chain (NF-L) protein interacts with MTMR2 in neurons [51]. It has to be noted that mutations in NF-L also cause Charcot–Marie–Tooth disease, as an axonal form of the disease or "intermediate" forms with mixed features of axonopathy and demyelination—rarely with excessive myelin resembling myelin outfoldings. NF-L is mainly expressed by neurons, while Schwann cells upregulates *NF-L* mRNA level after injury [51]. Thus, MTMR2/NF-L interaction might have a relevant role in neurons. Whether or not loss of the MTMR2/NF-L interaction contributes to the axonal pathology observed in human CMT4B1 is not clear.

To assess whether Mtmr2 might have a cell-autonomous role in both cell types, we also generated two conditional null mice with *Mtmr2*-specific ablation in either Schwann cells or motor neurons [59]. Only the Schwann-cell null mouse displayed a dys-myelinating phenotype very similar to that observed in human CMT4B1 and in the *Mtmr2*-null mouse. Dys-myelination and axonopathy were not observed in the motor neuron-null mouse. It is possible to conclude that loss of Mtmr2 phosphatase activity in Schwann cells is sufficient and necessary to provoke CMT4B1 with myelin outfoldings. Mtmr2 might still have a function in motor neurons, which is redundant or compensated by the presence of other MTMRs at the stage when Mtmr2 is inactivated in mice at E9.5. Alternatively, it has also to be considered that axonal phenotypes have been very rarely reproduced in mice.

MTMR2 and MTMR13 Subcellular Localization

Because MTMRs act on PtdIns3*P* and PtdIns(3,5)P_2, several efforts have been made to localize these proteins at membranes of early endosomes and late endosomes/lysosomes. Although MTM1 partial colocalization with early endosomes and late endosomes has been demonstrated, these studies have been mainly performed in overexpressing cells [12, 42, 62]. At physiological levels, a clear colocalization of MTMRs with intracellular vesicle markers has not been yet demonstrated.

Several overexpression studies also showed that MTMR2 localizes in the cytosol with a broad diffuse pattern [13, 19, 27, 51]. Enrichment in the perinuclear region was also reported [17, 18]. It has been then hypothesized that MTMR2 might localize to precise subcellular compartments under stimulated conditions that change the levels of intracellular poliphosphoinositides and/or after interaction with the inactive partner, such as MTMR13 in the nerve. Consistent with this, when hypoosmotic stress is induced in COS7 cells, a condition that increases PtdIns(3,5)P_2 levels, MTMR2 delocalizes to membranes of intracellular vacuoles [13]. Berger et al. [26] recently showed that MTMR2 and MTMR13 interact and form heterotetramers by the association of homodimers of two MTMR2 and MTMR13. When MTMR13 is coexpressed with MTMR2 at high levels in COS7 cells, MTMR13 preferentially binds to membranes of vacuoles formed under hypoosmotic stress, while if coexpressed at lower levels, both bind to intracellular membranes.

Interaction between MTMR2 and MTMR13 has been demonstrated also at physiological levels in HEK293 cells. In these cells, in differential fractionation experiments, MTMR2 and MTMR13 are detected in both the soluble and light membrane fractions [27]. In the heavier membrane fraction containing most of the markers for endosomal compartments, little MTMR2 was found, and only 20% of MTMR13 was detected. Therefore, under these conditions, MTMR2 and MTMR13 fail to cofractionate with markers for late endosomal compartments as predicted by the phosphatase activity toward PtdIns(3,5)P_2. In conclusion, the precise nature of intracellular vesicle compartments to which MTMR2 and MTMR13 bind as well as of the physiological event/stimulus that might lead to changes in PPIn composition and subsequent delocalization of MTMRs in Schwann cells remain to be assessed.

We demonstrated that endogenous MTMR2 is mainly cytosolic in neurons, axons, and Schwann cells [51]. In a myelinated fiber, MTMR2 is found where portions of Schwann cell cytoplasm are restricted, in the perinuclear region, paranodes, and in Schmidt–Lanterman incisures. Myelin outfoldings also arise from paranodes and incisures at 3–4 weeks after birth. Paranodes are known sites of

membrane remodeling and homeostasis during myelin assembly in postnatal nerve development. Notably, clathrin coated pits, endosomes, and lysosomes have been demonstrated in the paranodal cytoplasm—consistent with a role for these sites in membrane remodeling/homeostasis. Myelin assembly obviously relies on intracellular trafficking and membrane homeostasis in Schwann cells, and lipid rafts have been proposed as important macrodomains in the membrane that help to deliver lipids and proteins to the membrane [2]. However, how these macrodomains are formed and segregated is poorly understood. In this line, MTMR2 might constitute the first known phosphatase regulating these events at paranodal regions of myelin-forming Schwann cells.

MTMR2 and MTMR13 Interactors

MTMR2 dephosphorylates both PtdIns3*P* and PtdIns(3,5)P_2 in vitro and in vivo. However, this enzymatic activity in the nerve at physiological levels still remains to be assessed. Moreover, whether MTMR2 clearly colocalizes with markers of intracellular vesicle compartments in Schwann cells and neurons has not been demonstrated. To get insight into the cellular function and localization of the MTMR2 phosphatase, tissue-specific interactors were sought basically by yeast two-hybrid screening analysis using nerve cDNA libraries. Because *Mtmr2* ablation in Schwann cells causes the dys-myelinating CMT4B1 phenotype with myelin outfoldings, efforts have been concentrated on this cell type. We demonstrated interaction between Mtmr2 and Dlg1/SAP97 (discs large/synapse associated protein 97) in Schwann cells [58, 59]. Dlg1 is a scaffolding molecule belonging to the MAGUK (membrane-associated guanylate kinase-like) protein family, which links transmembrane proteins with the intracellular cytoskeleton. Multimeric protein complexes can be formed through multiple interaction domains of MAGUK proteins such as PDZ, SH3 (Src-homology-3), GuK (guanylate kinase-like), etc. Dlg1 is a known molecule located in vertebrates at adherens junctions of epithelial cells and at pre- and postsynaptic sites in neurons. In *Drosophila melanogaster*, loss of Dlg produces overgrowth of imaginal discs, loss of polarity, and adhesion because of septate junction disruption [63]. We first reported Dlg1 expression in Schwann cells. In myelinated fibers, Dlg1 is located at Schmidt–Lanterman incisures and paranodal loops, where myelin outfoldings also arise in CMT4B1 neuropathy. This localization pattern is lost in *Mtmr2*-null nerves, suggesting that loss of the Mtmr2/Dlg1 complex is at the basis of the myelin pathology.

Putative Role of MTMR2/MTMR13 in the Pathogenesis of CMT4B

Mtmr2 has a Schwann cell-autonomous role because (a) specific inactivation of Mtmr2 in these cells reproduces the phenotype of *Mtmr2*-null mice; (b) myelin outfoldings originate from Schwann cell structures; and (c) Dlg1, interactor of Mtmr2 in Schwann cells, is almost lost in paranodal regions where also myelin outfolding preferentially arise.

Mtmr2 dephosphorylates phospholipids implicated in intracellular trafficking events. Dlg1 is also involved in membrane addition in polarized cells in other organisms [64]. Thus, Mtmr/Mtmr13/Dlg1 might regulate membrane addition/homeostasis in Schwann cells in the nerve during postnatal maturation (Fig. 5). Consistently, in *Mtmr2*-null mice, myelin outfoldings arise around the third–fourth week after birth, a time when the architecture of the nerve is established, but the Schwann cells have to elongate, creating internodes of the final length around 1 mm. Mtmr2, targeted by Dlg1, might directly function in membrane remodeling or vice versa; Mtmr2 might favor the transport of Dlg1, which regulates membrane homeostasis. In both cases—whether Mtmr2 or Dlg1 is the principal effector—Mtmr2/Dlg1 might control (a) membrane addition; (b) membrane recycling, and (c) membrane degradation. Further studies will be necessary to prove these hypotheses and to assess whether the 3-phosphatase

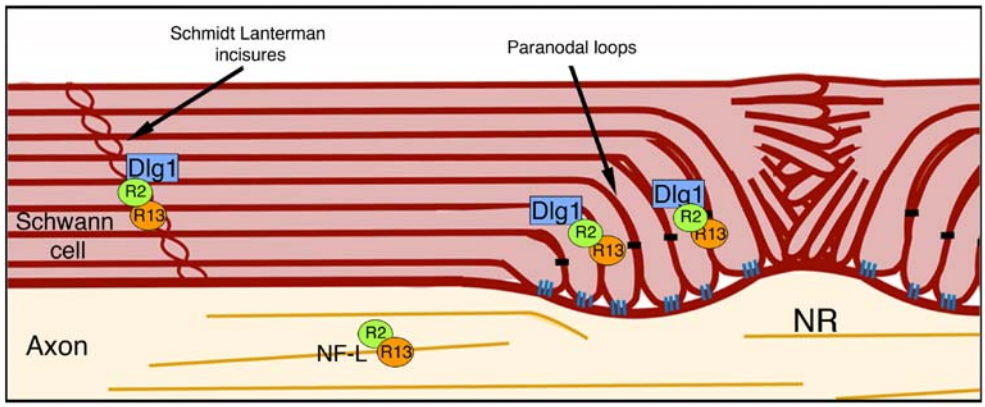

Fig. 5 MTMR2 interactors in the nerve. *Dlg1*, discs large 1, synapse associated protein 97; *R2*, MTMR2; *R13*, MTMR13, and *NF-L*, the neurofilament light chain protein

activity of MTMR2 at physiological levels contributes to this function in the nerve.

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