Cell expression of GDAP1 in the nervous system and pathogenesis of Charcot-Marie-Tooth type 4A disease

Laia Pedrola [a, b], **Antonio Espert** [a, b], **Teresa Valdés-Sánchez** [c, d],
Maribel Sánchez-Piris [a, b], **Erich E. Sirkowski** [e], **Steven S. Scherer** [e],
Isabel Fariñas [c, d], **Francesc Palau** [a, b, *]

[a] *Department of Genomics and Proteomics, Instituto de Biomedicina, CSIC, Valencia, Spain*
[b] *Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain*
[c] *Departamento de Biología Celular, Universidad de Valencia, Burjassot, Spain*
[d] *Centro de Investigaciones Biomédicas en Red de Enfermedades Neurodegenerativas (CIBERNED), Valencia, Spain*
[e] *Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*

Abstract

Mutations in the mitochondrial protein GDAP1 are the cause of Charcot-Marie-Tooth type 4A disease (CMT4A), a severe form of peripheral neuropathy associated with either demyelinating, axonal or intermediate phenotypes. GDAP1 is located in the outer mitochondrial membrane and it seems that may be related with the mitochondrial network dynamics. We are interested to define cell expression in the nervous system and the effect of mutations in mitochondrial morphology and pathogenesis of the disease. We investigated GDAP1 expression in the nervous system and dorsal root ganglia (DRG) neuron cultures. GDAP1 is expressed in motor and sensory neurons of the spinal cord and other large neurons such as cerebellar Purkinje neurons, hippocampal pyramidal neurons, mitral neurons of the olfactory bulb and cortical pyramidal neurons. The lack of GDAP1 staining in the white matter and nerve roots suggested that glial cells do not express GDAP1. In DRG cultures satellite cells and Schwann cells were GDAP1-negative. Overexpression of GDAP1-induced fragmentation of mitochondria suggesting a role of GDAP1 in the fission pathway of the mitochondrial dynamics. Missense mutations showed two different patterns: most of them induced mitochondrial fragmentation but the T157P mutation showed an aggregation pattern. Whereas null mutations of GDAP1 should be associated with loss of function of the protein, missense mutations may act through different pathogenic mechanisms including a dominant-negative effect, suggesting that different molecular mechanisms may underlay the pathogenesis of CMT4A.

Keywords: Charcot-Marie-Tooth type 4A disease ● GDAP1 ● peripheral neuropathy ● mitochondrial dynamics ● fusion and fission pathway ● CMT4A mutations and pathogenesis

Introduction

Charcot-Marie-Tooth disease (CMT) is the eponym for non-syndromic inherited neuropathies, and usually

*Correspondence to: Francesc PALAU,
Instituto de Biomedicina, CSIC, C/ Jaume Roig 11, 46010 Valencia, Spain.
Tel.: + 34-96-33 9-37 73
Fax: +34-06-36 9-08 00
E-mail: fpalau@ibv.csic.es*

affects both motor and sensory nerve fibres. CMT is one of the most common inherited neurological diseases, with a prevalence of 28 in 100,000 [1]. CMT can be classified by electrophysiology and nerve biopsy morphological analysis into two major types. In the demyelinating type (CMT1), the primary lesion appears to occur in myelinating Schwann cells, as nerve conduction velocities (NCV) are reduced and

nerve biopsies show evidence of de- and re-myelination. The axonal type (CMT2) is characterized by a reduced number of axons with otherwise normal or almost normal NCVs, indicating that the primary lesion is located in the neurons and/or their axons. CMT is inherited in different Mendelian patterns – autosomal dominant, autosomal recessive and X-linked.

Charcot-Marie-Tooth disease type 4A (CMT4A) is caused by mutations in *GDAP1* (ganglioside-induced differentiation associated protein 1) gene [2, 3]. Phylogenetic and structural analyses suggest that GDAP1 belongs to the glutathione S-transferases (GSTs) enzyme family [3, 4] but functional activity has not been demonstrated yet [5, 6]. GDAP1 is mainly expressed in neurons of the CNS [3, 5] but also in the Schwann cells, and is localized to mitochondrial outer membrane [7]; it may be involved in mitochondrial network dynamics [5], specifically in mitochondrial fission [7].

With regard to phenotypic expression and inheritance pattern there are two controversial points. Initially CMT4A was described as a demyelinating neuropathy [8, 9] but both axonal and intermediate phenotypes have also been associated with mutations in *GDAP1* [10–12]. On the other hand, CMT4A used to segregate within families as an autosomal recessive trait; however, a few families segregating CMT4A dominantly have been reported [13]. Here we show that GDAP1 is expressed in motor and primary sensory neurons, and in other large neurons such as cerebellar Purkinje neurons or hippocampal pyramidal neurons, but we did not observe expression in glial cells of the CNS. We also report two different mitochondrial morphology patterns associated with overexpression of *GDAP1* of either recessive or dominant missense mutations, respectively, suggesting that different mechanisms may underlay the disease pathogenesis.

Materials and methods

Antibodies

For immunocytochemistry, we used the following primary antibodies: rabbit polyclonal antibodies GDAP1-N and GDAP1-C raised against two different GDAP1 peptides in our laboratory (5), and mouse monoclonal antibodies to β-III-tubulin (Tuj1, Covance) and OxPhos Complex IV subunit I (Molecular Probes).

Immunohistochemistry

Adult mice and rats were overdosed with pentobarbital (Sigma-Aldrich) and perfused with freshly prepared 4% PFA in 0.1 M phosphate buffer, pH 7.4 (PB) or with Zamboni solution (4% paraformaldehyde [PFA], 15% picric acid (Sigma-Aldrich), in 0.1 M PB). The spinal cords, dorsal root ganglia (DRGs) and brains were removed and post-fixed for 30 min in the same fixative and rinsed in PB. Brains were embedded in 3% agarose, serially cut into 30 μm-thick coronal sections using a vibratome, and the sections were collected and stored in PB. The spinal cords and attached DRGs were infiltrated in 30% sucrose in PB and frozen at −20°C using tissue freezing medium (Triangle Biomedical Sciences, Durham, NC, USA). Cryostat sections were thaw-mounted on SuperFrost Plus glass slides (Fisher Scientific) and stored at −20°C until use. Sections were post-fixed and permeabilized by immersion in −20°C cold acetone for 10 min, blocked at room temperature for at least 1 hr in PB containing 5% fish skin gelatin, 0.5% Triton X-100 and 10% foetal bovine serum (FBS) [blocking buffer], and incubated for 24–48 hrs at 4°C with primary antibodies diluted in blocking solution. Afterwards, the sections were washed in PB and incubated with appropriate secondary antibodies. For immunoperoxidase detection, sections were incubated with HRP-conjugated anti-rabbit secondary antibodies (Sigma-Aldrich) in blocking buffer for one hour at room temperature and, after several washes, sections were reacted with 0.05% diaminobenzidine and 0.003% hydrogen peroxide in PB, washed thoroughly and mounted using Vectashield (Vector Laboratories, Burlingame, CA, USA). For fluorescent immunodetection we used: fluorescein-, rhodamine-, or Alexa 488 (1:500; Molecular Probes)-conjugated donkey antimouse antibodies or biotinylated goat anti-rabbit antibodies (diluted 1:200, 1:200, 1:100 or 1:500; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) followed by Cy3 and Cy2-conjugated streptavidin for one hour at room temperature each. After several washes, sections were counterstained with 4′, 6-diamidin-2-phenylindoldihydrochloride (DAPI) and mounted with Fluorsave (Calbiochem). The slides were examined on a Leica (Nussloch, Germany) DMR light microscope and photographed with a Hamamatsu (Tokyo, Japan) digital camera or with a Leica TCS laser-scanning confocal microscope. In control experiments in which the primary antibodies to GDAP1 were either omitted or pre-incubated with the corresponding immunizing peptide we did not observe any staining.

DRG neuron culture

Neonatal mice were decapitated and approximately 50 DRGs per animal were dissected and collected in L15

media. Ganglia were incubated at 37°C with 2 mg/ml collagenase (Worthington) followed by 0.05% trypsin (Sigma). Approximately 2000 neurons were plated in 1 ml of defined medium, consisting of Ham's F12 supplemented with 2 mM glutamine, 0.35% bovine serum albumin (Pathocyte-4, ICN, Irvine, CA, USA), 60 ng/ml progesterone, 16 (g/ml putrescine, 400 ng/ml L-thyroxine, 38 ng/ml sodium selenite, 340 ng/ml tri-iodo-thyronine, 60 μg/ml penicillin and 100 µg/ml streptomycin supplemented with nerve growth factor (NGF) at 10 ng/ml using 24-well plates (Orange) with 12 mm-coverslips that had been previously coated with poly-ornithine (0.5 mg/ml, overnight) and laminin (20 μg/ml, 4 hrs) [14]. After 48 hrs at 37°C in a humidified incubator under 5% CO_2 neurons were fixed for 20 min with 4% PFA in PB, followed by three rinses in PB, and an incubation with 10% FBS and 0.2% Triton X-100 in PB (blocking buffer) for 30 min at room temperature. For the detection of GDAP1-C and the neuronal marker anti-Tuj1, coverslips were incubated overnight at 4°C with rabbit anti-GDAP1-C (1:100) and mouse anti-Tuj1 (1:300) in blocking buffer, followed by secondary biotinylated anti-rabbit antibodies for 45 min (1:500; Jackson Immuno Research Laboratories). For double-labelling, Alexa 488-conjugate antimouse (1:500; Molecular Probes) and Cy3-conjugated streptavidin (1:200; Jackson ImmunoResearch Laboratories Inc.) were used, followed by DAPI staining and Fluorsave mounting. The slides were examined on a Leica TCS laser-scanning confocal microscope.

Constructs and site directed mutagenesis

We generated several constructs of *GDAP1* cDNA by PCR using appropriate primer pairs (available upon request): full length GDAP1, GDAP1 lacking the transmembrane domain (amino acids 1–320), and the transmembrane domain (amino acids 307–358). Point mutations (R120Q, R120W, R161H, T157P and R282C) and a non-pathologic polymorphism, G83A, were generated by site directed mutagenesis with primers including the corresponding nucleotide change. PCR conditions were set as follows: 4 min initial denaturation at 94°C, 12 denaturation cycles of 30 sec at 95°C, 1 min annealing at 55°C, 12 min elongation at 68°C and a 7 min final extension at 72°C. After that, the non-methylated parental strand which had not incorporated the nucleotide change was removed with *Dpn* I (Roche) digestion during 1 hr at 37°C. All constructs – wild-type and mutant – were cloned in frame with the green fluorescent protein gene, *gfp*, with the pEGFP-C1 and pEGFP-N1 vectors (Clontech).

Cell culture and transient transfection

COS-7 and HeLa cells were grown in DMEM containing 10% (v/v) FBS supplemented with 2 mM glutamine, 100 IU/ml penicillin and 100 mg/ml streptomycin (Invitrogen). Cells were seeded on glass coverslips in six-well plates and then transfected by calcium phosphate precipitation using 1$\times$ HEPES-buffered saline solution with 250 mM $CaCl_2$ and 2 μg of DNA. Twenty-four hours after transfection, cells were stained with the vital mitochondrial probe MitoTracker Orange CMTMRos (Molecular Probes) for 30 min at 37°C and 5% CO_2 and then, cells were washed twice with PBS buffer, fixed in 4% paraformaldehyde (PFA) in PBS, mounted in Fluoromount-G (Southern Biotech), and examined using a Leica DM RXA2 microscope.

Results

Neurons express GDAP1

To investigate the expression of GDAP1 protein, we examined frozen sections of adult mouse from different regions – DRGs, spinal cord, cerebellum, olfactory bulbs and other brain areas – with anti-GDAP1 specific antibodies [5]. GDAP1 was ubiquitously expressed in the grey matter of brain and spinal cord. In each region, abundant expression in neuronal cell bodies and a fainter neuropil staining was observed (Figs 1 and 2). The cellular staining was especially evident in largest neurons, such as mitral neurons of the olfactory bulb, Purkinje neurons of the cerebellum, pyramidal neurons of the hippocampus and cerebral cortex (Fig. 1), motor neurons of the lateral motor column (Fig. 2) and brainstem (not shown) and DRG neurons (Fig. 2). We also observed staining of smaller neurons, such as cerebellar granule cells (Fig. 1B) and hippocampal interneurons (Fig. 1C). Although the neuropil of the grey matter was GDAP1-positive, an indication that dendrites and/or axons were also labelled (Fig. 1), the white matter and peripheral nerves originating from these heavily labelled neurons were relatively unlabeled. The finding that the olfactory glomeruli were strongly GDAP1-positive (Fig. 1A) suggests that GDAP1 is also targeted to the synaptic terminals.

The lack of GDAP1 staining in the white matter and nerve roots suggested that glial cells do not express GDAP1. To explore this issue, we cultured dissociated DRGs from neonatal mice for two days *in vitro*, and then immunostained for GDAP1. As shown in Fig. 3, all cells in the culture that were immunopositive for GDAP1 also stained for β-III-tubulin, a neuronal marker.
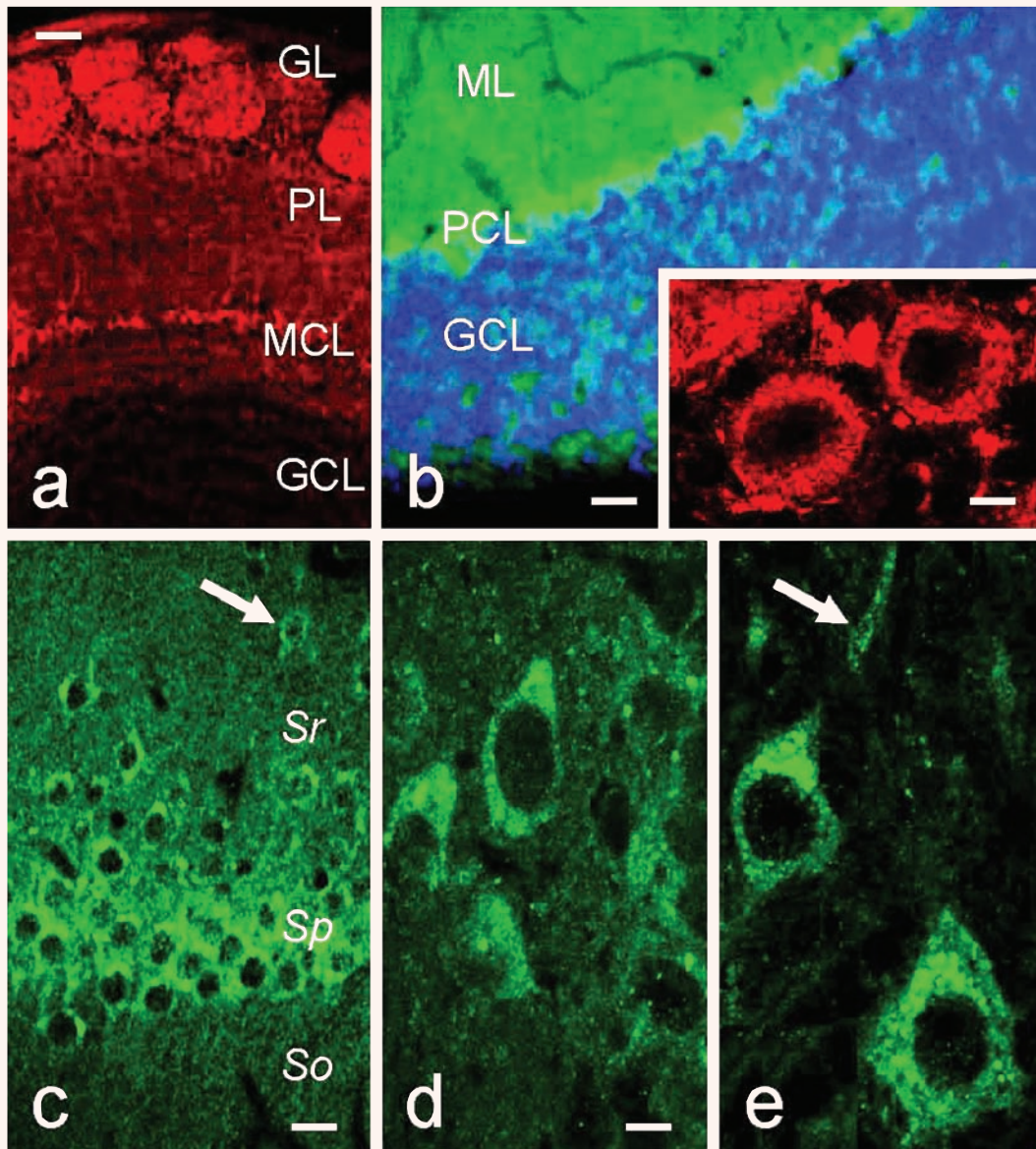
Fig. 1 Immunohistochemical detection of GDAP1 in various adult mouse brain regions. (**a**) Olfactory bulb. GDAP1 is strongly expressed in the glomerular layer (GL) and in the large neurons located in the mitral cell layer (MCL). The neuropil of the plexiform layer (PL) is more strongly labelled than is the granule cell layer (GCL). (**b**) Cerebellum. GDAP1 is strongly expressed in Purkinje neurons whose somata are located in the Purkinje cell layer (PCL); two cells are shown in the insert. Some staining is also observed in the granule cell layer (GCL), were nuclei of the small granule neurons stained with the DNA marker DAPI, and in the molecular layer (ML) where the dendrites of the Purkinje neurons and arriving axons are intermingled. (**c** and **d**) Hippocampus. (**c**) Confocal image showing immunopositive pyramidal neurons in the *stratum pyramidale* (SP) and interneurons (white arrow) located in the *stratum radiatum (SR). SO, stratum oriens.* (**d**) Confocal image showing immunopositive CA1 pyramidal neurons exhibiting a punctate staining in the cell body and in the surrounding neuropil. (**e**) Cerebral cortex. Confocal image showing immunopositive pyramidal neurons with punctate staining in the cytoplasm and dendrites (white arrow). Scale bars: (a) & (b) = 100 μm, other images = 10 μm.

Fig. 2 Immunohistochemical detection of GDAP1 in adult mouse spinal cord and dorsal root ganglia (DRG). (**a**–**d**) Spinal cord. (**a**) GDAP1 is found in the grey matter (GM) in the ventral spinal cord more than in the white matter (WM). The DAPI counterstain labels nuclei; the arrow marks the midline. (**b**) Confocal image showing punctate GDAP1 staining in motoneurons. (**c**) Differential interference contrast image of motor neuron-immunostained for GDAP1 using peroxidase showing the punctate cytoplasmic staining for GDAP1 (**d**) Differential interference contrast image of a sensory neuron immunostained for GDAP1 using peroxidase showing the characteristic punctate cytoplasmic staining. (**e**) In the DRG, sensory neurons but not the nerve fibres crossing and exiting the ganglion (surrounded by white dotted lines), show strong GDAP1 expression. (**f**) Confocal image showing punctate GDAP1 staining in DRG neurons . Scale bars: (a), (c), and (e) = 100 µm, other images = 10 µm.
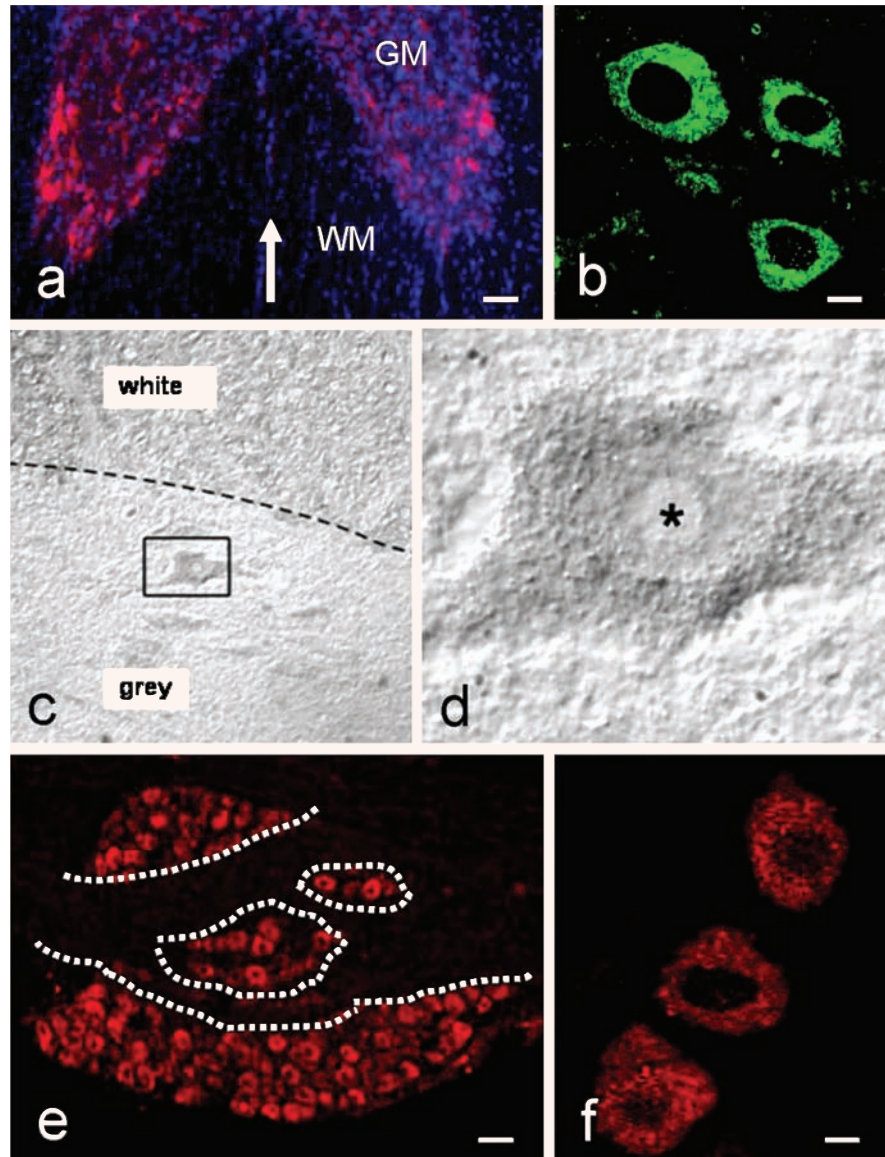
Non-neuronal cells, mostly satellite cells and Schwann cells, were GDAP1-negative. GDAP1 staining in these cultured neurons, moreover, had a characteristic punctuate pattern, also noted for neurons *in vivo* (Figs 1 and 2). Because GDAP1 is localized in mitochondria in cultured cell lines [5, 7], we co-labelling cultured DRG neurons for GDAP1 and cytochrome c oxidase, a protein located in the inner mitochondrial membrane. As shown in Fig. 3C, GDAP1 and cytochrome c oxidase were co-localized, confirming that GDAP1 is localized to mitochondria in neurons.

GDAP1 transmembrane domains do not lead to mitochondrial fragmentation

GDAP1 is expressed in the outer membrane of mitochondria [7]. Overexpression of the whole protein induces fragmentation of mitochondria suggesting participation of GDAP1 in the fusion and fission pathways of the mitochondrial dynamics [7]. We have previously demonstrated that transmembrane domains are necessary to target the protein to
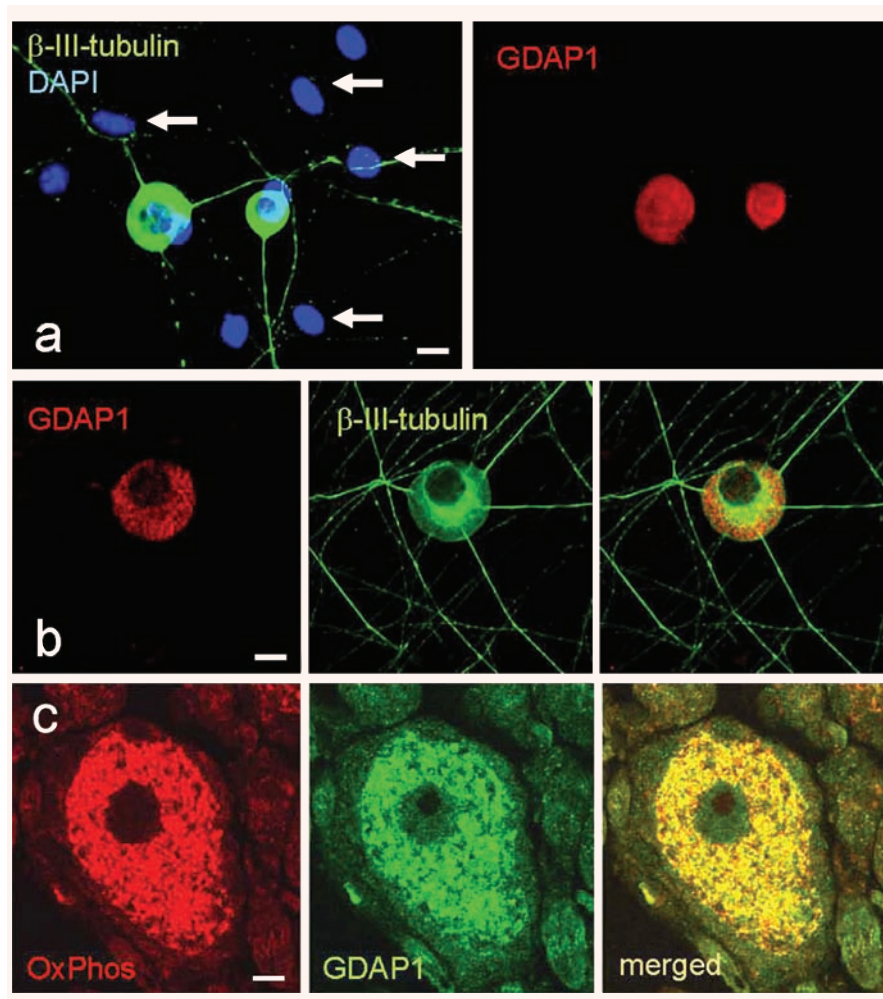
Fig. 3 Immunohistochemical detection of GDAP1 in cultured DRG neurons. (**a**) Beta-III-tubulin-positive neurons (green), but not non-neuronal cells (visualized by DAPI; blue), are GDAP1 positive (red). (**b**) Confocal image shows cytoplasmic GDAP1-positive puncta. Scale bars = 10 μm. (**c**) Co-localization of GDAP1 with a mitochondrial marker. These are confocal images of a section of a rat DRG immunostained for cytochrome C oxidase (red) and GDAP1 (green). Note their co-localization in the merged image, showing that GDAP1-positive puncta are mitochondria. Scale bar = 10 μm.

mitochondrial membranes [5]. We, therefore, tested whether transmembrane domains and the C-terminal region participated in the fission process. We generated a construct in which the sequence encoding C-terminal amino acids 307–358, containing the transmembrane domains, were cloned into both pEGFP-C1 and pEGFP-N1 vectors (*pEGFP:GDAP1*³⁰⁷⁻³⁵⁸). Transient overexpression of these constructs showed that the resulting proteins were correctly located in mitochondria. Moreover, the overexpressing cells showed a rather normal pattern of mitochondrial architecture with mostly small and medium sized tubular mitochondria, a result suggesting that transmembrane domains do not have a direct role in the fission process of mitochondrial network dynamics (Fig. 4).

Morphology patterns of *GDAP1* missense mutations

To investigate the pathogenic effect of missense mutations in the mitochondrial dynamics we analysed the mitochondrial morphology in COS7 cells after transient expression of *gfp-GDAP1* constructs. All the expressed mutations showed a normal cell trafficking with no retention at the endoplasmic reticulum or Golgi system. To describe the potential changes in mitochondrial shape and organization, we defined five different mitochondrial architectures within the cell: perinuclear aggregated ('aggregated'), predominantly tubular ('tubular'), tubular and vesicular ('mixed'), predominantly vesicular ('vesicular') and completely fragmented ('fragmented') (Fig. 5). Most
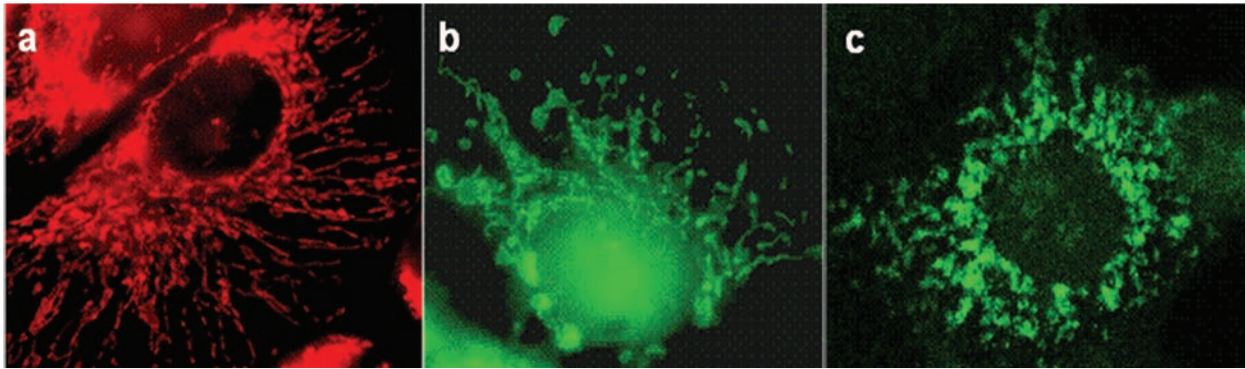
Fig. 4 Mitochodrial morphology analysis of GDAP1. (**a**) Fluorescent mitochondrial marker (MitoTracker) shows that in COS-7 cells mitochondria are tubular. (**b**) Transmembrane domain (*pEGFP:GDAP1$^{307-358}$*) transiently transfected in HeLa cells show that this domain is sufficient to target the protein to mitochondria, and these are small and medium tubular mitochondria. (**c**) Wild-type GDAP1 transiently expressed in COS-7 cells shows fragmented pattern of mitochondria.

control empty vector-transfected cells mainly exhibited a tubular pattern although some cells belonged to the mixed type. As mentioned above, overexpression of the full length GDAP1 results in most cells showing the fragmented pattern. Expression of R120Q, R120W, R161H and R282C mutations alleles and also the G83A polymorphism resulted in a fragmented mitochondrial distribution very similar to that found in cells overexpressing the wild-type protein (Fig. 6). By contrast, the T157P mutation, originally described as a *de novo* dominant mutation in a sporadic case [13], induced an aggregated morphology in a large proportion of cells, although the fragmented pattern was observed as well.

Discussion

Mutations in *GDAP1* have been associated with either demyelinating [2, 8], axonal [3, 15], or intermediate forms of CMT [16–20]. Thus, in some patients it is difficult to determine the primary lesion of GDAP1 neuropathy from electrophysiological and histopathological findings. Moreover, experimental data are contradictory. Niemann *et al.* [7] have detected GDAP1 in both sensory neurons and sciatic nerve Schwann cells using GDAP1-specific antibodies in both Western blot and tissue histochemistry. By contrast, in RNA experiments based on Northern blot and real-time quantitative PCR we

could not demonstrate GDAP1 expression in either sciatic nerve or cultured Schwann cells [5]. To address this relevant point to understand the pathogenesis of CMT4A disease, we have performed a more extensive survey of GDAP1 expression in the nervous system in general and in the peripheral nervous system (PNS) in particular. We observed a wide expression of GDAP1 including not just DRG sensory neurons and motor neurons from anterior horn of spinal cord but also in large neurons in the brain. On the contrary, we could not observe any detectable levels of GDAP1 in peripheral glial cells, such as satellite or Schwann cells neither in tissue sections through adult mouse DRGs nor in neonatal DRG cultures. Interestingly, expression in the CNS was restricted to the grey matter, suggesting that CNS glial cells may not express detectable levels of GDAP1 either. Although we obtained identical results using polyclonal antibodies either the N-terminal or the C-terminal portions of the protein, a possibility exists that our antibodies can not detect very low levels of the protein. However, previous analyses by our group with more sensitive techniques could not detect GDAP1 mRNA in glial cells [5]. Thus, our results together suggest that cells primarily affected in the pathogenesis of CMT4A may be motor and sensory neurons. However, there is not a clear relationship between the wide expression of GDAP1 in the nervous system and the specific pathophysiology of CMT4A which only affects neurons with peripheral myelinated fibres. It remains unknown whether
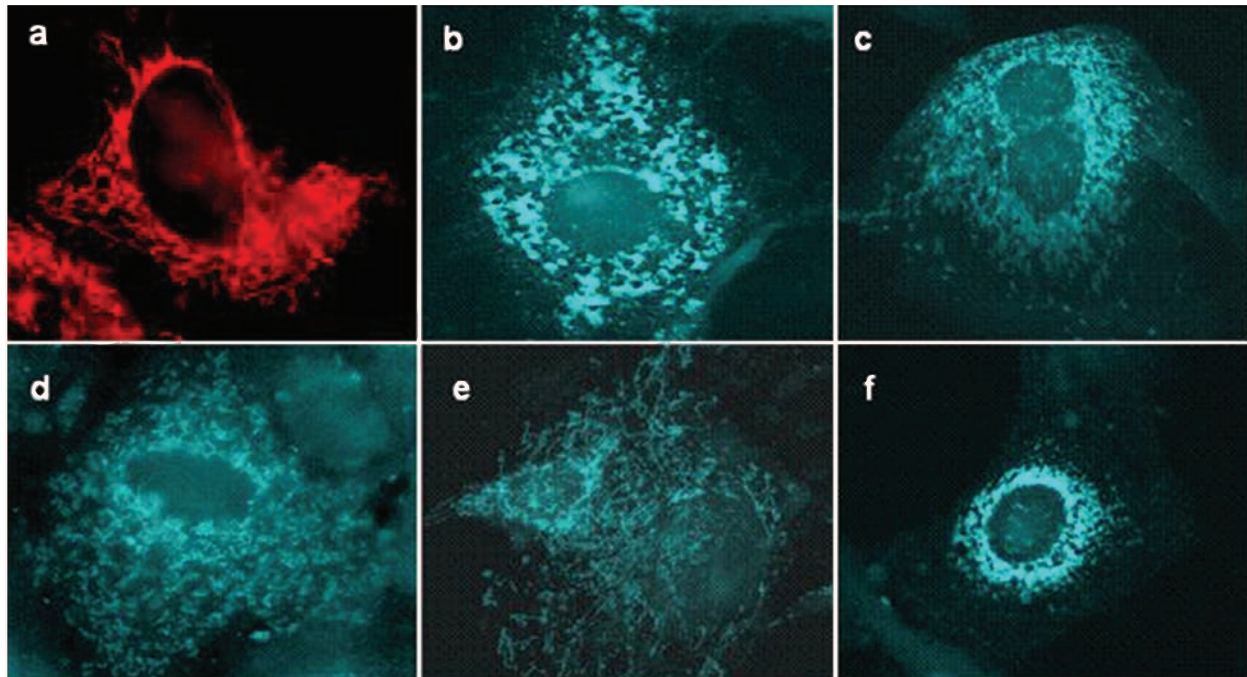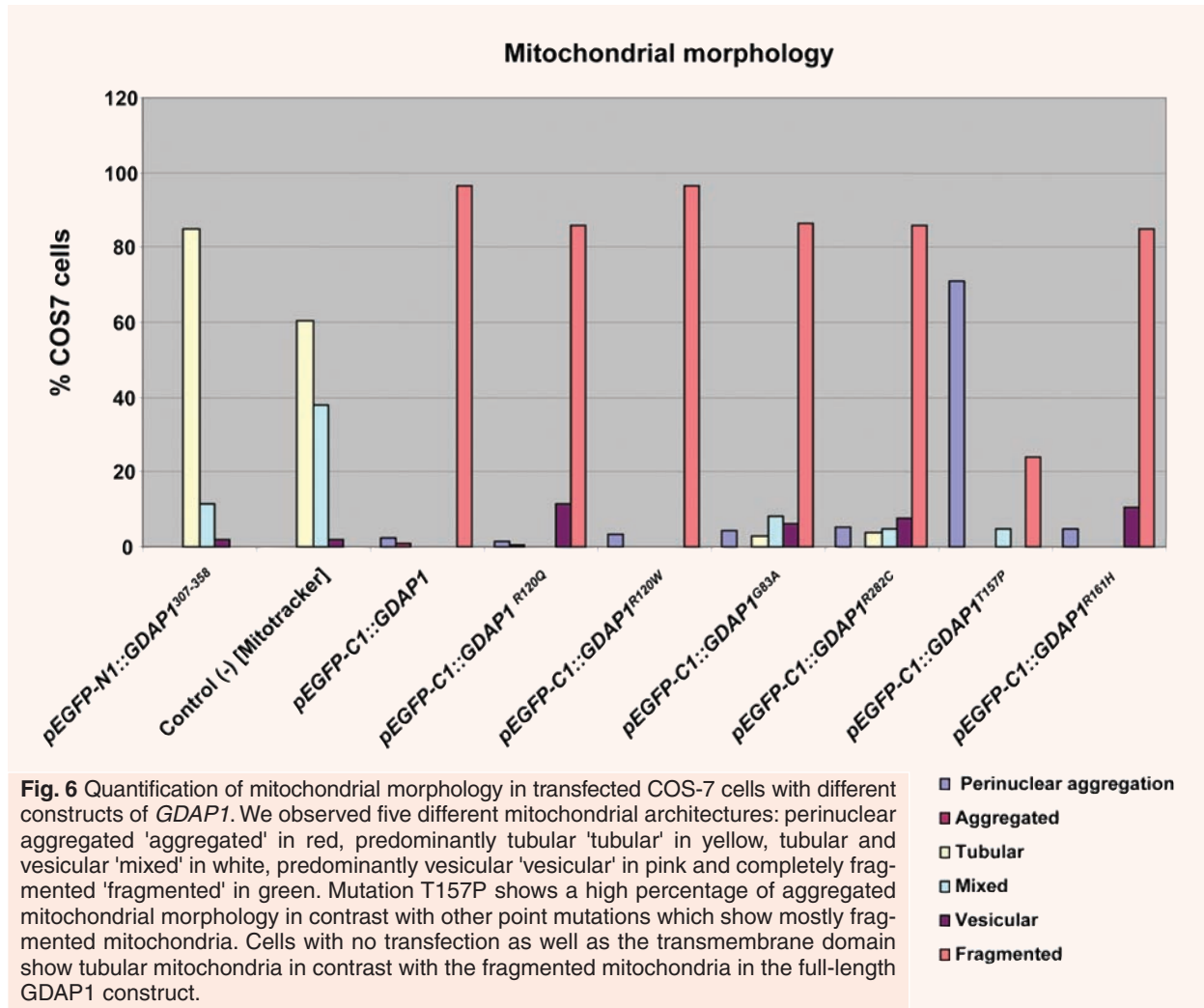
Fig. 5 Morphological patterns of mitochondria observed after overexpression of different mutations in COS-7 cells. (**a**) Mitochondrial stain with MitoTracker Orange CMTMRos showing the tubular mitochondrial morphology in red. (**b–f**) Overexpression of GFP contructs with different *GDAP1* missense mutations show five different mitochondrial morphologies named as (**b**) 'fragmented', (**c**) 'vesicular', (**d**) 'tubular', (**e**) 'mixed' and (**f**) 'aggregated'.

there are any other genes replacing GDAP1 function in these other neural regions.

In our experiments GDAP1 immunoreactivity colocalized with mitochondrial markers and the protein has been shown to be located at the mitochondrial membrane. Our results further indicate that the COOH-terminus is necessary and sufficient to target the protein to its mitochondrial localization. GDAP1 seems to be involved in the regulation of the mitochondrial network dynamics in mammalian cells [21, 22] as a component of the physiological fission machinery. In fact, overexpression of GDAP1 leads to fragmentation of mitochondria, and reduction of GDAP1 induces the elongation of mitochondria resulting in a characteristic tubular architecture [7, 23].

Both nonsense and missense mutations have been associated with autosomal recessive inheritance in CMT4A families suggesting a loss of function mechanism [2, 3, 13]. Whereas, the mechanism involved in the effects of nonsense and frameshift mutations may be related to the production of nonfunctional truncated proteins, pathogenesis of missense mutations is more difficult to explain. A number

of the missense mutations that we have analysed by overexpression experiments resulted in a pattern of fragmented mitochondria identical to that caused by overexpression of the full length GDAP1 [7, present study], which suggest that other loss of function mechanisms may cause the disease. In this way, an interesting mutation is R120W. This mutation has been reported in a compound heterozygote patient carrying also a G271R missense change in the other mutant allele. The patient showed a severe phenotype with disease onset at the age of 3 years, she became wheelchair dependent at the age of 20 years and she had slowed NCV in the median nerve (23 m/s) and ulnar nerve (19 m/s) whereas motor and sensory action potentials were severely reduced [24]. By contrast, in patients of the two families were R120W mutation segregated as a dominant mutation reported by Claramunt *et al.* [13] the phenotype was milder. In fact, the probands' age of onset was in the second decade and they were still walking at the age of 35 years. In spite of the R120W mutation show identical expression pattern than the wild type protein and other missense mutations, it is tentative to speculate

Fig. 6 Quantification of mitochondrial morphology in transfected COS-7 cells with different constructs of *GDAP1*. We observed five different mitochondrial architectures: perinuclear aggregated 'aggregated' in red, predominantly tubular 'tubular' in yellow, tubular and vesicular 'mixed' in white, predominantly vesicular 'vesicular' in pink and completely fragmented 'fragmented' in green. Mutation T157P shows a high percentage of aggregated mitochondrial morphology in contrast with other point mutations which show mostly fragmented mitochondria. Cells with no transfection as well as the transmembrane domain show tubular mitochondria in contrast with the fragmented mitochondria in the full-length GDAP1 construct.

that the pathogenic mechanism may be different when the mutation is unique or when a second mutant allele is present. More intriguing is the T157P mutation. Cells overexpressing this mutation showed the aggregation pattern of mitochondria, especially perinuclear; this finding suggests that this mutation may be acting through a dominant negative effect and, then, increasing fusion of mitochondria. Any molecular situation producing an unbalance in the mitochondrial network dynamics may lead to abnormal trafficking and transport of mitochondria through axons to reach the synapses where a high level of energy is required to maintain proper activity. One likely possibility is that the different mutations cause similar apparent morphology with the wild-type form

but different mitochondrial physiology, resulting in abnormal/reduced bioenergetics or an increase in oxidative stress. The lack of energy at synapses of the affected neurons might produce a dying-back axonal dysfunction resulting in the characteristic peripheral nerve pathology found in CMT4A patients.

In summary, we report here a specific expression of GDAP1 in neurons. We could demonstrate expression not just in DRG sensory neurons and motor neurons of the anterior horn of the spinal cord but also in many other neuronal types of the CNS. However, the expression pattern has no correlation with the CMT4A pathology, which involves only the peripheral nerves. By contrast, we could not demonstrate expression of GDAP1 in Schwann and other

glial cells. We propose that CMT4A primarily involves neurons and the Schwann cell pathology observed in some cases may be a secondary, although relevant, event in the mechanism causing peripheral neuropathy by *GDAP1* mutations. Moreover, GDAP1 may be important for the Schwann cell-axon interaction physiology, which also could explain the paradox between cell expression in both PNS neurons and CNS neurons and pathology that just involves the peripheral nerves. It could be argue that GDAP1 has not any physiological role in cell interaction between glial cells and neurons in the CNS. The pathogenic effect of these mutations seems to dysbalance the mitochondrial dynamics mainly involving the fission process. However, different mutations may affect the mitochondria fusion and fission pathways in the same way: loss of function mutations will favour the fusion process but fusion may also be favoured by the presence of a dominant-negative mutation. Further studies in the biology of GDAP1 and other proteins involved in the fusion and fission pathway will help us to understand the molecular mechanism of the mitochondrial network dynamics and the role of mitochondria in the pathophysiology of neuropathies.

Acknowledgements

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