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Mutations in the *ganglioside-induced differentiation-associated protein-1* (*GDAP1*) gene in intermediate type autosomal recessive Charcot–Marie–Tooth neuropathy

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Summary

Mutations in the gene for the ganglioside-induced differentiation-associated protein-1 (GDAP1) on 8q21 recently were reported to cause autosomal recessive Charcot–Marie–Tooth (CMT) sensorimotor neuropathy. Neurophysiology and nerve pathology were heterogeneous in these cases: a subset of *GDAP1* mutations was associated with peripheral nerve demyelination, whereas others resulted in axonal degeneration. In this study, we identified two novel mutations disrupting the *GDAP1* reading frame. Homozygosity for a single base pair insertion in exon 3 (c.349_350insT) was observed in affected children from a Turkish inbred pedigree. The other novel allele detected in a German patient was a homozygous mutation of the intron 4 donor splice site (c.579 + 1G>A). Patients with *GDAP1* mutations displayed severe, early childhood-onset CMT neuropathy with prominent *pes equinovarus* deformity and impairment of hand muscles. Nerve conduction velocities were between 25 and 35 m/s and peripheral nerve pathology showed axonal as well as demyelinating changes. These findings fitted the definition of intermediate type CMT and further support the view that GDAP1 is vital for both, axonal integrity and Schwann cell properties.

Keywords: hereditary motor and sensory neuropathy; Charcot–Marie–Tooth neuropathy; autosomal recessive; intermediate; ganglioside-induced differentiation-associated protein-1; GDAP1

Abbreviations: ARCMT = autosomal recessive Charcot–Marie–Tooth neuropathy; CMT = Charcot–Marie–Tooth neuropathy; GDAP1 = ganglioside-induced differentiation-associated protein-1; GST = glutathione *S*-transferase; MNCV = motor nerve conduction velocity; NCV = nerve conduction velocity; SSCP = single-strand conformation polymorphism

Introduction

Hereditary motor and sensory neuropathy (HMSN) or Charcot–Marie–Tooth (CMT) disease comprises a group of clinically and genetically heterogeneous disorders of the peripheral nervous system. With an overall prevalence of 1 in 2500, CMT is the most common inherited neuromuscular disorder in man (Skre, 1974). The clinical features of CMT include progressive distal muscle weakness and atrophy, starting in the legs and spreading to the upper extremities, foot deformities, steppage gait, distal sensory loss and decreased or absent tendon reflexes (Harding and Thomas, 1980). On nerve biopsy and neurophysiology, CMT falls into two main subtypes, the demyelinating form, CMT1, and the axonal type, CMT2 (Dyck *et al*., 1993). CMT1 is characterized by reduced nerve conduction velocities (NCVs) with values <38 m/s for the median motor nerve, segmental de- and remyelination, and onion bulb formation. These

changes differentiate CMT1 from CMT2 in which NCVs are near normal and nerve pathology shows axonal loss and regenerative sprouting. It has been suggested that these differences originate from distinct aetiologies, with a primary Schwann cell defect in CMT1, and axonal dysfunction in CMT2.

The majority of CMT cases are autosomal dominantly or X-linked dominantly inherited (Vance, 2000). Autosomal recessive CMT (ARCMT) is a much rarer disorder and clinically similar to the dominant forms, but usually more severe with an earlier age of onset (Thomas, 2000). At least 10 loci responsible for ARCMT and four candidate genes have been identified so far (Warner *et al*., 1998; Bolino *et al*., 2000; Kalaydjieva *et al*., 2000; Boerkoel *et al*., 2001; Guilbot *et al*., 2001; Nelis, 2001). The first recognition of a recessive CMT locus (*CMT4A*) was by Ben Othmane *et al*. (1993) who mapped the responsible gene to chromosome 8q13–q21 in four Tunisian inbred kindreds. This locus turned out to be a major cause of ARCMT, making up ~25% of cases (Nelis *et al*., 2001). Recently, Baxter *et al*. (2002) and Cuesta *et al*. (2002) found that the gene for the ganglioside-induced differentiation-associated protein-1 (GDAP1) resides within the 8q21 candidate region. They subsequently sequenced this gene in ARCMT families positive for linkage to 8q21 and identified several causative mutations (Baxter *et al*., 2002; Cuesta *et al*., 2002). A subset of the so far described patients with *GDAP1* mutations presented with a demyelinating neuropathic phenotype (Baxter *et al*., 2002), while other families displayed axonal type ARCMT (Cuesta *et al*., 2002).

Table 1 *ARCMT patients*

Family	Affected	Consanguinity	Origin	Nerve biopsy
AC531	2	+	Turkey	Intermediate
CMT117	3	–	Germany	Demyelinating
CMT425	2	–	Germany	Demyelinating
CMT32	1	+	Turkey	Intermediate
CMT53	1	+	Turkey	Demyelinating
CMT413	1	+	Turkey	Demyelinating
CMT48	1	–	Germany	Intermediate
AC49	1	–	Germany	Intermediate
CMT409	1	–	Germany	Axonal
CMT543	1	–	Germany	Demyelinating

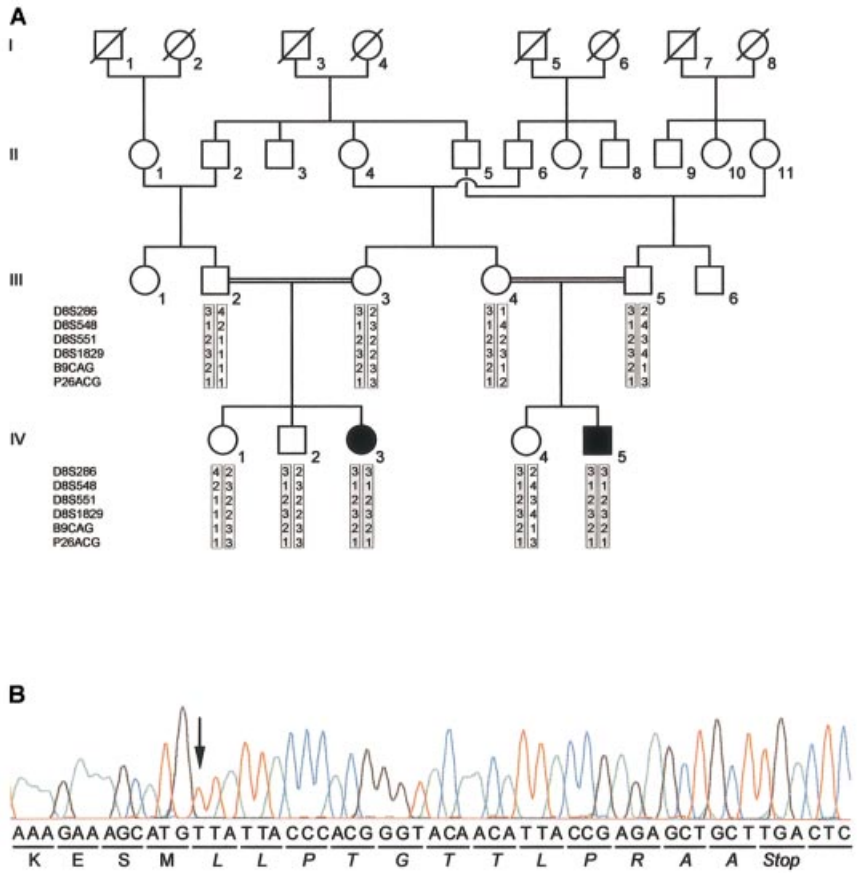

Fig. 1 Family AC531. (**A**) Pedigree of the family with two loops of consanguinity. Haplotype analysis with 8q21 markers showed homozygous haplotypes in both affected patients. (**B**) Sequencing analysis of patients' *GDAP1* exon 3. The homozygous T insertion is marked by an arrow in the chromatogram. Amino acid positions altered consecutive to the reading frame shift are printed in italics.

Here we report two novel *GDAP1* mutations which presumably result in a shortened non-functional peptide. These alleles were found to cause a phenotype which combines both pathologies, demyelinating and axonal. This is the first report on *GDAP1* mutations causing an intermediate type of ARCMT. Detailed fine structural findings in the diseased nerves will be presented.

Methods and subjects

A series of 10 families with the inferred diagnosis of ARCMT was selected (Table 1). Selection criteria were (i) progressive sensorimotor neuropathy with onset in the first decade; (ii) absence of clinical symptoms and neurophysiological signs in the parents; and (iii) exclusion of mutations in dominant CMT genes. Clinical details, the results of nerve conduction studies, and those for nerve biopsy if carried out were obtained in all cases. One Turkish consanguineous family presented with affected children in two independent sibships (AC531). Two German pedigrees included at least two affected siblings (CMT117 and CMT425). No consanguinity was known in the latter families. Three Turkish inbred families with a single affected child were also included in the study (CMT32, CMT53 and CMT413). The remaining patients were single affected children from German families. Neurophysiology and nerve biopsy indicated a demyelinating, axonal or intermediate type of CMT. Mutation analysis for dominant CMT genes *PMP22*, *Cx32*, *MPZ* and *EGR2* had been negative prior to testing for ARCMT.

Genotyping for the ARCMT locus on 8q21 was performed in families AC531, CMT117, CMT425, CMT32, CMT53 and CMT413. The following microsatellite markers were used: D8S286, D8S548, D8S551, D8S1829, B9CAG and P26ACG. Primers for polymerase chain reaction (PCR) amplification were as published by the Genome Database (http://www.gdb.org) and by Ben Othmane *et al*. (1998). Sense primers were labelled with FAM fluorophores (Pharmacia, Uppsala, Sweden) for electrophoresis and analysis on an ABI PRISM 310 genetic analyser (Applied Biosystems, Weiterstadt, Germany). Individuals with possible linkage to the *CMT4A* locus were screened for *GDAP1* mutations. All patients for whom haplotype analysis was not applicable were subjected directly to sequencing of the *GDAP1* gene.

For mutation analysis, primers were constructed outside the predicted six GDAP1-coding exons using the Primer3 program (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi). These primers were used to PCR amplify genomic DNA templates from the index patients. PCR products were gel purified with the QiaEx-kit (Qiagen, Hilden, Germany) and sequenced using ABI BigDye chemistry (Applied Biosystems). Samples were run and analysed on an ABI PRISM 310 genetic analyser (Applied Biosystems). Appropriate conditions for single-strand conformation polymorphism (SSCP) analysis were established for exons bearing mutations to test for the segregation of the altered allele. In the case of PCR products >300 bp in length, exonic primers were designed to yield smaller products suitable for SSCP. DNA samples from 100 apparently unrelated normal control subjects (58 females and 42 males) were also tested under the same SSCP conditions.

Sural nerve biopsies were performed and processed for light and electron microscopic examination using standard methods. Morphometric, optic-electronic evaluation was performed on a KS300 system of Zeiss/Kontron (Eching/Munich, Germany). Five representative endoneurial areas per nerve (measuring 5412 μm^2 each) were analysed. In selecting these areas, care was taken to avoid perineurial spaces or septa, blood vessels, distortions and fixation artefacts.

Written informed consent was obtained from all family members included in the study for use of blood and DNA samples for diagnostic purposes.

Results

CMT4A markers were homozygous in affected probands from Turkish inbred family AC531 (Fig. 1A). In family CMT117, there is an unaffected sib who shares haplotypes with an affected child. Therefore, family CMT117 is excluded for linkage to *CMT4A*. In family CMT425, positive but insignificant lod scores were obtained. Patients CMT32, CMT53 and CMT413 from consanguineous marriages did not present with a homozygous 8q21 haplotype and were therefore regarded as unlikely to have *CMT4A*.

Two previously unreported mutations of the *GDAP1* gene were identified; these mutations were present in the homozygous state and were not found in healthy controls. Another mutation, a heterozygous substitution of Arg282 with cysteine, was detected in family CMT48. The allele was inherited by the clinically normal father. This mutation was not found in 100 control individuals. However, no mutation of the second allele could be identified by *GDAP1* sequencing. A c.507G>T transversion in exon 4 was present in the homozygous and heterozygous state in ARCMT patients as well as in normal controls. This DNA variant did not alter the corresponding serine codon 169 (Table 2).

Family AC531

This family originates from Southern Turkey, near the Syrian border. The parents in the two nucleus families (III.2 and III.3; III.4 and III.5) with affected individuals were first cousins (Fig. 1A). A homozygous *GDAP1* mutation was detected in the index case, AC531-IV.5, and his female cousin, AC531-IV.3. This mutation is a T insertion in *GDAP1* exon 3 (c.349_350insT) resulting in a frameshift and premature stop of translation 12 GDAP1 missense amino acid residues downstream (Fig. 1B, Table 2). For index patient AC531-IV.5, pregnancy and delivery were reported as uneventful. The neonatal period and early motor milestones had also been normal. He started to walk independently at the age of 14 months and gait was noted to have been clumsy since then. He progressively developed bilateral *pes equino-*

Table 2 *Reported GDAP1 mutations and variants*

Exon	Mutation	Amino acid change	Pathogenic character	Present in controls	Reference
1	c.92G>A	Trp31stop	Yes	No	Baxter *et al*. (2002
3	c.349_350insT	Thr117fs	Yes	No*	This study
3	c.482G>A	Arg161His	Yes	No	Baxter *et al*. (2002)
4	c.487C>T	Gln163stop	Yes	No	Cuesta *et al*. (2002)
4	c.507G>T	No change of Ser169	No	GG:24/TG:58/TT:18*	This study
4	c.579 + 1G>A	Exon skipping supposed	Yes	No*	This study
5	c.581C>G	Ser194stop	Yes	No	Cuesta *et al*. (2002); Baxter *et al*. (2002)
6	c.844C>T	Arg282Cys	Supposed	No*	This study
6	c.863insA	Thr288fs	Yes	No	Cuesta *et al*. (2002

*100 control individuals.

Table 3 *Nerve conduction data*

Case	Motor conduction				Sensory conduction			
	Median		Peroneal		Median		Sural	
	CV (m/s)	Amp (mV)	CV (m/s)	Amp (mV)	CV (m/s)	Amp (μV)	CV (m/s)	Amp (μV)
AC531-IV.5	31	0.5	23.7	0.2	32	4	–	–
AC49-II.2	26.4	0.3	–	–	30	5	–	–

– = not recordable.

varus deformity so that he had to walk on the outer edges of the feet. At age 3 years, bilateral foot drop and steppage gait became apparent. Bilateral lengthening of the achilles tendons was performed at the age of 4 years and brought about only temporary amelioration. Examination at age 7 years revealed atrophy of the calves, foot muscles and, to a lesser extent, of the thenar and hypothenar muscles. Muscle hypotonia was noted. Lower limb weakness and clubfoot deformity impaired ambulation, which was only possible with the aid of braces. Heel and toe walking was impossible. Fine motor capacities of the hands were severely disturbed, and he was not able to grab a pen. Upper limb tendon reflexes were diminished and lower limb tendon reflexes were not obtainable. Sensory qualities for vibration, touch and pain were reduced in the feet and lower legs, but preserved in the hands. Electromyography revealed neurogenic changes. At age 6 years, median motor nerve conduction velocity (MNCV) was reduced to 31 m/s, with severely decreased motor action potential amplitude. Motor conduction of the peroneal nerve was also slowed. Sensory action potential of the median nerve was severely decreased while stimulation of the sural nerve resulted in no response (Table 3). Both parents had normal NCVs. His cousin, patient AC531-IV.3, showed similar clinical features. Clubfoot deformity was even more prominent in her and required repeated surgery. Moreover, severe wasting of hand muscles with claw hand deformity could be noted.

Family AC49

The 6-year-old girl II-2 is the second child of healthy German parents. No consanguinity was known in this family. *GDAP1* sequencing disclosed a homozygous mutation of the intron 4 donor splice site (c.579 + 1G>A) in the affected patient (Fig. 2, Table 2). Haplotype analysis proved homozygosity for all 8q21 markers tested. Thus, the parents might well be at least distantly related. On the mRNA level, the c.579 + 1G>A mutation will most probably result in skipping of exon 4 (Nakai and Sakamoto, 1994). Unfortunately, frozen tissue from the nerve and muscle biopsy was not left and a fresh blood sample was unobtainable for mRNA isolation to prove this hypothesis. Clinical records of patient AC49-II.2 included an uneventful pregnancy and neonatal period, and normal early motor milestones. Since she started to walk at the age of 18 months, both feet were noted to be twisted and she could not place the sole flat on the ground. *Equinovarus* deformity was first diagnosed at age 2 years and subsequently required orthopaedic correction. Examination at age 6 years revealed severe wasting of the calves, foot muscles and, to a lesser extent, of the hand muscles. Clubfoot deformity was prominent. Heel and toe walking was impossible. She still could ambulate independently over a distance of 100 m, but required use of crutches for longer distances. Fine motor capacities of the hands were disturbed due to claw hand deformity, and she was not able to tie her shoes. Upper limb

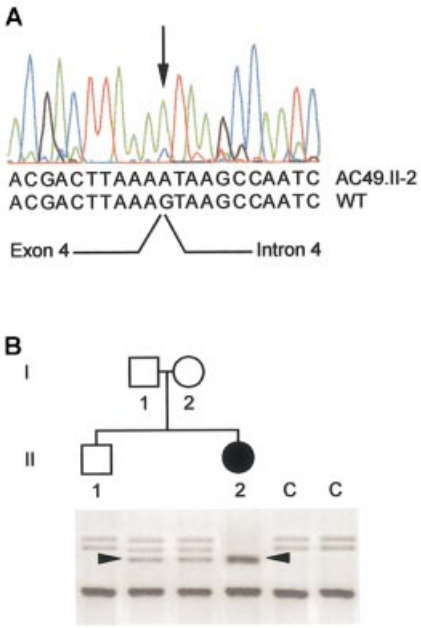

Fig. 2 Family AC49. (**A**) Sequencing analysis of the patient's *GDAP1* exon 4 displays a homozygous G to A transition of position +1 of the donor splice site (arrow). (**B**) Segregation of the mutant allele in the family. SSCP analysis of the exon 4 PCR product reveals heterozygosity in both parents. The healthy brother inherited the normal alleles (C = healthy controls).

tendon reflexes were diminished and lower limb tendon reflexes could not be elicited. All sensory qualities were diminished in the feet and lower legs, and to a lesser degree in the hands. Neurophysiological studies were performed at age 5 years. MNCV of the median nerve was 26.4 m/s, with severely decreased amplitude of the evoked muscle action potential. No muscle action potentials could be obtained after stimulation of the tibial and peroneal nerves. Sensory action potential of the median nerve was low, and no response to stimulation of the sural nerve was obtained (Table 3). Nerve conduction studies in the parents and in the healthy brother were normal.

Histology

Patients AC531-IV.5 and AC49-II.2 presented with comparable findings on sural nerve biopsies (Fig. 3A and B). Patient AC531-IV.5 was biopsied at the age of 3.5 years. The sural nerve specimen showed marked loss of large myelinated axons and numerous clusters of small, closely adjacent regenerated fibres. Occasional demyelinated fibres and some isolated, thinly remyelinated fibres with surrounding flat cell processes were also apparent. In patient AC49-II.2, biopsies were performed at the age of 2 years. The sural nerve appeared to be affected similarly to patient AC531-IV.5, but there were more isolated, thinly remyelinated fibres with incipient onion bulb formation. Clusters of regenerated fibres were less frequent. Morphometric assessment confirmed the reduction of the number of large diameter fibres in both patients with a relative increase of small myelinated fibres. The myelin area per endoneurial area, the mean total fibre area and the mean myelin area were decreased (Table 4). Assessment of muscle specimens showed neurogenic atrophy.

Electron microscopy (Fig. 3C–E) revealed numerous degenerating axons, bands of Buengner and groups of regenerated myelinated fibres combined with not yet remyelinated fibres. Some of these clusters were surrounded by supernumerary Schwann cell processes forming onion bulb-like structures. Demyelinated axons and incipient onion bulb formations with up to three Schwann cell layers were apparent in both cases, but were encountered more frequently in patient AC49-II.2. Few fibres displayed non-compacted innermost myelin lamellae with adaxonal vacuoles. Analysis of myelin periodicity did not reveal further changes. The unmyelinated fibres appeared to be better preserved than the myelinated ones, yet their diameter varied considerably. Specific fine structural changes were not detectable.

Discussion

We have identified two pathogenic mutations in the *GDAP1* gene in a Turkish and a German family, both with autosomal recessive inheritance of CMT neuropathy. We also performed haplotyping of the *GDAP1* locus and sequencing of the gene in eight further families compatible with autosomal recessive neuropathies. One further presumably pathogenic mutation was observed; however, no mutation of the second allele could be detected in this case. The clinical phenotype of patients with *GDAP1* mutations was similar, and neurophysiology and nerve biopsy indicated a combined axonal and demyelinating lesion.

Our findings extend the spectrum of known mutations in the *GDAP1* gene (Baxter *et al*., 2002; Cuesta *et al*., 2002). Two mutations severely disrupt the gene and are likely to result in loss of function of the protein by significantly altering its structure. Thus, their pathogenic character seems to be very likely. The c.349_350insT mutation in exon 3 (family AC531) causes a frameshift and creates a truncated protein, with the last 12 amino acids being missense (Thr117fs). The c.579 + 1G>A mutation (family AC49) destroys the sequence of the donor splice site of intron 4. Three different consequences could be expected for a splice site mutation, i.e. skipping of the entire preceding exon, read-through of the retained intron or use of a cryptic splice donor site. Exon skipping is the most frequently observed result of a splice site mutation in mammals (Nakai and Sakamoto, 1994). As to the present case, absence of exon 4 from the mature *GDAP1* transcript would result in a premature termination codon in the aberrantly spliced mRNA as the junction between exon 3 and exon 5 is not in-frame. Unfortunately, this could not be proven to be the case as no appropriate source for mRNA isolation was accessible. The Arg282Cys (c.844C>T) missense mutation (family CMT48)

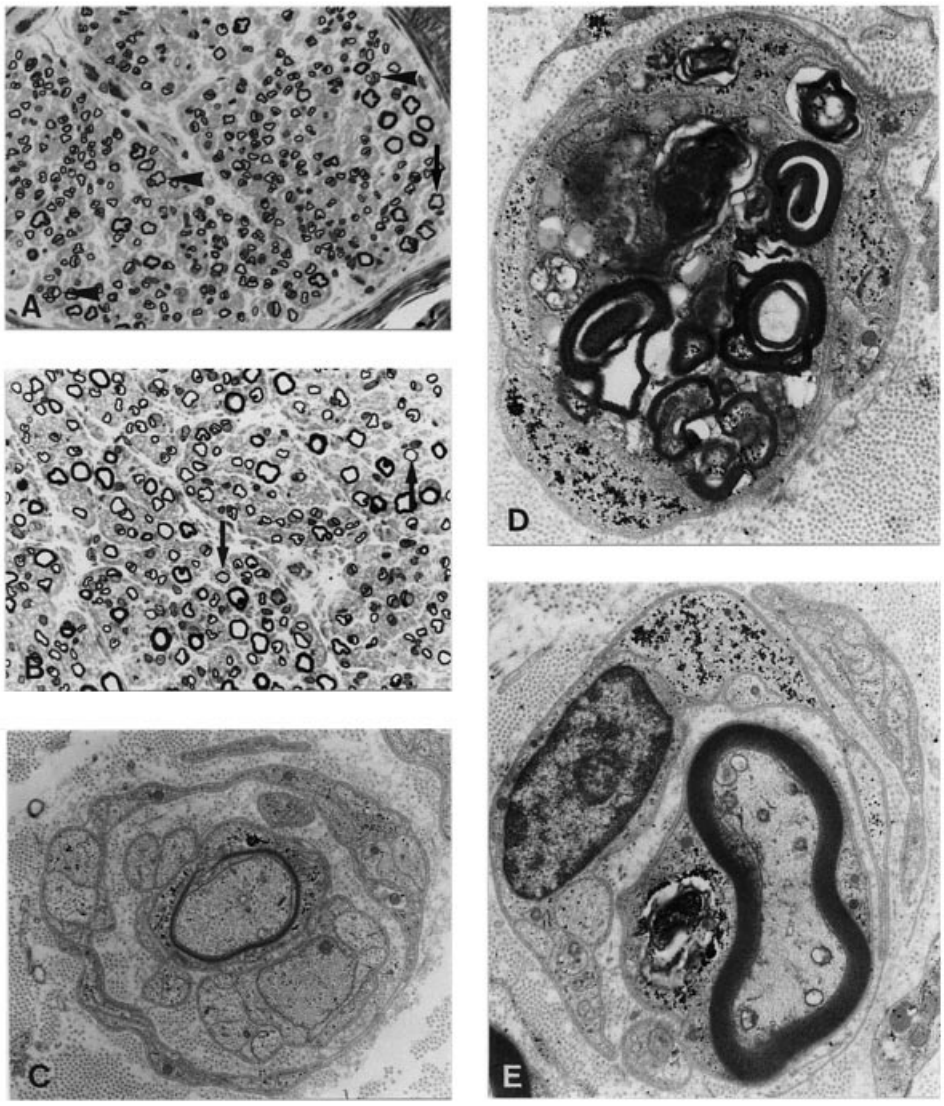

Fig. 3 Light microscopic (**A** and **B**) and ultrastructural (**C**–**E**) findings. (**A**) Sural nerve biopsy from patient AC531-IV.5 at the age of 3.5 years. The number of large myelinated fibres is severely reduced, and numerous small clusters of regenerated fibres are encountered (arrowheads). Some isolated axons have disproportionately thin myelin sheaths (arrow). Semi-thin section, toluidine blue stain, ×266. (**B**) Sural nerve biopsy from patient AC49-II.2 at the age of 2 years. There is a similar decrease of large myelinated fibres, but isolated, thinly myelinated axons (arrows) are more prevalent compared with **A**. Semi-thin section, toluidine blue stain, ×287. (**C**) A cluster of two regenerated axons from patient AC49-II.2 is surrounded by supernumerary Schwann cell processes. One axon shows an early stage of remyelination, ×6580. (**D**) Sural nerve biopsy from patient AC531-IV.5: myelin degradation products in an early band of Buengner indicate preceding axonal decay, ×9450. (**E**) Early stage of an onion bulb formation in the nerve biopsy from patient AC531-IV.5, ×7700.

represents either a rare polymorphism or a causative substitution if the corresponding patient has autosomal recessive disorder and a second mutation in *GDAP1* that we were unable to detect. Interestingly, the clinical course as well as the electrophysiological and histopathological phenotype of family CMT48's propositus were similar to those observed in the other two families with *GDAP1* mutations. Arg282Cys affects an amino acid residue that is highly conserved in the GDAP1 sequences of various mammals (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). It is contained in the C-terminal glutathione *S*-transferase (GST)-like domain which is probably important for GDAP1 function (see below). Moreover, this variant could not be detected in healthy controls that were analysed under appropriate SSCP conditions. Consequently, Arg282Cys might be a pathogenic mutation rather than a harmless variant, but more definite proof has to come from functional studies or identification of the mutation *in trans* in this family. Since the allele is

Table 4 *Morphometric data*

Case	Sex	Age at biopsy (years)	Nerve fibre density ($1/mm^2$)+	Myelin area per endoneurial area (%)+	Mean total fibre area (μm^2)	Mean myelin area ($\mu m^2 \pm$ SD)+
AC531-IV.5	M	3.5	9423	13.5	450	14.3 ± 9.8
Control 29*	F	4	9570	17	919	17.8 ± 15.0
AC49-II.2	F	2	8204	10.9	382	9.2 ± 7.9
Control 27*	F	2.5	10 716	19	1006	17.3 ± 16.1

*Data for the age-matched controls were taken from Bertram and Schröder (1993).
+Altogether, 255 nerve fibres were evaluated in case AC531-IV.5, 259 in control 29, 222 in case AC49-II.2 and 290 nerve fibres in control 27.

inherited from the clinically normal father, there is no evidence for a dominant mutation. The c.507G>T transversion in exon 4 does not change the corresponding serine codon 169 and occurs with a high frequency in the general population. Therefore, it is most likely to be a polymorphism without phenotype.

The *GDAP1* gene consists of six exons which give rise to a 4.1 kb transcript encoding an open reading frame of 358 amino acids (Liu *et al*., 1999; Baxter *et al*., 2002; Cuesta *et al*., 2002). Little is known about the function of GDAP1. The GDAP1 amino acid sequence has strong similarity to GSTs that serve as antioxidant systems preventing degenerative cellular processes (Baez *et al*., 1997). GDAP1 is expressed in various tissues, with the highest levels in brain and spinal cord. Amplification of *GDAP1* transcripts from human and mouse peripheral nerve and cauda equina suggested that GDAP1 expression occurs not only in neurons but also in Schwann cells, the primary source of mRNA in the peripheral nervous system (Cuesta *et al*., 2002). Mutated GDAP1 might impair the correct catalysing *S* conjugation of reduced glutathione, resulting in progressive attrition of the Schwann cell and/or the axon.

In the present study, all patients with *GDAP1* mutations showed severe early childhood-onset neuropathy with prominent weakness beginning in the lower limbs, spreading proximally and affecting the distal upper limbs within the first decade of life. Sensory disturbance was less prominent. All three patients had clubfoot deformity which severely impaired ambulation. Clinical phenotypes reported so far in patients with *GDAP1* mutations (Baxter *et al*., 2002; Cuesta *et al*., 2002) have been similar to those seen in our patients. Progressive sensorimotor neuropathy started in infancy and led to loss of independent ambulation at the beginning of the second decade. *GDAP1*-related ARCMT seems to be clinically homogeneous without manifestation other than peripheral neuropathy. Hoarsening of the voice due to vocal cord paresis as observed in the families reported by Cuesta *et al*. (2002) might also be noted in CMT patients with other genetic causes.

GDAP1 mutations have been found to cause both demyelinating and axonal peripheral nerve lesion (Baxter *et al*., 2002; Cuesta *et al*., 2002). In the present cases with *GDAP1* mutations, nerve pathology was consistent with both axonal degeneration and demyelination. This intermediate form of CMT has already been observed in cases with autosomal dominant inheritance and occurs with median MNCVs between 25 and 45 m/s (Davis *et al*., 1978; Rouger *et al*., 1997). In our patients, median MNCVs were at the lower extreme of this range. This might be attributed to a more rapid progression of this autosomal recessive disease. In addition to segmental demyelination, NCV slowing would be aggravated by decay of large, fast-conducting axons. Prominent fibre loss was reflected by severely decreased motor/sensory nerve action potential amplitudes and by nerve histopathology. The finding of phenotypic variability with *GDAP1* mutations adds to reports that mutations in the known CMT1 genes for the structural myelin protein MPZ (P0) and for the gap junction protein connexin32 (Cx32) can also set off axonal CMT2 (Vance, 2000). Obviously, the axonal and demyelinating delineation does not imply unique aetiologies.

It has been noted previously that ARCMT linked to 8q21 makes up ~25% of all ARCMT cases (Nelis *et al*., 2001), which is in line with the detection rate observed in the present study. Up to now, without knowing the gene responsible, diagnosis of *CMT4A* was dependent on linkage analysis that—in the setting of genetic heterogeneity—was restricted to extended pedigrees. Identification of *GDAP1* as the causative gene defect now allows direct mutation analysis in individual patients also from small, non-consanguineous families with only one affected child. A molecular genetic diagnosis eliminates the need for invasive techniques (e.g. sural nerve biopsy), prevents fruitless therapeutic approaches (e.g. for chronic inflammatory demyelinating polyneuropathy) and allows accurate genetic counselling.

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