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## Dominant *GDAP1* mutations cause predominantly mild CMT phenotypes

M. Zimoń, MSc\*, J. Baets, MD, PhD\*, G.M. Fabrizi, MD, PhD, E. Jaakkola, MD, PhD, D. Kabzińska, PhD, J. Pilch, MD, PhD, A.B. Schindler, MS, CGC, D.R. Cornblath, MD, K.H. Fischbeck, MD, M. Auer-Grumbach, MD, PhD, C. Guelly, PhD, N. Huber, MSc, E. De Vriendt, BSc, V. Timmerman, PhD, U. Suter, PhD, I. Hausmanowa-Petrusewicz, MD, PhD, A. Niemann, PhD, A. Kochanski, MD, PhD, P. De Jonghe, MD, PhD, and A. Jordanova, PhD Neurogenetics Group (M.Z., J.B., E.D.V., P.D.J., A.J.) and Peripheral Neuropathy Group (V.T.), VIB Department of Molecular Genetics, University of Antwerp; Neurogenetics Laboratory (M.Z., J.B., V.T., E.D.V., P.D.J., A.J.), Institute Born-Bunge, University of Antwerp; Division of Neurology (J.B., P.D.J.), University Hospital Antwerp, Belgium; Department of Neurological, Neuropsychological, Morphological and Motor Sciences (G.M.F.), University of Verona, Italy; Department of Clinical Genetics (E.J.), Oulu University Hospital, Oulu, Finland; Neuromuscular Unit (D.K., I.H.-P., A.K.), Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw; Department of Child Neurology (J.P.), Medical University of Silesia, Katowice, Poland; Neurogenetics Branch (A.B.S., K.H.F.), NINDS, NIH, Bethesda; Department of Neurology (D.R.C.), Johns Hopkins University, Baltimore, MD; Department of Internal Medicine, Division of Endocrinology and Metabolism (M.A.-G.), and Center of Medical Research (C.G.), Medical University Graz, Graz, Austria; and Institute of Cell Biology (N.H., U.S., A.N.), Department of Biology, ETH Zurich, Switzerland.

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Address correspondence and reprint requests to Prof. Dr. Alben Jordanova, VIB Department of Molecular Genetics, Molecular Neurogenetics Group, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium, alben.jordanova@molgen.vib-ua.be.

\*These authors contributed equally to this work.

### AUTHOR CONTRIBUTIONS

M. Zimo : drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data. Dr. Baets: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision. Dr. Fabrizi: analysis or interpretation of data, acquisition of data. Dr. Jaakkola: drafting/revising the manuscript, acquisition of data. Dr. Kabzińska: study concept or design, analysis or interpretation of data, acquisition of data, obtaining funding. Dr. Pilch: drafting/revising the manuscript, contribution of vital reagents/tools/patients, acquisition of data. A.B. Schindler: drafting/revising the manuscript, acquisition of data. Dr. Cornblath: drafting/revising the manuscript, analysis or interpretation of data, contribution of patients. Dr. Fischbeck: analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data. Dr. Auer-Grumbach: analysis or interpretation of data, acquisition of data. Dr. Guelly: analysis or interpretation of data. N. Huber: analysis or interpretation of data. E. De Vriendt: analysis or interpretation of data, acquisition of data. Dr. Timmerman: study concept or design, study supervision, obtaining funding. Dr. Suter: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, study supervision, obtaining funding. Dr. Hausmanowa-Petrusewicz: analysis or interpretation of data, acquisition of data. Dr. Niemann: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data, statistical analysis. Dr. Kochanski: study concept or design, analysis or interpretation of data, acquisition of data. Dr. De Jonghe: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision, obtaining funding. Dr. Jordanova: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision.

### DISCLOSURE

M. Zimo , Dr. Baets, Dr. Fabrizi, Dr. Jaakkola, Dr. Kabzińska, Dr. Pilch, and A.B. Schindler report no disclosures. Dr. Cornblath serves/has served on scientific advisory boards for Ardea Biosciences, Inc., Avigen, Inc., Pfizer Inc, Johnson & Johnson, GlaxoSmithKline, Abbott, Acorda Therapeutics Inc., Alexion Pharmaceuticals, Inc., Astellas Pharma Inc., Baxter International Inc., Bionevia Pharmaceuticals Inc., Bristol-Myers Squibb, Cebix, CSL Behring, Eisai Inc., Exelixis Inc., FoldRx, Genzyme Corporation, Keryx Biopharmaceuticals, Inc., Mitsubishi Tanabe Pharma Corporation, Octapharma AG, Sangamo BioSciences, Inc., sanofi-aventis, and Talecris Biotherapeutics; holds and has received license fee payments for patents re: Total neuropathy score (nurse, clinical), Methods to assess neuropathy. Dr. Fischbeck receives intramural research support from the NIH/NINDS. Dr. Auer-Grumbach, Dr. Guelly, N. Huber, E. De Vriendt, and Dr. Timmerman report no disclosures. Dr. Suter receives research support from the Swiss National Science Foundation and the NCCR Neural Plasticity and Repair. Dr. Hausmanowa-Petrusewicz, Dr. Niemann, Dr. Kochanski, Dr. De Jonghe, and Dr. Jordanova report no disclosures.

## Abstract

**Objective**—Ganglioside-induced differentiation associated-protein 1 (*GDAP1*) mutations are commonly associated with autosomal recessive Charcot-Marie-Tooth (ARCMT) neuropathy; however, in rare instances, they also lead to autosomal dominant Charcot-Marie-Tooth (ADCMT). We aimed to investigate the frequency of disease-causing heterozygous *GDAP1* mutations in ADCMT and their associated phenotype.

**Methods**—We performed mutation analysis in a large cohort of ADCMT patients by means of bidirectional sequencing of coding regions and exon-intron boundaries of *GDAP1*. Intragenic *GDAP1* deletions were excluded using an allele quantification assay. We confirmed the pathogenic character of one sequence variant by in vitro experiments assaying mitochondrial morphology and function.

**Results**—In 8 Charcot-Marie-Tooth disease (CMT) families we identified 4 pathogenic heterozygous *GDAP1* mutations, 3 of which are novel. Three of the mutations displayed reduced disease penetrance. Disease onset in the affected individuals was variable, ranging from early childhood to adulthood. Disease progression was slow in most patients and overall severity milder than typically seen in autosomal recessive *GDAP1* mutations. Electrophysiologic changes are heterogeneous but compatible with axonal neuropathy in the majority of patients.

**Conclusions**—With this study, we broaden the phenotypic and genetic spectrum of autosomal dominant *GDAP1*-associated neuropathies. We show that patients with dominant *GDAP1* mutations may display clear axonal CMT, but may also have only minimal clinical and electrophysiologic abnormalities. We demonstrate that cell-based functional assays can be reliably used to test the pathogenicity of unknown variants. We discuss the implications of phenotypic variability and the reduced penetrance of autosomal dominant *GDAP1* mutations for CMT diagnostic testing and counseling.

Charcot-Marie-Tooth (CMT) disease forms a clinically and genetically heterogeneous group of inherited peripheral neuropathies affecting 1 in 2,500 individuals.<sup>1</sup> Mutations in ganglioside-induced differentiation-associated protein 1 (*GDAP1*) cause autosomal recessive demyelinating,<sup>2</sup> axonal,<sup>3</sup> and intermediate forms of CMT.<sup>4,5</sup> Autosomal recessive *GDAP1* patients develop distal muscle weakness and wasting, typically with early childhood onset and a severe disease course. Proximal muscles become affected later, often leading to a wheelchair-dependency in the second or third decade of life. In the majority of patients, sensory impairment is evident on physical examination.<sup>6,7</sup> Development of unilateral or bilateral vocal fold paresis (VFP) in the later stages of disease may be indicative of phenotype severity.<sup>8</sup> Clinical heterogeneity was documented among patients with the same mutation, even within one kinship.<sup>4</sup>

Besides numerous recessive *GDAP1* mutations (<http://www.molgen.ua.ac.be/.CMTMutations/>), 6 amino acid substitutions were shown to be pathogenic in a heterozygous state, indicating that *GDAP1* mutations can be transmitted also as an autosomal dominant trait. Families were described with Ser34Cys, Arg120Trp, Gln218Glu, Arg226Ser, and Cys240Tyr mutations, while Thr157Pro occurred de novo in a single patient.<sup>9-12</sup> The phenotype of the autosomal dominant *GDAP1* patients described so far is consistent with mild axonal neuropathy, with later disease onset and slow progression, unlike most patients with recessive *GDAP1* mutations.

Here we provide genetic and functional evidence for the pathogenicity of 4 heterozygous *GDAP1* mutations and present detailed clinical description of the patients. Our study substantially broadens the understanding of autosomal dominant *GDAP1*-associated neuropathies.

## METHODS

### Patient cohort

We recruited 7 CMT2 families with dominant *GDAP1* mutations via a call for autosomal dominant *GDAP1* families within the International CMT Consortium. To ascertain dominant *GDAP1* mutation frequency we additionally assembled a cohort of 97 index patients, belonging to unrelated families with dominantly inherited CMT. Families were selected if there were affected members in at least 2 generations. Our cohort included 38 patients diagnosed with CMT2, 17 with CMT1, and 9 with intermediate CMT. No distinct electrophysiologic categorization could be made for 33 patients. Routine mutation screening of the common dominant CMT genes was uneventful in the majority of patients.

### Standard protocol approvals, registrations, and patient consents

All of our patients or their legal representatives signed an informed consent form prior to enrollment. This study was approved by the local institutional review board.

### Mutation analysis

Total genomic DNA isolated from peripheral blood samples of patients with CMT and control individuals served as a template in the PCR reactions. All coding exons and exon-intron boundaries of *GDAP1* were amplified using primer oligonucleotides described previously<sup>3</sup> or redesigned with Primer3<sup>13</sup> (primer sequences and PCR conditions are available upon request). Subsequently, PCR products were purified with the Exonuclease I-Shrimp Alkaline Phosphatase enzymes (USB, Cleveland, OH) and bidirectionally sequenced using the BigDye<sup>®</sup> Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Electrophoretic separation of fragments was performed on an ABI3730xl DNA Analyzer (Applied Biosystems). Mutation analysis was conducted with the SeqMan<sup>™</sup>II (DNASTAR Inc., Madison, WI) program. Mutations were described according to the HGVS nomenclature (<http://www.hgvs.org/mutnomen>) with nucleotide numbering based on the published online protein (NP\_061845) and mRNA (NM\_018972) sequence of *GDAP1* ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). All sequence variants were confirmed by an independent PCR and resequencing of the original or newly obtained DNA samples. Segregation analysis of the mutations with the disease phenotype was performed in all available family members. For the newly identified His123Arg, Ala156Gly, and Pro274Leu mutations, 280 control individuals of European descent were screened. Additionally, 96 control individuals of Finnish origin were sequenced for the His123Arg mutation. The in silico prediction of the functional effect of mutations was performed with PolyPhen-2 algorithm (<http://genetics.bwh.harvard.edu/pph2/index.shtml>). Score 1 is the highest score in PolyPhen-2.

### Multiplex amplicon quantification assay

We investigated the presence of intragenic deletions on the second allele of *GDAP1* by the Multiplex Amplicon Quantification (MAQ) assay ([www.multiplicon.com](http://www.multiplicon.com)). A multiplex PCR was performed including 10 fluorescently labeled amplicons targeting the genomic region of *GDAP1* and 6 reference amplicons located at randomly selected genomic positions outside the *GDAP1* region and other known copy number variations. PCR fragments were mixed with a formamide and GeneScan<sup>™</sup> 500 Liz<sup>®</sup> Size Standard (Applied Biosystems) solution (ratio 1:30) and size-separated on ABI3730xl DNA Analyzer. The ratio of peak areas between target and reference amplicons was calculated. Comparison of the normalized peak area values between patients and control individuals allowed determination of a dosage quotient (DQ) for each target amplicon, calculated by the MAQ-S package ([www.multiplicon.com](http://www.multiplicon.com)). DQ values below 0.75 were considered indicative for an amplicon deletion.

## Haplotype and paternity testing

We ascertained haplotype sharing between families with common *GDAP1* mutations with 6 highly informative short tandem repeat (STR) markers surrounding *GDAP1* (D8S279, D8S286, D8S551, D8S1144, D8S548, D8S1829) and one exonic SNP (rs11554166). Paternity was examined with 15 STRs distributed throughout the genome (ATA38A05, D1S1646, D1S1653, D1S1360, D2S2256, D3S3037, D4S2382, D4S3240, D7S509, D8S1759, D9S1118, D12S1056, D12S2082, D16S2619, and GATA152H04). STRs were first PCR-amplified with fluorescently labeled primer pairs (sequences are available at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)); fragments were subsequently combined with a formamide and GeneScanTM 500 Liz<sup>®</sup> Size Standard (Applied Biosystems) mixture (ratio 1:30) and size-separated on an ABI3730xl DNA Analyzer. Genotyping results were analyzed with Local Genotype Viewer, an in-house developed software program (<http://www.vibgeneticservicefacility.be/>).

## Functional assessment of pathogenicity of the Ala156Gly mutation

We transfected COS-7 or HeLa cells with cDNA constructs containing wild-type or mutant human *GDAP1* forms as outlined previously.<sup>14</sup> For transfection we used Fugene 6 reagent, according to the manufacturer's recommendations (Roche). The detailed experimental procedures for evaluating the mitochondrial morphology of the transfected cells, the polyethylene glycol (PEG) cell fusion assay, and the cytochrome *c* release assay are as described previously.<sup>14,15</sup>

## RESULTS

### Genetic findings

In this study, we identified 4 different heterozygous missense *GDAP1* mutations in 8 unrelated CMT families (figure 1). We collected 7 of these families through collaborative efforts. One additional Finnish family (CMT-129) was identified through a *GDAP1* mutation analysis of a heterogeneous cohort of 97 dominant CMT families and carried a new heterozygous c.358A>G (His123Arg) mutation. The same sequence variant occurred de novo in a patient of Tunisian origin (CMT-1060.01). Affected members of 2 Polish families (CMT-1123 and CMT-1124) carried a new heterozygous c.467C>G transversion in *GDAP1* resulting in alanine to glycine change at codon 156 (Ala156Gly). Another novel missense variant c.821C>T (Pro274Leu) segregated with the CMT phenotype in an Italian family (CMT-1058). We identified a previously reported c.358C>T (Arg120Trp) mutation in families from Italy (CMT-1057), the United States (CMT-1130), and Austria (CMT-1121).<sup>10</sup> All mutations target conserved amino acids and in silico analysis with PolyPhen-2 algorithm predicted a "probably damaging" effect on the protein function with score values ranging from 0.945 for Pro274Leu to 0.996 for His123Arg. The Pro274Leu mutation is localized in the C-terminal domain of GDAP1 protein, homologous to glutathione S-transferase domains (GST-N and GST-C) (figure 2). The other 3 mutations target the interdomain between GST-N and GST-C, which is in comparison to other GST families enlarged and therefore structurally characteristic for members of the GDAP1-family of GST proteins.<sup>16</sup> The 3 novel mutations (His123Arg, Ala156Gly, Pro274Leu) were absent from 280 control individuals. We ruled out partial intragenic deletions on the second *GDAP1* allele and thus a possible recessive inheritance by performing a MAQ assay.

We traced segregation of the *GDAP1* mutations in all available family members and all clinically affected individuals carried a mutant allele. This analysis further identified 4 unaffected carriers of the His123Arg mutation in the first generation of the Finnish family (CMT-129). The same His123Arg mutation however arose de novo and on a different chromosomal background in the isolated Tunisian patient CMT-1060.01 (table 1).

Nonpenetrance was also documented for individual CMT-1124.02 carrying the Ala156Gly mutation. This mutation resided on the same haplotype in the affected members of the 2 Polish families (CMT-1123, CMT-1124), thus indicating a common ancestor (table 1). Additionally, we found one clinically normal but electrophysiologically affected carrier of the Pro274Leu mutation in family CMT-1058.

The 3 families (CMT-1057, CMT-1121, CMT-1130) carrying the Arg120Trp mutation share a disease haplotype and therefore represent one mutational event (table 1).

### Pathogenicity of the Ala156Gly mutation

The Ala156Gly variation was found in 2 related Polish families and in one of them we identified an unaffected mutation carrier. We performed a cell fusion assay<sup>15</sup> in order to rule out the possibility of Ala156Gly being a private, benign sequence variant cosegregating with the phenotype. Wild-type human GDAP1-transfected hybrids and hybrids expressing the recessively inherited mutation behaved in similar fashion as control transfected cells with no obvious inhibition of mitochondrial fusion (figure 3). These data were consistent with previous observations with the mouse GDAP1 and mutated forms hereof.<sup>15</sup> Conversely, the fusion process was dramatically impaired in cells expressing the Ala156Gly variant or the known dominant Arg120Trp *GDAP1* mutation (figure 3). Furthermore, impaired fusion process resulted in fragmented mitochondria in cells expressing the Ala156Gly mutant (figure e-1A on the *Neurology*® Web site at [www.neurology.org](http://www.neurology.org)) and increased sensitivity to proapoptotic agents, as quantified by a cytochrome *c* release assay, which was also in line with our previous findings for other dominant *GDAP1* mutations (figure e-1, B and C).<sup>15</sup>

### Clinical findings

Detailed clinical and electrophysiologic studies were performed in a total of 15 patients from 8 families with dominant *GDAP1* mutations. Six additional patients were affected by history; however, either DNA samples or clinical details were not available for this study (see table 2, figure 1, table e-1, and table e-2).

We observed considerable phenotypic variability among these individuals even though many carried the same mutation. Based on electrophysiologic data, the majority of our autosomal dominant *GDAP1* patients can be diagnosed as axonal CMT; patients CMT-1060.01 and CMT-1058.01 had nerve conduction velocities (NCVs) in the intermediate range. Examination of a sural nerve biopsy in the same patient showed mixed axonal and demyelinating features.

Disease onset varied widely, ranging from childhood to late adulthood. Walking difficulties were the most common initial symptoms. Disease progression was slow and as a rule patients remained ambulatory. Four patients (CMT-129.03, CMT-129.07, CMT-1060.01, and CMT-1130.03) required an ankle foot orthosis; patient CMT-1058.01 became wheelchair-dependent at the age of 61 years. Weakness and atrophy was mainly restricted to distal muscles of the lower and upper limbs except for patients CMT-129.03, CMT-129.07, CMT-1058.01, and CMT-1130.03, who had mild signs of proximal weakness.

We did not observe vocal fold involvement in this study; patient CMT-1124.03 presented with hoarse-ness but a laryngoscopic evaluation could not be performed. We noted no additional clinical features in our patients apart from thoracic scoliosis seen in 2 sibs, CMT-1123.03 and CMT-1123.04. Noteworthy are several mildly affected or even asymptomatic mutation carriers at an advanced age (CMT-129.05, CMT-129.11, CMT-129.12, CMT-129.13, CMT-1058.02, CMT-1124.02, and CMT-1130.01).

## DISCUSSION

Mutations in *GDAP1* are most commonly associated with ARCMT; however, rare disease-causing heterozygous missense mutations have been reported in recent years.<sup>9-12</sup> *GDAP1* is thus one of the few CMT-associated genes for which both autosomal dominant and recessive inheritance patterns can be observed. Other notable examples are early growth response 2 (*EGR2*) and neurofilament light polypeptide (*NEFL*).<sup>17-23</sup> Interestingly, the mode of mutation inheritance was demonstrated to be dependent on the disease mechanism for all 3 genes.<sup>15,18,23,24</sup>

Here, we present genetic and functional evidence and comprehensive clinical data in a large collection of patients with CMT with dominant *GDAP1* mutations. In addition to the dominant *GDAP1* families gathered through international collaboration, we screened a group of 97 dominant CMT families and identified one new heterozygous mutation. This indicates that the frequency of dominant *GDAP1* mutations in larger cohorts is relatively low, at about 1%. Conversely, mutations in *GDAP1* are common in cohorts of recessive CMT families.<sup>25</sup> The recently reported mutation frequency of 27% in a small Italian ADCMT cohort is considerably higher than in this study, possibly because our study population is larger and more heterogeneous both clinically and ethnically.<sup>9</sup>

We identified 4 autosomal dominant *GDAP1* mutations in 8 families. Disease-haplotype sharing suggested that the previously described Arg120Trp mutation is a founder mutation. In a Finnish pedigree we identified a novel His123Arg mutation that also arose as a de novo event in an isolated Tunisian patient. Haplotype analysis showed that this mutation occurred independently on different genetic backgrounds thus underscoring its pathogenicity (table 1). Conversely, a novel heterozygous Ala156Gly variation (figure 1) was found in 2 Polish families that were distantly related (table 1). In addition, pathogenicity was uncertain due to an asymptomatic female mutation carrier in one of these families (CMT-1124) and the cosegregation of a non-CMT specific clinical feature (cardiac arrhythmia) in the second (CMT-1123). Therefore we performed functional characterization of the Ala156Gly mutation.<sup>15</sup>

*GDAP1* has sequence similarity to GSTs but neither binding to glutathione nor GST activity could be demonstrated.<sup>26,27</sup> Notably, *GDAP1* is an integral mitochondrial outer membrane protein playing an important role in mitochondrial dynamics and functioning.<sup>14,26</sup> Overexpression of *GDAP1* induces mitochondrial fission, a process abrogated to some extent by recessive *GDAP1* missense mutations, resulting in a mixture of fragmented and enlarged tubular mitochondria.<sup>14</sup> Conversely, dominant mutations in *GDAP1* alter the mitochondrial fusion process, as we have recently shown.<sup>15</sup> These clear differences in mitochondrial dynamics between dominant and recessive mutations prompted us to test the effect of the novel Ala156Gly variant. We demonstrated that this variant impairs the mitochondrial fusion process (figure 3) leading to fragmented mitochondria (figure e-1A) and increased cell sensitivity to apoptosis (figure e-1, B and C) in a similar fashion as other known dominant *GDAP1* mutations, thus supporting the pathogenic nature of this variant.

The mutations identified in our study target highly conserved residues that are predicted to be located in  $\alpha$ -helices.<sup>16</sup> The Arg120Trp mutation was previously identified in other studies, making it the most frequently observed dominant *GDAP1* mutation so far. Surprisingly, in our study all families with this variant shared a common ancestor. Conversely, His123Arg was proven to be recurrent, as it occurs on different chromosomal backgrounds. The recurrent character of some dominant *GDAP1* mutations suggests presence of mutational hotspots in the *GDAP1* gene. Interestingly, all known dominant *GDAP1* mutations target residues within the GST-homology region of the *GDAP1*-protein

family. Recessive *GDAPI* mutations in contrast are spread throughout the protein sequence. These data under-score the functional importance of the GST-like domain and may imply that the location of the mutation has an impact on the molecular mechanism leading to the ADCMT phenotype.

The dominant *GDAPI* mutations described so far are usually associated with mild axonal neuropathy with late onset in the second or beginning of the third decade of life and slow disease evolution.<sup>10-12</sup> The age at onset in half of our patient group was before the end of the first decade of life, which is earlier than noted previously. Disease progression in the majority of the patients in the current study is slow and most of them remain ambulatory to date. Although we observed proximal weakness in some patients, generally its extent and severity is less pronounced than for typical recessive *GDAPI* mutations. A notable exception is patient CMT-1058.01 who developed symptoms only at the age of 47 years, but nonetheless displayed a rapid deterioration resulting in wheelchair dependency at the age of 61 years. An acquired neuropathy was suspected; however, CSF studies remained negative and no clinical improvement was seen after various immunomodulating treatments.

*GDAPI* is one of the few CMT genes<sup>28-33</sup> that can be associated with VFP,<sup>8,34</sup> a severity hallmark for autosomal recessive *GDAPI* neuropathies.<sup>8</sup> One mildly affected individual (CMT-1124.03) from our cohort presented with a hoarse voice but VFP was not formally diagnosed. The general absence of VFP in autosomal dominant *GDAPI* is another indication of the generally milder phenotype.

In our study, electrophysiologic findings are quite heterogeneous. We observed a clear axonal neuropathy (i.e., moderately slowed NCVs >38 m/s and decreased CMAP amplitudes) in several patients but not in others. In addition, clinical variability is very striking, even within one family. In that respect pedigree CMT-129 (His123Arg mutation) is exemplary; the phenotype varied from asymptomatic individuals (CMT-129.05, CMT-129.11, CMT-129.12, and CMT-129.13) to patient CMT129.03, who displayed a clinically and electrophysiologically more pronounced phenotype. Overall, however, individuals from this family had the least abnormal NCVs. Reduced penetrance, though not uncommon for dominant *GDAPI* mutations, is generally rare in the context of CMT and is only occasionally observed for mutations in *MPZ*, *GARS*, *BSCL2*, and *TRPV4*.<sup>33</sup> Pronounced phenotypic variability is thus an important common characteristic of dominant *GDAPI* mutations. Clinicians evaluating patients in terms of consideration of this diagnosis should therefore take into account that some mutation carriers only have mild or minimal clinical and electrophysiologic abnormalities. A diagnosis should be based on detailed electrophysiologic findings in combination with the clinical and genetic data in the proband and extended to the proband's family.

In this study, we present a large cohort of patients with dominant *GDAPI* mutations, broadening the current knowledge about the phenotypic and genetic spectrum of this type of neuropathy. Based on our findings, it is advisable to screen for mutations in *GDAPI* in ARCMT patients, but also ADCMT families or isolated mildly affected patients with variable electrophysiology in the absence of additional clinical features like VFP or scoliosis. Sequencing *GDAPI* may yield heterozygous sequence variants without clear autosomal dominant segregation due to the common occurrence of reduced penetrance. In fact, several additional missense variations of unknown significance were encountered when performing diagnostic *GDAPI* screening in isolated patients or small families (P. De Jonghe, personal communication, 2010). Identified sequence variants can be regarded as disease-causing when a clear segregation with the phenotype is present or other strong genetic evidence of pathogenicity such as recurrent character, absence from controls, and targeting conserved and functionally relevant residues. If the genetic data remain

unconvincing, a functional assay may be considered in order to distinguish truly pathogenic sequence variants from benign polymorphisms, like for the Ala156Gly mutation.

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## GLOSSARY

<b>ADCMT</b>	autosomal dominant Charcot-Marie-Tooth
<b>ARCMT</b>	autosomal recessive Charcot-Marie-Tooth
<b>CMT</b>	Charcot-Marie-Tooth
<b>DQ</b>	dosage quotient
<b>GDAP1</b>	ganglioside-induced differentiation-associated protein 1
<b>MAQ</b>	Multiplex Amplicon Quantification
<b>NCV</b>	nerve conduction velocity
<b>PEG</b>	polyethylene glycol
<b>STR</b>	short tandem repeat
<b>VFP</b>	vocal fold paresis

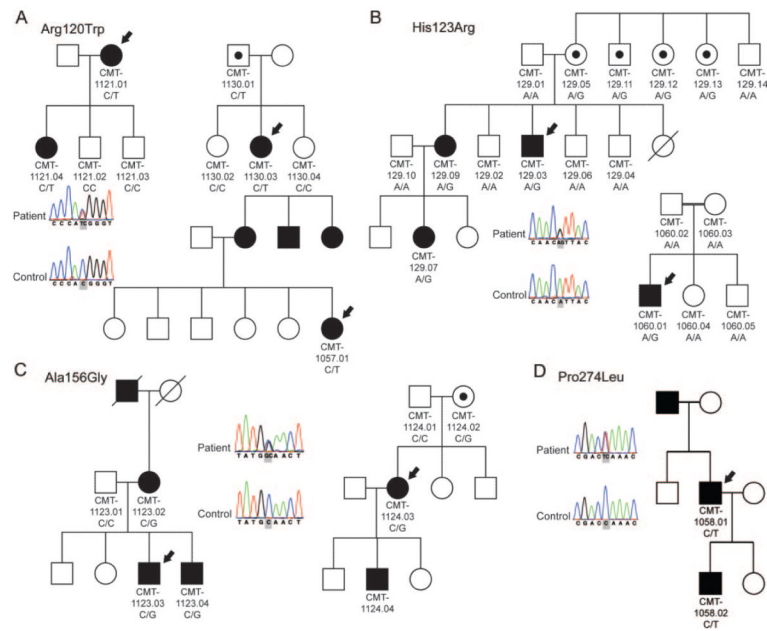
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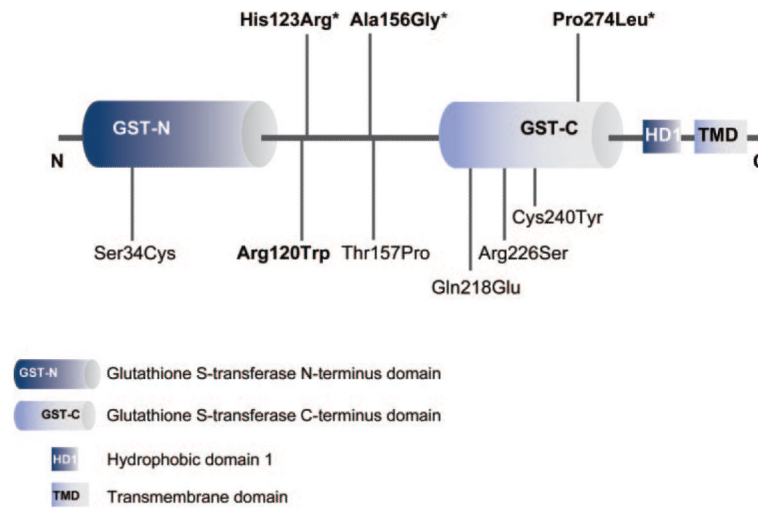
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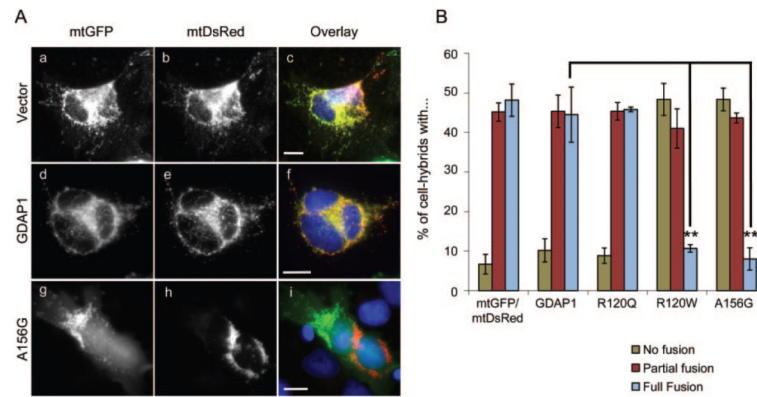
**Figure 1. Pedigrees of families with *GDAP1* mutations**

Segregation analysis and sequence trace files are shown for 7 families. (A) Families CMT-1057, CMT-1121, and CMT-1130 carrying the Arg120Trp mutation. (B) Families CMT-129 and CMT-1060 with the His123Arg mutation. (C) Families CMT-1123 and CMT-1124 with the Ala156Gly mutation. (D) Family CMT-1058 with the Pro274Leu mutation. Square = male, circle = female, black filled symbol = affected, empty symbol = unaffected, empty symbol with black dot = unaffected mutation carrier, black arrow = proband; genotype is indicated under each individual from whom the DNA was available for testing.



**Figure 2. Location of mutations in the GDAP1 protein**

The relative position of the mutations to the glutathione S-transferase (GST) homology region and interdomain region between GST-N and GST-C domains is indicated; bold = mutations identified in this study, \* = novel mutations.



**Figure 3. Dominant Ala156Gly mutation in *GDAP1* impairs fusion of mitochondria**

(A) HeLa cells were transiently cotransfected with expression constructs encoding either mtDsRed or mtGFP (a-c), and wild-type human protein (d-f), autosomal recessively inherited (Arg120Gln), or dominantly inherited (Arg120Trp, Ala156Gly) (g-i) alleles of *GDAP1*. The cells were coplated and fused with PEG. Cell hybrids were stained for *GDAP1* and analyzed for fusion of the mitochondrial markers mtGFP and mtDsRed. Bars, 10  $\mu$ m.

(B) Quantitation analysis revealed that fusion was similar for controls, *GDAP1*-transfected hybrids, and hybrids expressing the recessively inherited Arg120Gln. A dramatically impaired fusion was observed for cell hybrids expressing Arg120Trp and Ala156Gly ( $n = 3$ , average and standard error are shown, statistically significant levels are shown for the category full fusion; Student *t* test *p* value \*\* <0.005).

**Table 1**  
**Haplotype analysis of 8q21 markers in patients with autosomal dominant GDAP1 mutations<sup>a</sup>**

Marker	Arg120Trp			His123Arg			Ala156Gly		
	CMT-1121.01	CMT-1130.03	CMT-1057.01	CMT-129.03	CMT1060.01	CMT-1123.03	CMT-1124.03	CMT-1123.03	CMT-1124.03
D8S279	251	229	229/245	241	238	235	229	235	229
D8S286	232	230	232	232	242	230	230	230	230
D8S551	263	253	263	253	253	275	275	275	275
rs11554166	G	G	G	G	T	G	G	G	G
D8S1144	161	161	157/165	169	157	157	157	157	157
D8S548	230	230	230	230	242	234	234	234	234
D8S1829	111	111	111	107	111	115	115	115	115

<sup>a</sup>The alleles of the short tandem repeats are sized in base pairs (bp). The position of the markers is according to the reference assembly of NCBI genome build 36.3.

**Table 2**  
**Overview of clinical findings in families with dominant *GDAP1* mutations identified in this study**

Mutation and family	Ethnicity	Diagnosis	AAO	No. of affected	No. of asymptomatic mutation carriers	Remarks
<b>Arg120Trp</b>						
<b>CMT-1057</b>	Italian	CMT2	20 y	4	–	–
<b>CMT-1121</b>	Austrian	CMT2	10–45 y	2	–	Proximal weakness in UL/LL in patient CMT-1121.01
<b>CMT-1130</b>	American (Ashkenazi Jewish)	CMT2	Childhood	1	1	Proximal weakness LL in patient CMT-1130.03
<b>His123Arg</b>						
<b>CMT-129</b>	Finnish	CMT2	3 to >32 y	3	4	Proximal weakness LL in patient CMT-129.07
<b>CMT-1060</b>	Tunisian	CMT-INT	Childhood	1	–	Delayed early motor milestones (independent gait at 24 mo), severely affected
<b>Ala156Gly</b>						
<b>CMT-1123</b>	Polish	CMT2	8–13 y	4	–	Cardiac arrhythmia cosegregating in family
<b>CMT-1124</b>	Polish	CMT2	13–18 y	2	1	–
<b>Pro274Leu</b>						
<b>CMT-1058</b>	Italian	CMT-INT, CMT2	47 y	3	–	Fast disease progression, wheelchair-bound at 61 y (CMT-1058.01); CMT-1058.02 clinically normal, electrophysiology: clear axonal neuropathy

Abbreviations: AAO = age at onset; INT = intermediate; LL = lower limb; UL = upper limb.