

# A Novel GDAP1 Mutation 439delA is Associated with Autosomal Recessive CMT Disease

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**ABSTRACT: Background:** Charcot-Marie-Tooth (CMT) disease is the most common form of inherited motor and sensory neuropathy. Based on neurophysiological and neuropathological criteria CMT has been sub-classified into two main types: demyelinating and axonal. Furthermore, it is genetically heterogeneous with autosomal dominant, autosomal recessive (AR) and X-linked modes of inheritance. Thus far, seven genes have been identified in association with the demyelinating AR-CMT disease. We hereby report our clinical and molecular genetic findings in a consanguineous family with AR-CMT. **Methods:** Two young sisters with AR-CMT and other non-affected family members were clinically and electrophysiologically evaluated and then molecular genetic investigation was carried out in order to identify the pathogenic mutation. **Results:** Following an initial indication for linkage of the family to the CMT4A locus on chromosome 8, we sequenced the Ganglioside-induced differentiation-associated protein 1 (GDAP1) gene and identified a single nucleotide deletion in exon 3 that is associated with AR-CMT in the family. **Conclusions:** We identified a novel GDAP1 439delA mutation that is associated with AR-CMT in a consanguineous family of Iranian descent with two affected young girls and a history in other members of the family.

**RÉSUMÉ: Une nouvelle mutation 439delA du gène GDAP1 associée à la maladie de CMT récessive autosomique.** **Contexte :** La maladie de Charcot-Marie-Tooth (CMT) est la forme la plus fréquente de neuropathie sensitivomotrice héréditaire. La maladie de CMT a été sous divisée en deux types principaux, le type démyélinisant et le type axonal, selon des critères neurophysiologiques et neuropathologiques. De plus, la maladie est hétérogène au point de vue génétique avec hérédité dominante autosomique, récessive autosomique (RA) et liée à l’X. Jusqu’à maintenant, sept gènes associés à la maladie de CMT-RA démyélinisante ont été identifiés. Nous rapportons nos observations cliniques et moléculaires chez une famille consanguine atteinte de maladie de CMT-RA. **Méthodes :** Deux jeunes sœurs atteintes de maladie de CMT-RA et les membres non atteints de la famille ont subi une évaluation clinique et électrophysiologique ainsi qu’une étude de génétique moléculaire afin d’identifier la mutation en cause. **Résultats :** Les études initiales indiquaient un linkage au locus CMT4A sur le chromosome 8 dans la famille. Nous avons donc séquencé le gène GDAP1 et nous avons identifié une délétion d’un seul nucléotide dans l’exon 3 associée au CMT-RA dans cette famille. **Conclusions :** Nous avons identifié une nouvelle mutation, 439delA dans le gène GDAP1, associée au CMT-RA dans une famille consanguine de descendance iranienne ayant une histoire familiale positive et dont deux jeunes filles sont atteintes de la maladie.

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Charcot-Marie-Tooth (CMT) disease is the most common inherited peripheral neuropathy with an incidence of ~1/2500 in the general population. It is characterized by progressive weakness due to muscular atrophy with sensory loss more severely affecting the hands and feet.<sup>1</sup> There are two major pathophysiological types of CMT: the demyelinating form (CMT1, CMT4) characterized by decreased motor nerve conduction velocities (MNCV) and the axonal form (CMT2) that is characterized by normal or slightly reduced MNCV(s).<sup>2</sup> Both

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**Table: Autosomal recessive Charcot-Marie-Tooth clinical types, loci and genes**

Type	OMIM	Chromosome	Gene	Clinical characteristics	References
CMT4A	#214400	8q13-q21.1	GDAP1	demyelinating severe neuropathy of childhood	5, 22
CMT4B1	#601382	11q22	MTMR2	demyelinating neuropathy - presence of focally folded myelin sheaths	23-25
CMT4B2	#604563	11p15	SBF2	early-onset demyelinating CMT with focally folded myelin sheaths	26-28
CMT4C	#601596	5q32	SH3TC2	demyelinating CMT, nerve biopsy findings of large cytoplasmic extensions of Schwann cells	29-31
CMT4D	#601455	8q24.3	NDRG1	demyelinating CMT with deafness (Lom)	32, 33
CMT4E	#605253	10q21.1-q22.1	EGR2	demyelinating congenital hypomyelinating neuropathy	34
CMT4F	#145900	19q13.1-q13.3	PRX	demyelinating CMT	35, 36
CMT4G	%605285	10q23.2	unknown	demyelinating severe disabling form of CMT (Russe)	37
CMT4H	%609311	12p11.21-q13.11	unknown	severe demyelinating CMT	38
CMT2B1	#605588	1q21.2-q21.3	LMNA	axonal CMT	39, 40
CMT2B2	%605589	19q13.3	unknown	axonal CMT	41, 42
CMT2H	607731	8q21.3	unknown	axonal CMT with pyramidal features	43
CMT2K	#607831	8q13-q21.1	GDAP1	early onset axonal CMT	7, 8
CMT2 + VCP	#607706	8q13-q21.1	GDAP1	axonal CMT with vocal cord paresis	6, 44
CMTRIA	#608340	8q21	GDAP1	intermediate CMT	9, 10

conditions can have autosomal dominant, X-linked or autosomal recessive (AR-CMT) modes of inheritance.<sup>3,4</sup> The various clinical forms of AR-CMT that have been described and the corresponding genes that have been identified or mapped are shown in the Table.

Ganglioside-induced differentiation-associated protein 1 (GDAP1) gene mutations were initially reported in two phenotypically different groups of AR-CMT families. Baxter et al<sup>5</sup> reported GDAP1 gene mutations in demyelinating AR-CMT families, whereas Cuesta et al<sup>6</sup> reported GDAP1 gene mutations in axonal AR-CMT families. Since then, additional GDAP1 mutations in families from various ethnic groups were reported.<sup>7-18</sup> Electrophysiological and neuropathological findings of patients revealed that GDAP1 mutations contribute to axonal, demyelinating or intermediate AR-CMT occasionally associated with hoarseness or vocal cord paralysis. Phenotypic variability within the same family has also been reported.<sup>12</sup> Ganglioside-induced differentiation-associated protein 1 mutations are currently reported in OMIM under demyelinating AR-CMT (CMT4A; MIM 214400), axonal AR-CMT (CMT2K; MIM 607831), axonal AR-CMT with vocal cord paresis (CMT2 + VCP; MIM 607706) and intermediate A AR-CMT (CMTRIA; MIM 608340).

We hereby report a novel GDAP1 gene mutation in a consanguineous AR-CMT family of Iranian descent.

Electrophysiological and neuropathological findings of the proband are suggestive of a demyelinating polyneuropathy whereas the electrophysiological data of her younger sister are indicative of an axonal polyneuropathy.

#### PATIENTS AND METHODS

The proband (IV-2) as well as five other members of the family (III-1, III-2, IV-1, IV-3 and IV-4) were clinically examined. Nerve conduction studies were performed on the proband (IV-2), her younger affected sister (IV-3) and the parents (III-1 and III-2). A sural nerve biopsy investigation was done on the proband.

The proband is a 9-year-old girl who was born following an uncomplicated pregnancy and delivery. At the age of 2<sup>1/2</sup> years she was noted to walk with dropped feet and difficulties in running with frequent falls. At 3 years she was noted to have contractures of her fingers although at that stage she could still open her hands fully and use her fingers. Electrophysiological studies done on the median nerve at the age of 3<sup>3/12</sup> revealed slow nerve conduction velocity (NCV) with left posterior tibial motor conduction velocity of 28.7 m/sec (amplitude: 0.02 mV), left deep peroneal showing no response with supramaximal current, left sural sensory conduction of 32.5 m/sec (amplitude: 7.9  $\mu$ V) and left median motor conduction velocity of 33.7 m/sec

(amplitude: 0.7 mV). The pattern was consistent with demyelinating sensory motor polyneuropathy with diffuse slowing, and temporal dispersion, but no conduction blocks. An MRI of the whole spine done at 4 years of age showed slight enhancement of several left-sided nerve roots in the region of the cauda equine consistent with polyneuropathy. No other spinal cord abnormalities were detected. Her CSF showed normal protein and glucose.

Over the next few years she continued to lose her muscle strength mostly distally with mild atrophy and clawing of her hands. She also developed mild ankle contractures bilaterally with prominent calf atrophy and required ankle bracing for walking. Her gait became unstable and she developed Gower's sign. Left sural nerve biopsy at the age of 3<sup>9/12</sup> showed evidence of thinly myelinated axons with several axons showing more than one layer of Schwann cells processes suggestive of early onion bulb. However, overall onion bulbs were not prominent. There was also a sparse inflammatory infiltrate; the findings were suggestive of a demyelinating neuropathy.

Her sister (IV-3) who is 6-years-old was also born following an uncomplicated pregnancy and delivery. Initial symptoms were at the age of 2 years with dropped feet and clumsiness while walking and running with difficulties in climbing and descending stairs. She also had difficulties in grasping objects, buttoning and zipping. Overall her clinical manifestations were the same as in her sister and the progression of her condition was at the same pace as in her sister. Electrophysiological studies carried out at the age of 1<sup>8/12</sup> revealed relatively preserved NCV of the median motor nerve (42.3 m/sec) with decreased amplitude (0.78 mV). The tibial nerve studies showed low response of 0.64 mV with normal velocity at 45 m/sec suggestive of developing neuropathy. Nerve conduction studies of both parents (III-1 and III-2) were normal.

### Genotyping

Individuals III-1, III-2, IV-1, IV-2, IV-3 and IV-4 were genotyped at microsatellite polymorphic marker loci, spanning the candidate AR-CMT loci (CMT1A: D17S122, D17S261; CMT1B: CRP, APOA, SPTA; CMT4A: D8S551, D8S548, D8S164, D8S64; CMT4B1: D11S1332, D11S919, D11S917; CMT4B2: D11S4149, D11S1329, D11S1999, D11S4189; CMT4C: D5S643, D5S463, D5S402, D5S638; CMT4D: D8S558, D8S378, D8S529, D8S256; CMT4E: D10S1220, D10S1225, D10S1432; CMT4F: D19S897, D19S223, D19S420), following previously described methodology.<sup>19</sup>

### Linkage and Haplotype Analyses

Two-point linkage analysis between the disease and each of the above marker loci was performed, using MLINK of the LINKAGE package of programs.<sup>20</sup> Haplotypes of individuals were constructed manually following the order in which the marker loci are mapped at the corresponding chromosomal region.

### Mutation Analysis

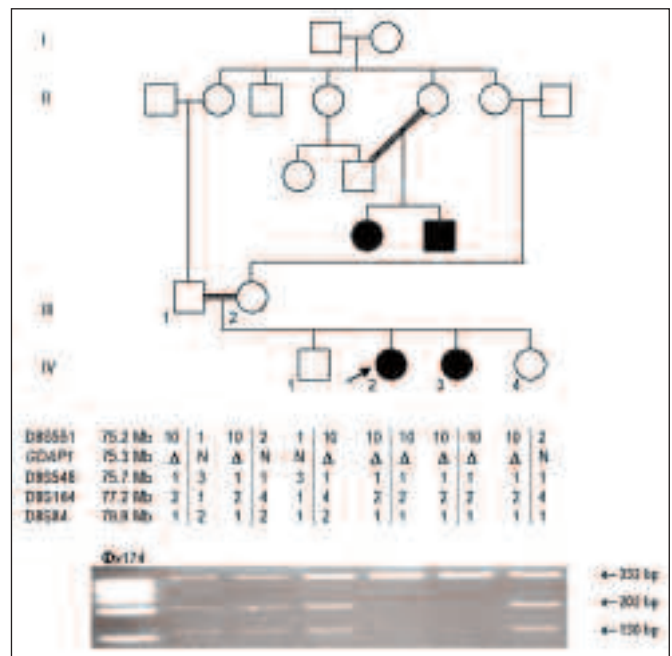
Mutation screening of the GDAP1 gene was performed using previously described PCR primer sets covering the six coding exons.<sup>6</sup> Amplification products were sequenced in both

directions using the CEQ DTCS kit and the Beckman Coulter CEQ analyser. The resulting data were automatically compared with the normal GDAP1 sequence as listed in the GenBank database (Accession: NT\_008183, Region: 27105194..27121658, Version: NT\_008183.16, GI: 29824571 of 28 April 2003), using the CEQ8000 investigator software. Direct mutation detection in all available family members was performed by PCR amplification of exon 3 and HincII restriction enzyme analysis.

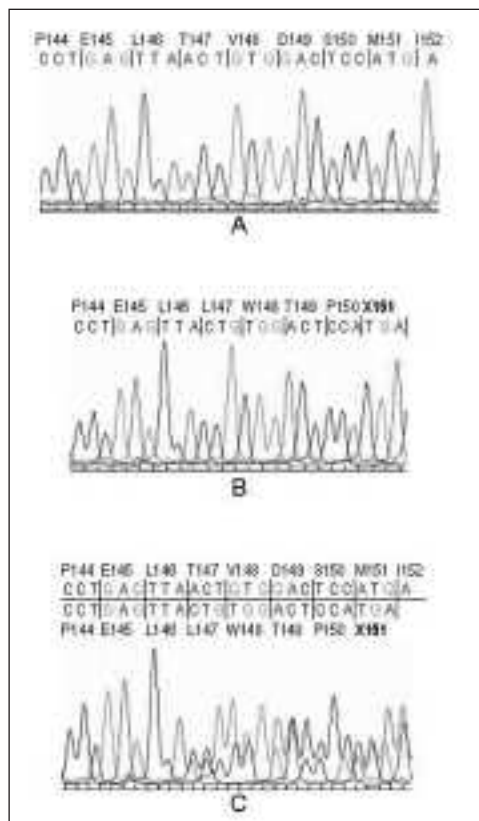
### RESULTS

The family was initially screened for linkage at the known CMT1 and CMT4 loci. Linkage to the CMT1A, CMT1B, CMT4B1, CMT4B2, CMT4C, CMT4D, CMT4E and CMT4F loci was excluded and an indication for linkage to the CMT4A1 locus was observed with a maximum lod score of 1.67 at  $\theta=0.00$  for locus D8S551. Haplotype analysis also supports linkage to the CMT4A locus (Figure 1).

Mutation screening of the entire coding and promoter regions of the GDAP1 gene revealed a homozygous one base-pair deletion of A at position 439 (439delA) in the third exon of the gene. It is predicted that this deletion leads to a frameshift mutation at codon Thr147 and a premature stop at codon 151



**Figure 1:** Pedigree of the consanguineous AR-CMT family of Iranian descent. Haplotypes are shown under the corresponding available individuals (III-1, III-2, IV-1, IV-2, IV-3 and IV-4). Mutation detection by PCR amplification of GDAP1 exon 3 and HincII restriction enzyme digestion is depicted under the haplotypes. Homozygous 439delA individuals (patients IV-2 and IV-3) give a single band of 332 bp, whereas heterozygous N/439delA individuals (III-1, II-2, IV-1, IV-4) give three bands of 332 bp, 202 bp and 130 bp. Homozygous normal individuals (not shown here) give two bands of 202 bp and 130 bp.



**Figure 2:** Sequence analysis data of the relevant GDAP1 region. Part A shows the DNA and amino acid sequence of a normal control individual. Part B shows the DNA and amino acid sequence of the proband, homozygous for the 439delA mutation. Part C shows the sequence data obtained from a carrier of the 439delA mutation (individual III-1) and the predicted amino acid sequences.

(T147fsX151) (Figure 2). The deletion at the DNA level destroys a HincII restriction endonuclease cutting site; therefore it was used to trace the mutation in all family members. PCR amplification of the corresponding region (exon 3) and HincII restriction enzyme analysis of all available members proved that there is complete co-segregation of the mutation with the disease in this family (Figure 1).

## DISCUSSION

Ganglioside-induced differentiation-associated protein 1 is a ubiquitously expressed gene with predominant expression in neurons and Schwann cells. The protein product of the GDAP1 has a strong similarity to glutathione S-transferases (GSTs) and two such domains are recognized spanning amino acid residues 26-119 and 210-287. Two putative transmembrane domains are predicted at the C-terminal of the protein. A putative role of GDAP1 in the interaction between Schwann cell and axon has been suggested.<sup>6</sup> Niemann et al<sup>21</sup> (2005) have recently demonstrated that GDAP1 is an integral membrane of the outer

mitochondrial membrane and is a regulator of the mitochondrial network.

Twenty-three other GDAP1 mutations have thus far been reported in CMT patients spanning the gene region from codon 92 to 844.<sup>5-18</sup> These include single base substitutions leading to the introduction of a stop codon (c.92G>A [Trp31stop], c.487C>T [Gln163stop], c.581C>G [Ser194stop], c.668T>A [Leu223stop]), single base substitutions leading to a splice site mutation (c.311-1G>A, c.579+1G>A), single base substitutions leading to a missense mutation (c.347T>G [Met116Arg], c.358C>T [Arg120Trp], c.359G>A [Arg120GLN], c.389C>G [Ser130Cys], c.445G>T [Asp149Tyr], c.469A>C [Thr157Pro], c.482G>A [Arg161His], c.715C>T [Leu239Phe], c.811G>A [Gly271Arg], c.844C>T [Arg282Cys], c.929G>A [Arg310Gln]) and small deletions or insertions leading to a frame shift mutation (c.341\_344delAAAG [Glu114fs], c.349\_350insT [Tyr117fs], c.485-2A>G [Ser162fsX166], c.558delT [Ile186fs], c.786delG [Gly262fs], c.863insA [Thr288fs]). The position and type of the above mutations does not appear to have any obvious correlation with the CMT phenotype (ie axonal, demyelinating, intermediate or axonal with vocal paresis).

We report a novel GDAP1 gene mutation (439delA) in a consanguineous AR-CMT family, which most probably results in an alteration in GDAP1 from amino acid 147 and it is predicted that a premature termination occurs subsequent to amino acid 151. The reported mutation T147fsX151 does not lie in a known functional domain of GDAP1, however it appears to be within a conserved part of the protein, and it most probably truncates GDAP1 removing the C terminal GST and the two putative transmembrane domains. Niemann et al<sup>21</sup> (2005) demonstrated that the T288fs290X and S194X truncated GDAP1 proteins were not targeted efficiently to mitochondria. The shortest Q163X construct was not detectable and the authors speculate that a highly unstable protein is generated by the mutation. We assume that similarly, the hereby reported truncating GDAP1 mutation most probably results in a protein that lacks the C terminal mitochondrial-targeting signal.

The reported novel mutation is associated with AR-CMT in our family. Although phenotypic variability caused by a GDAP1 mutation within the same family has been previously reported, the variability of neurophysiological data observed between these two patients may also be related to different stages in the course of the disease.

The mechanism by which the same mutation causes a different pathology within the same family remains to be elucidated. The number of known GDAP1 mutations is relatively small to be able to make reliable genotype-phenotype correlations. Further reported cases with GDAP1 mutations, along with detailed clinical, electrophysiological and pathological data, may provide an explanation for the intrafamilial and interfamilial variability in the pathological and electrophysiological findings associated with the different mutations in the GDAP1 gene.

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