Journal of the Peripheral Nervous System

WILEY

RESEARCH REPORT

GDAP1-Related Charcot–Marie–Tooth Disease: Axonal or Demyelinating Subtype? Autosomal Recessive or Autosomal Dominant Inheritance?

Moez Ravanbod[1] | Mahsa Mohammadi[1] | Aida Ghasemi[2] | Solmaz Jabbarzadeh[2,3,4] | Ali Asghar Okhovat[2,3] | Marzieh Khani[2,5] | Elahe Elahi[2,5] | Mahtab Ramezani[2] | Shahriar Nafissi[2,3] | Afagh Alavi[1,2]

[1]Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran | [2]Neuromuscular Research Center, Tehran University of Medical Sciences, Tehran, Iran | [3]Neurology Department, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran | [4]Department of Neurology, Urmia University of Medical Sciences, Urmia, Iran | [5]School of Biology, College of Science, University of Tehran, Tehran, Iran

Correspondence: Shahriar Nafissi (nafissishahriar@gmail.com) | Afagh Alavi (afaghalavi@gmail.com)

Received: 9 April 2025 | **Revised:** 10 June 2025 | **Accepted:** 11 July 2025

Funding: This work was supported by Tehran University of Medical Sciences (Grant number: 1403-4-499-79679).

Keywords: Charcot–Marie-Tooth | *GDAP1* gene | intronic variant | whole exome sequencing

ABSTRACT

Background and Aims: The *GDAP1* gene encodes a mitochondrial outer membrane protein crucial for mitochondrial function. Mutations in this gene are associated with different subtypes of Charcot–Marie-Tooth (CMT) disease, inherited in either an autosomal recessive or dominant manner. In this study, we discuss the clinical and genetic aspects of 11 unrelated Iranian *GDAP1*-related CMT families.

Methods: The probands were selected from a large CMT cohort after whole exome sequencing (WES) analysis. 11 *GDAP1*-related CMT families–16 patients—were included in this study. Co-segregation analysis was performed to confirm the candidate variants.

Results: In total, eight exonic variants in *GDAP1* were identified; two were novel. Among all known variants, a deep intronic variant, c.311-23A>G, was found in two families. 11/16 patients were AR-CMT2K, three were CMT4A, and only two had AD-CMT2K.

Interpretation: Among our variants, two were more significant: c.311-23A>G, which has only been documented in another Iranian family and may represent a founder mutation within our population, and c.347T>G, which has exclusively been reported within the Italian population and is recognized as a founder mutation in that country. We found this variant in three unrelated families, suggesting that this variant is not confined to Italy and that codon 347 may be a hotspot codon. Our findings extend the clinical and genetic aspects of *GDAP1*-related CMT and emphasize the need to consider intronic variants in genetic analysis. Additionally, we highlight that AD-CMT2K has a milder phenotype than other *GDAP1*-related disease types, which could result in an underestimation of the number of AD-CMT2K cases.

Moez Ravanbod and Mahsa Mohammadi have contributed equally.

1 | Introduction

Hereditary motor and sensory neuropathy (HMSN), commonly referred to as Charcot–Marie-Tooth (CMT) disease, is the most prevalent hereditary peripheral neuropathy, affecting approximately 1 in 2500 people worldwide. This group of disorders is clinically and genetically heterogeneous, impacting both motor and sensory nerves. Patients typically experience progressive distal limb weakness, foot deformities such as pes cavus, sensory impairments, and reduced deep tendon reflexes [1, 2].

Clinically, CMT can be categorized into three forms based on electromyography (EMG) results, classified by nerve conduction velocity (NCV) in the upper limbs. These categories include demyelinating (NCV < 38 m/s), axonal (NCV > 45 m/s), and intermediate (35 < NCV < 45) forms. This parameter, along with the pattern of inheritance, helps classify CMT into four major subtypes: CMT1, the demyelinating subtype with an autosomal dominant (AD) inheritance; CMT2, the axonal subtype inherited in either an AD or autosomal recessive (AR) manner; CMTX, inherited in an X-linked (XL) manner with intermediate NCV; and CMT4, another demyelinating subtype with an AR inheritance. In recent years, genetic knowledge of CMT has expanded, and causal genes are now represented by alphabetic letters, such as CMT1A [1, 3, 4]. Though more than 120 genes have been related to CMT, some are more prevalent. These comprise the most prevalent genetic subtype, CMT1A, which is caused by the duplication of the *PMP22* gene, followed by pathogenic variants in the *GJB1* (CMTX1), *MPZ* (CMT1B), *MFN2* (CMT2A), and *GDAP1* (CMT4A, CMT2K) genes [4, 5].

The *GDAP1* gene, containing six exons, located on chromosome 8q21, encodes ganglioside-induced differentiation-associated protein 1, an integral protein residing in the mitochondrial outer membrane (MOM). GDAP1 plays a crucial role in regulating mitochondrial function and calcium homeostasis. This protein consists of two major glutathione-S-transferase (GST) domains: GST-N and GST-C. Other important domains of this protein include a hydrophobic domain (HD) and a transmembrane domain (TMD) located near the C-terminal and an alpha 4–5 loop located near the N-terminal (Figure 1) [6, 7]. Mutations in the *GDAP1* gene can lead to a spectrum of phenotypes, including AR CMT4A, AD or AR CMT2K, and an intermediate form known as CMTRIA (CMT recessive intermediate A), exhibiting variable degrees of phenotypic severity. Previous studies suggest that AR forms generally result in a more severe, early-onset neuropathy, whereas AD forms present with a milder phenotype and a later age at onset. Additionally, some symptoms like vocal cord palsy are more commonly reported in certain forms of the disease such as *GDAP1*-related CMT [2, 4].

In the present study, we discuss the genetic and clinical features of 11 unrelated Iranian families afflicted by *GDAP1*-related CMT.

2 | Methods and Materials

This study was conducted in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS: IR.TUMS.SHARIATI.REC.1404.022) in Iran. Written informed consent was obtained from all participants.

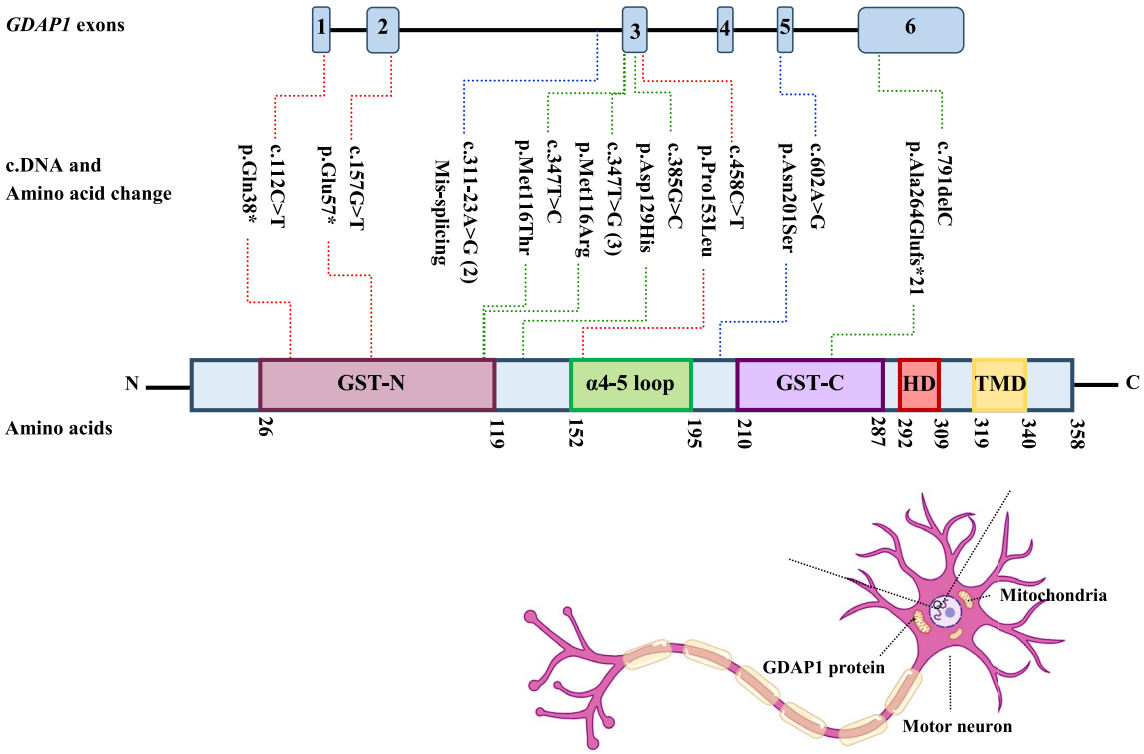

FIGURE 1 | Schematic structure of the human *GDAP1* gene and protein, and the variants found in this study. The number in parenthesis indicates the number of families with this variant. Red dotted lines: Pathogenic variants, Green dotted lines: Likely pathogenic variants, Blue dotted lines: Variants of uncertain significance (VUS), N: Amino terminal of the protein, GST: Glutathione-S-transferase, HD: Hydrophobic domain, TMD: Transmembrane domain, C: Carboxyl terminal of the protein. The numbers below the protein indicate the number of amino acids.

2.1 | Subjects

In the past 10 years, more than 200 CMT families were referred to the Neuromuscular Research Center (NRC) at the Department of Neurology of Shariati Hospital, affiliated with Tehran University of Medical Sciences. The probands underwent thorough clinical and paraclinical evaluations. Genetic analysis was recommended by the neurologist to establish a definitive diagnosis. Therefore, all cases with demyelinating neuropathy were initially assessed for the *PMP22* duplication/deletion using the multiplex ligation-dependent probe amplification (MLPA) kit (MRC Holland, Amsterdam, the Netherlands) according to the manufacturer's protocol. Demyelinating cases with a negative *PMP22* duplication/deletion and all axonal neuropathy cases were evaluated by whole exome sequencing (WES). In total, 16 patients from 11 unrelated Iranian families with variants in the *GDAP1* gene were identified and included in this study.

2.2 | Genetic Analysis

DNA was extracted from the peripheral blood of all participants, including probands and their family members, using the salting-out protocol [8]. The exon enrichment process was carried out using the SureSelect V6-Post kit, and sequencing was conducted with the Illumina HiSeq 4000 system for all probands. The sequences were aligned to the human reference genome (UCSC NCBI38/hg38). Various tools, including Burrows-Wheeler Aligner (BWA), SAMTools, Picard, and the Genome Analysis Toolkit (GATK), were utilized for the variant calling process. Variants with a minor allele frequency (MAF) greater than 0.01 in population databases (e.g., gnomAD, 1000 Genomes, and IRANOME) were excluded. In the second step, variants located in non-coding regions (intronic, intergenic, and UTRs) as well as synonymous exonic variants with no effect on splicing were filtered out. The remaining variants were assessed to identify those located in neuropathy-associated genes. It should be mentioned that in families 101 and 102, where we did not find any mutations in the CMT-related genes, the intronic regions of these genes were evaluated more closely. *In silico* tools such as PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (https://sift.bii.a-star.edu.sg/), MutationAssessor (http://mutationassessor.org/r3/), MutationTaster (https://www.mutationtaster.org/), and combined annotation dependent depletion 1.7 (CADD) (https://cadd.gs.washington.edu/) were used to predict the pathogenicity of candidate variants. Subsequently, variants were classified based on the guidelines from the American College of Medical Genetics and Genomics (ACMG) [9]. Primers were designed for each candidate variant, and fragments containing these variants were amplified using polymerase chain reaction (PCR). Primer sequences are available upon request. The PCR products were subjected to Sanger sequencing using the ABI3130 genetic analyzer (Applied Biosystems, Foster City, CA) to confirm the presence of the candidate variants, first in the probands and later in their family members to co-segregate analysis [10].

Copy number variations (CNVs) were also analyzed in genes associated with CMT disease, as CNVs are frequent in some of these genes. The DetermineGermlineContigPloidy module within GATK was specifically used to assess autosomal and allosomal contig ploidy. Finally, potential CNVs in CMT-related genes were evaluated through a read-depth detection method [11, 12].

3 | Results

The current study includes 16 patients from 11 unrelated Iranian families, four males and 12 females. 11/16 patients (~68.7%) were born to consanguineous parents (the total number of patients was considered because some patients were from different generations and had different parents). A positive family history of CMT was observed in 5/11 families (~45%). Detailed demographic and clinical data of all patients were provided in Table 1.

3.1 | Clinical and EMG Results

All patients presented symptoms before the age of 15 years except CMT101-III2, who manifested the disease at age 45 years. Distal limb weakness and frequent falling were the most common initial symptoms (each one was observed in ~31% of total cases). It should be noted that in most patients, distal limb weakness was also present indirectly and manifested itself as an abnormal gait. Pes cavus deformity, distal limb atrophy, abnormal gait, distal limb paresthesia, and foot drop were observed in 4/16 (25%), 12/16 (75%), 15/16 (~93.7%), 5/16 (~31%), and 4/16 (25%), respectively. All patients exhibited upper and lower limb weakness except CMT101-III2, who did not have upper limb weakness by the time of the last examination (50 years old). None of our patients had vocal cord palsy, even though it is a relatively common symptom of *GDAP1*-related CMT.

Some patients manifested unusual symptoms. For example, unilateral scapular winging and seizures were observed in CMT102-III9 and CMT107-IV3, respectively. None of our patients had Babinski sign.

Nerve conduction studies (NCS) were consistent with axonal neuropathy for all patients except CMT109-III5, CMT111-IV1, and CMT111-IV2, who showed a severe demyelinating type of neuropathy. Detailed NCS results, as well as the CMT neuropathy score (CMTNS) of all patients were summarized in S1 and S2, respectively.

3.2 | Genetic Findings

In this study, nine *GDAP1* variants were detected in our patients; two were novel, including c.157G>T:p.Glu57*, and c.791delC:p.Ala264Glufs*21. The patients of three families, CMT105, CMT107, and CMT109, shared the same known variant, c.347T>G. Additionally, the subjects belonging to families CMT101 and CMT102 carried an identical noncanonical splice site variant, c.311-23A>G, which has been documented in another Iranian family [1].

The disease-causing variants within the *GDAP1* gene were found to be homozygous in eight families, while in one family, CMT103, variants were compound heterozygous (c.112C>T and c.347T>C); in another family, CMT104, the variant was

TABLE 1 | Clinical characteristics of Iranian patients affected with *GDAP1*-related CMT.

Family ID	CMT101		CMT102				CMT103	CMT104	CMT105	CMT106	CMT107	CMT108	CMT109	CMT110	CMT111	
Patient ID	III2	IV3*	III2	III3	III5	III9*	III1*	IV2*	III2*	IV3*	IV3*	IV3*	III5*	IV5*	IV1*	IV2
Gender	F	F	M	F	M	F	M	F	M	F	F	F	F	F	F	F
Consanguinous parents	No	Yes	Yes				No	Yes	No	Yes	No	Yes	No	Yes	Yes	
Present age (year)	50	26	52	49	42	33	14	27	7	26	26	39	26	22	13	12
Age at onset (year)	45	6	8	~15	7	Early childhood	2	Early childhood	3	7	5	10	5	4	Infancy	Infancy
Family history	+		+				−	−	−	−	+	−	+	−	+	
Mean motor NCV (M/S) (median)	63.25	NR	NA	41	NA	49.3	44.7	41.35	43.2	42.2	54.25	NR	22.8	NR	NA	NA
DTRs	0	0	NA	NA	NA	0	0	0	Triceps, Biceps, Brachioradialis: 2+, Patellar: 1+, Achill's: 0	0	0	0	0	0	0	0
Neuropathy type	Axonal	Axonal	Axonal	Axonal	Axonal	Axonal	Axonal	Axonal	Axonal	Axonal	Axonal	Axonal	Demyelinating	Axonal	Demyelinating	Demyelinating
Initial symptom	Distal limb weakness	Frequent falling	Frequent falling	Frequent falling	Frequent falling	Difficulty in climbing stairs	Distal limb weakness	Delay in walking	Abnormal gait	Back pain	Frequent falling	Distal limb weakness	Distal limb weakness	Toe walking	Delay in walking	Distal limb weakness
Pes cavus deformity	−	+	−	−	+	−	−	−	−	−	+	−	+	−	−	−
Upper limb weakness	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lower limb weakness	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distal limb paresthesia	−	−	+	−	+	−	−	+	−	−	+	−	−	+	−	−
Distal limb atrophy	−	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−
Claw hands	−	+	−	−	−	−	+	−	−	+	+	−	−	−	−	−
Abnormal gait	−	+ (Ataxic)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Foot drop	−	+	−	−	−	−	−	−	+	−	−	−	+	−	+	−
Hammer toes	−	+	−	−	+	−	−	−	−	−	−	−	+	−	−	−

(Continues)

TABLE 1 | (Continued)

Family ID	CMT101		CMT102			CMT103	CMT104	CMT105	CMT106	CMT107	CMT108	CMT109	CMT110	CMT111	
Patient ID	III2	IV3*	III2	III3	III5	III9*	III1*	IV2*	III2*	IV3*	IV3*	III5*	IV5*	IV1*	IV2
Other complication(s)	Hand tremor	–	–	–	Hand tremor	Scapular winging	Contracture (knee in 90 degree flexion), upper extremity distal joint laxity and bilateral wrist drop	Scoliosis, left side monocular diplopia, lower limb cramps	Calcaneal protrusion, bilateral ulnar nerve conduction block through elbow	Lordosis, tremor, equinovarus deformity of feet and joint laxity	Lordosis, seizure, equinovarus deformity of feet	Lordosis	–	–	Impaired vibration sensation in distal lower limbs	Impaired vibration sensation in distal lower limbs

Abbreviations: *, Proband of family; CMT, Charcot-Marie-Tooth; DTRs, Deep tendon reflexes; F, Female; M/S, Meter per second; M, Male; ms, Millisecond; mV, Millivolt; NA, Not available; NCV, Nerve conduction velocity; NR, No response.

heterozygous (c.602A>G; no additional mutant allele, including a single nucleotide variant (SNV) or a copy number variation (CNV), was detected in this family). Notably, in family CMT101, both homozygous and heterozygous variants were present. In this family, the individual CMT101-IV3 carried a *GDAP1* variant in a homozygous state, whereas her affected mother, CMT101-III2, harbored the variant in a heterozygous state.

The identified variants were distributed across various exons within the *GDAP1* gene, with exon 3 exhibiting the highest frequency. Correspondingly, at the protein level, the majority of these variants were found within the GST-N domain. Figure 1 shows the distribution of all variants at the gene and protein levels.

One of the notable points in the WES study of these families was the absence of a disease-causing variant found during the preliminary analysis, mentioned in the "Methods and Materials" section, of families CMT101 and CMT102. In the preliminary analysis of the proband CMT101-IV3, two heterozygous variants in CMT-related genes, *DHTKD1*: NM_018706.7, c.1756+1G>T and *DYNC1H1*: NM_001376.5, c.7203A>C:p.Lys2401Asn, were detected; however, these variants did not co-segregate with the disease in the family. Consequently, we analyzed intronic variants within the probands of CMT101 and CMT102 families and detected an identical intronic variant in the *GDAP1* gene, c.311-23A>G, in these families. This variant was located in intron 2 of *GDAP1* and co-segregated with the disease status in both families.

According to ACMG guidelines, of the nine variants identified in this study, three variants were classified as pathogenic (P), four were considered as likely pathogenic (LP), and two were classified as variants of uncertain significance (VUS). Details of all variants were summarized in Table 2.

4 | Discussion

The ganglioside-induced differentiation-associated protein 1 is a 387-amino-acid protein that is encoded by the *GDAP1* gene and localized in the MOM. It is expressed ubiquitously throughout the body but mainly in neurons. Although the protein is a member of the GST superfamily, it lacks glutathione binding activity and only shares 20% sequence homology with other canonical GSTs. Thus, it is considered a noncanonical GST [13, 14]. Mutations in the *GDAP1* gene lead to abnormal mitochondrial activity and are associated with some different subtypes of CMT, including AR-CMT4A, AD-CMT2K, AR-CMT2K, and CMTRIA. Mitochondrial dysfunction in *GDAP1*-related CMT cases is different based on the mode of inheritance. Monoallelic mutations in *GDAP1* induce an abnormal gain of function, which leads to defective mitochondrial fusion, increased production of reactive oxygen species (ROSs), and ultimately causes apoptosis initiation. On the other hand, biallelic mutations act through a loss of function mechanism that reduces mitochondrial fission activity and leads to the formation of abnormal mitochondria [15, 16]. According to earlier research in the literature, AD-CMT2K manifests as a mild sensory predominant neuropathy with a slow disease progression, whereas AR types of *GDAP1*-related CMT (AR-CMT2K and CMT4A) typically

TABLE 2 | Genetic findings of patients with *GDAP1* variants (NM_018972.4).

Family ID	CMT101		CMT102				CMT103		CMT104	CMT105	CMT106	CMT107	CM108	CMT109	CMT110	CMT111	
Patient ID	IV3	III2	III2	III3	III5	III9	III1		IV2	III2	III3	IV3	IV3	III5	IV5	IV1	IV2
c.DNA change	c.311-23A>G		c.311-23A>G	c.311-23A>G	c.311-23A>G	c.311-23A>G	c.112C>T	c.347T>C	c.602A>G	c.347T>G	c.458C>T	c.347T>G	c.157G>T	c.347T>G	c.791delC	c.385G>C	
Amino acid change	Mis-splicing		Mis-splicing	Mis-splicing	Mis-splicing	Mis-splicing	p.Gln38*	p.Met116Thr	p.Asn201Ser	p.Met116Arg	p.Pro153Leu	p.Met116Arg	p.Glu57*	p.Met116Arg	p.Ala264Glufs*21	p.Asp129His	
Rs #	–	–	–	–	–	–	rs761035569	rs281865060	rs1586806110	rs281865060	rs538412810	rs281865060	–	rs281865060	–	rs1279013936	
Zygosity	Hom	Het	Hom	Hom	Hom	Hom	Com het		Het	Hom	Hom	Hom	Hom	Hom	Hom	Hom	Hom
Novelty	Known	Known	Known	Known	Known	Known	Known	Known	Known	Known	Known	Known	Novel	Known	Novel	Known	
Exon/Intron #	Int 2	Int 2	Int 2	Int 2	Int 2	Int 2	Ex 1	Ex 3	Ex 5	Ex 3	Ex 3	Ex 3	Ex 2	Ex 3	Ex 6	Ex 3	
Protein domain	–	–	–	–	–	–	GST-N	GST-N	Between alpha 4-5 loop and GST-C	GST-N	Alpha 4-5 loop	GST-N	GST-N	GST-N	GST-C	Between GST-N and alpha 4-5 loop	
Conservity	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	
CADD score	21.4	21.4	21.4	21.4	21.4	21.4	47	19.89	24.2	21.4	32	21.4	46	21.4	34	30	
ACMG classification	VUS	VUS	VUS	VUS	VUS	VUS	P	LP	VUS	LP	P	LP	P	LP	LP	LP	

Abbreviations: #, number; ACMG, American college of medical genetics and genomics; CADD, combined annotation dependant depletion; Com het, compound heterozygous; Ex, exon; GST, glutathione-S-transferase; Het, heterozygous; Hom, homozygous; Int, intron; LP, likely pathogenic; P, pathogenic; VUS, variant of uncertain significance.

present as an early-onset severe neuropathy, frequently accompanied by vocal cord palsy. A moderate neuropathy that starts in early childhood has also been documented in certain cases, albeit this is uncommon. The presence of both affected and asymptomatic carriers in a single family is evidence of decreased penetrance in the dominant *GDAP1* variants, according to some studies [2, 7, 17–19].

Among 16 patients included in this study (File S3), 11 patients were AR-CMT2K, three were CMT4A, and only two had AD-CMT2K (subject CMT104-III2 is also affected with AD-CMT2K, but since she was unavailable for a thorough examination, she was not included in Table 1). Based on research conducted in different countries including China, America, Spain, and Italy, CMT2K appears to be more frequent than CMT4A [20–24]. This was also evident in our cases, and CMT4A was only observed in 3/16 (~18.7%) of affected individuals with *GDAP1*-related CMT.

The clinical and genetic data of our patients encompassed significant aspects that were delineated below.

CMT104-IV2 and CMT101-III2, both exhibiting the AD-CMT2K phenotype of CMT, demonstrated considerable variable expressivity. In contrast to the typical features of AD-CMT2K previously elucidated, the CMT104-IV2 case exhibited delayed ambulation during early childhood. Her disease progression was rapid, and she developed distal weakness and atrophy, muscle cramps, scoliosis, and visual problems before the age of 15. According to the proband, her heterozygote mother also manifested similar symptoms such as severe distal limb weakness and pain since the age of 42 (she was unavailable for further examination by a neurologist). Although her parents are consanguine, it is still possible that she has a compound heterozygous mutation, which could explain her severe disease pattern. The second mutation in the proband might be in a part of the gene that the WES does not detect. Or, it is possible that other genetic, epigenetic, and environmental factors contribute to this severe disease pattern.

Conversely, the CMT101-III2 case presented with the disease at the age of 45 years, characterized by a milder phenotype and typical features of AD-CMT2K; however, by the age of 50, she exhibited no sensory involvement. These observations underscore the clinical variability, encompassing the age at onset, symptoms, and severity associated with AD-CMT2K disease. This clinical variability is pronounced to such an extent that within the majority of our cohorts, heterozygote parents exhibit exceedingly mild manifestations, leading them to refrain from seeking consultation with a neurologist, despite their having AD-CMT2K. Of course, it should be mentioned that because of their relatively young ages or the low penetrance of mutations in this specific gene, some of these parents are actually asymptomatic. Consequently, our findings emphasize the underestimation of the prevalence of AD-CMT2K cases and the low penetrance of mutations in the *GDAP1* gene.

Diaphragmatic dysfunction and vocal cord palsy are notable symptoms that can be highlighted in our cases. Although these symptoms are not specific to any one CMT subtype, they have been more frequently found in some CMT subtypes, including AR types of *GDAP1*-related CMT, *DCTN1*-related neuropathy, and CMT2C (linked to *TRPV4* variants) [25]. Of our 16 patients, none had vocal cord problems by the time of the last examination. The absence of vocal cord issues might be due to the young age of these cases, and some of them may develop vocal cord palsy later in life.

To our knowledge, unilateral scapular winging has not been widely reported as a typical feature of *GDAP1*-related CMT. While most of these cases focus on distal limb weakness, atrophy, and sensory loss, shoulder girdle involvement has not been systematically documented. Therefore, the unilateral scapular winging we observed in patient CMT102-III9 may reflect an atypical presentation, secondary mechanical factors, or possibly an underrecognized manifestation. As for seizures, based on current literature, seizures are not a known or common feature of *GDAP1*-related CMT. However, one patient in our cohort (CMT107-IV3) had a confirmed epilepsy diagnosis, treated with ethosuximide and valproate, and has remained seizure-free for the past 6 years. Given GDAP1's role in mitochondrial function and oxidative stress regulation, pathways implicated in some neurological disorders, including epilepsy, it is theoretically plausible that *GDAP1* variants could have broader neurological effects. Nonetheless, without direct evidence, we consider this an incidental finding but highlight it as a novel observation warranting further investigation.

In our study, a total of 9 variants were identified in *GDAP1*, of which two were common: variants c.347 T>G and c.311-23A>G, which were observed in three and two families, respectively. The 347T>G variant was first described in three unrelated Italian families with the same haplotype. This mutation was therefore proposed as a potential founder mutation in the Italian population [26]. The likelihood of a founder mutation was further supported when this specific variant was later discovered in four Italian patients [23]. Interestingly, this variant was found in 3/11 (~27%) of our families, indicating that this variant is not confined to the Italian population and has the potential to be detected in other populations as well. Notably, another family, CMT103, had a different known variant in the same codon 347, c.347T>C. Given that different mutations in codon 347 have been found in multiple cases from other populations [23, 26, 27], this codon may also function as a hotspot codon.

The second common variant, c.311-23A>G, has been previously reported in a consanguineous Iranian family [1]. Interestingly, this family and both our families, CMT101 and CMT102, originated from a province in the West of Iran. So, we suggest this variant may be a founder mutation in our population. Based on WES data of the CMT101-IV3 individual, a total of nine variants were identified throughout the *GDAP1* gene, seven of which were also found in CMT102-III9 (including the causal variant, c.311-23A>G), revealing a shared haplotype between these two probands. Upon further examination of WES data from an in-house database (approximately 100 WES files) no such haplotype was found. These findings further support the notion of a founder mutation (File S4). The c.311-23A>G variant was predicted to create a new splice acceptor site by various prediction tools, including NNSPLICE 0.9 and human splice finder version 3.1 (HSF 3.1). These tools indicated that this new acceptor site (NNSPLICE score 0.86 out of 1; HSF score 91.99 out of 100) is probably stronger than the canonical splice acceptor site

(NNSPLICE score: 0.80; HSF score: 77.16). From the data taken from these tools, it can be assumed that this new splice acceptor site would potentially introduce 22 additional nucleotides to the mature mRNA. Upon translation, the mutation would lead to 15 altered amino acids after aspartic acid 103, followed by two consecutive premature stop codons. As a result, the majority of the 358 amino acids in the wild-type protein would be missing. Functional studies by Khani et al. have confirmed the presence of the additional 22 nucleotides in the mature transcript [1]. The mother of family CMT101, individual III2, was a heterozygote carrier of this variant. She manifested distal limb weakness at age 45 years, and her NCS showed mild axonal neuropathy. She did not develop any other features by the age of 50 years. The fact that the proband's carrier father (CMT101-III1), carrier brother (IV2), and carrier sister (IV4) did not exhibit any peripheral neuropathy features, while the carrier mother did, suggests variable expressivity and reduced penetrance. In these cases, their symptoms may appear later in life. On the other hand, the c.311-23A>G variant highlights the importance of studying intronic regions, particularly among CMT patients who have not yet been given a genetic diagnosis.

Comparing all families carrying the c.311-23A>G variant, despite sharing some similar symptoms, marked clinical heterogeneities were noticed. This was also evident in the case of families with the c.347T>G variant. This heterogeneity was evident to such an extent that patients with the same variant showed different types of neuropathy [1, 26]. The detailed clinical information was provided in File S5.

Other notable variants included c.157G>T:p.Glu57* and c.791delC:p.Ala264Glufs*21, which were considered novel. c.157G>T results in a premature stop codon and was classified as pathogenic based on ACMG criteria (PS4, PVS1, PM2), with prediction tools unanimously indicating a damaging effect. Whereas, the c.791delC variant caused a frameshift deletion and it was considered a likely pathogenic (PVS1, PM2) variant. This variant alters a conserved nucleotide (PhyloP100 score = 7.41) and according to a nonsense-mediated decay (NMD) prediction tool (https://nmdpredictions.shinyapps.io/shiny/), the variant undergoes mRNA degradation by NMD.

In this study, in total, three additional known variants were also identified. C.112C>T and c.458C>T were categorized as pathogenic [(PS4, PVS1, PM2) and (PS4, PM1, PP2, PM2, PM5, PP3), respectively] and c.385G>C was a likely pathogenic variant (PM1, PP2, PM2, PP3) according to ACMG recommendations that expands the mutational spectrum of the *GDAP1* gene.

5 | Conclusion

In the present study, we describe the genetic and clinical features of 11 Iranian families affected by *GDAP1*-related CMT to increase our understanding of this specific CMT subtype. These patients showed genetic heterogeneity with different inheritance patterns. We identified two novel and six known exonic variants in the *GDAP1* gene as well as a previously reported intronic variant resulting in mis-splicing in two families. Probands also presented with phenotypic heterogeneity, and mutations in the *GDAP1* gene were linked to three phenotypes of CMT4A, AR-CMT2K, and AD-CMT2K. Of these CMT subtypes, AD-CMT2K cases exhibited reduced penetrance and a mild form of the disease, meaning that this form of the disease may be underestimated. Together, these findings highlight the importance of genetic testing to consolidate a definite diagnosis in peripheral neuropathies.

Acknowledgments

We acknowledge the Tehran University of Medical Sciences for funding the research (Grant number: 1403-4-499-79679) and thank the patients and their family members for participating in the study.

Ethics Statement

This research was performed by the Declaration of Helsinki and with the approval of the ethics board of the Tehran University of Medical Sciences in Iran (TUMS: IR.TUMS.SHARIATI.REC.1404.022). We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. M. Khani, H. Taheri, H. Shamshiri, et al., "Deep Geno- and Phenotyping in Two Consanguineous Families With CMT2 Reveals HADHA as an Unusual Disease-Causing Gene and an Intronic Variant in GDAP1 as an Unusual Mutation," *Journal of Neurology* 268, no. 2 (2021): 640–650, https://doi.org/10.1007/s00415-020-10171-4.

2. N. U. Jerath, "Mild Late-Onset Sensory Neuropathy Associated With Heterozygous Missense GDAP1 Variants, (In Eng)," *Case Reports in Medicine* 2022 (2022): 7492077, https://doi.org/10.1155/2022/7492077.

3. J. Morena, A. Gupta, and J. C. Hoyle, "Charcot-Marie-Tooth: From Molecules to Therapy," *International Journal of Molecular Sciences* 20, no. 14 (2019): 3419, https://doi.org/10.3390/ijms20143419.

4. U. Manzoor, A. Ali, S. L. Ali, et al., "Mutational Screening of GDAP1 in Dysphonia Associated With Charcot-Marie-Tooth Disease: Clinical Insights and Phenotypic Effects, (In Eng)," *Journal, Genetic Engineering & Biotechnology* 21, no. 1 (2023): 119, https://doi.org/10.1186/s43141-023-00568-9.

5. R. Sivera Mascaró, T. G. Sobrino, A. H. Hernández, et al., "Clinical Practice Guidelines for the Diagnosis and Management of Charcot-Marie-Tooth Disease," *Neurologia (Engl ed)* 40, no. 3 (2024): 290–305, https://doi.org/10.1016/j.nrleng.2024.02.008.

6. A. Sutinen, D. Paffenholz, G. T. T. Nguyen, S. Ruskamo, A. E. Torda, and P. Kursula, "Conserved Intramolecular Networks in GDAP1 Are Closely Connected to CMT-Linked Mutations and Protein Stability," *PLoS One* 18, no. 4 (2023): e0284532, https://doi.org/10.1371/journal.pone.0284532.

7. Z. Li, S. Zeng, Y. Xie, et al., "Genetic and Clinical Profile of 15 Chinese Families With GDAP1-Related Charcot–Marie–Tooth Disease and Identification of H256R as a Frequent Mutation," *Journal of the Peripheral Nervous System* 29, no. 2 (2024): 232–242, https://doi.org/10.1111/jns.12628.

8. J. Gaaib, A. F. Nassief And, and A. Al-Assie, "Simple Salting – Out Method for Genomic DNA Extraction From Whole Blood," *Tikrit Journal of Pure Science* 16 (2011): 9–11.

9. S. Richards, N. Aziz, S. Bale, et al., "Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, (In Eng)," *Genetics in Medicine* 17, no. 5 (2015): 405–424, https://doi.org/10.1038/gim.2015.30.

10. M. Khojasteh, P. Soleimani, A. Ghasemi, P. Taghizadeh, M. Rohani, and A. Alavi, "JAM2 Variants Can Be More Common in Primary Familial Brain Calcification (PFBC) Cases Than Those Appear; May Be due to a Founder Mutation," *Neurological Sciences* 45, no. 8 (2024): 3829–3844, https://doi.org/10.1007/s10072-024-07419-6.

11. A. Ghasemi, Z. Sadr, M. Babanejad, M. Rohani, and A. Alavi, "Copy Number Variations in Hereditary Spastic Paraplegia-Related Genes: Evaluation of an Iranian Hereditary Spastic Paraplegia Cohort and Literature Review, (In Eng)," *Molecular Syndromology* 14, no. 6 (2023): 477–484, https://doi.org/10.1159/000531507.

12. K. A. Benson, S. L. Murray, S. R. Senum, et al., "The Genetic Landscape of Polycystic Kidney Disease in Ireland, (In Eng)," *European Journal of Human Genetics* 29, no. 5 (2021): 827–838, https://doi.org/10.1038/s41431-020-00806-5.

13. M. R. Googins, A. O. Woghiren-Afegbua, M. Calderon, C. M. St Croix, K. Kirill, I, and A. P. VanDemark, "Structural and Functional Divergence of GDAP1 From the Glutathione S-Transferase Superfamily," *FASEB Journal* 34, no. 5 (2020): 7192–7207, https://doi.org/10.1096/fj.202000110R.

14. G. T. T. Nguyen, A. Sutinen, A. Raasakka, G. Muruganandam, R. Loris, and P. Kursula, "Structure of the Complete Dimeric Human GDAP1 Core Domain Provides Insights Into Ligand Binding and Clustering of Disease Mutations," *Frontiers in Molecular Biosciences* Original Research 7 (2021): 631232, https://doi.org/10.3389/fmolb.2020.631232.

15. A. Niemann, K. M. Wagner, M. Ruegg, and U. Suter, "GDAP1 Mutations Differ in Their Effects on Mitochondrial Dynamics and Apoptosis Depending on the Mode of Inheritance," *Neurobiology of Disease* 36, no. 3 (2009) (in eng): 509–520, https://doi.org/10.1016/j.nbd.2009.09.011.

16. A. Niemann, M. Ruegg, V. La Padula, A. Schenone, and U. Suter, "Ganglioside-Induced Differentiation Associated Protein 1 Is a Regulator of the Mitochondrial Network: New Implications for Charcot-Marie-Tooth Disease, (In Eng)," *Journal of Cell Biology* 170, no. 7 (2005): 1067–1078, https://doi.org/10.1083/jcb.200507087.

17. T. García-Sobrino, P. Blanco-Arias, F. Palau, et al., "Phenotypical Features of a New Dominant GDAP1 Pathogenic Variant (p.R226del) in Axonal Charcot-Marie-Tooth Disease, (In Eng)," *Neuromuscular Disorders* 27, no. 7 (2017): 667–672, https://doi.org/10.1016/j.nmd.2017.01.008.

18. M. Zimoń, J. Baets, G. M. Fabrizi, et al., "Dominant GDAP1 Mutations Cause Predominantly Mild CMT Phenotypes, (In Eng)," *Neurology* 77, no. 6 (2011): 540–548, https://doi.org/10.1212/WNL.0b013e318228fc70.

19. C. Crimella, A. Tonelli, G. Airoldi, et al., "The GST Domain of GDAP1 Is a Frequent Target of Mutations in the Dominant Form of Axonal Charcot Marie Tooth Type 2K," *Journal of Medical Genetics* 47, no. 10 (2010): 712–716, https://doi.org/10.1136/jmg.2010.077909.

20. J. Fu, S. Dai, Y. Lu, et al., "Similar Clinical, Pathological, and Genetic Features in Chinese Patients With Autosomal Recessive and Dominant Charcot-Marie-Tooth Disease Type 2K, (In Eng)," *Neuromuscular Disorders* 27, no. 8 (2017): 760–765, https://doi.org/10.1016/j.nmd.2017.04.001.

21. A. S. Saporta, S. L. Sottile, L. J. Miller, S. M. Feely, C. E. Siskind, and M. E. Shy, "Charcot-Marie-Tooth Disease Subtypes and Genetic Testing Strategies, (In Eng)," *Annals of Neurology* 69, no. 1 (2011): 22–33, https://doi.org/10.1002/ana.22166.

22. R. Sivera, T. Sevilla, J. J. Vílchez, et al., "Charcot-Marie-Tooth Disease: Genetic and Clinical Spectrum in a Spanish Clinical Series, (In Eng)," *Neurology* 81, no. 18 (2013): 1617–1625, https://doi.org/10.1212/WNL.0b013e3182a9f56a.

23. F. Manganelli, S. Tozza, C. Pisciotta, et al., "Charcot-Marie-Tooth Disease: Frequency of Genetic Subtypes in a Southern Italy Population, (In Eng)," *Journal of the Peripheral Nervous System* 19, no. 4 (2014): 292–298, https://doi.org/10.1111/jns.12092.

24. I. Pezzini, A. Geroldi, S. Capponi, et al., "GDAP1 Mutations in Italian Axonal Charcot-Marie-Tooth Patients: Phenotypic Features and Clinical Course, (In Eng)," *Neuromuscular Disorders* 26, no. 1 (2016): 26–32, https://doi.org/10.1016/j.nmd.2015.09.008.

25. T. Sevilla, T. Jaijo, D. Nauffal, et al., "Vocal Cord Paresis and Diaphragmatic Dysfunction Are Severe and Frequent Symptoms of GDAP1-Associated Neuropathy," *Brain* 131, no. 11 (2008): 3051–3061, https://doi.org/10.1093/brain/awn228.

26. E. Di Maria, R. Gulli, P. Balestra, et al., "A Novel Mutation of GDAP1 Associated With Charcot-Marie-Tooth Disease in Three Italian Families: Evidence for a Founder Effect, (In Eng)," *Journal of Neurology, Neurosurgery, and Psychiatry* 75, no. 10 (2004): 1495–1498, https://doi.org/10.1136/jnnp.2003.028100.

27. D. Kabzińska, A. Kochański, H. Drac, et al., "A Novel Met116Thr Mutation in the GDAP1 Gene in a Polish Family With the Axonal Recessive Charcot-Marie-Tooth Type 4 Disease, (In Eng)," *Journal of the Neurological Sciences* 241, no. 1–2 (2006): 7–11, https://doi.org/10.1016/j.jns.2005.10.002.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.