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Clinical and Neuroimaging Features in Charcot-Marie-Tooth Patients with *GDAP1* Mutations

Hyun Su Kim[a*], Hye Jin Kim[b,c*]
Soo Hyun Nam[c], Sang Beom Kim[d]
Yu Jin Choi[e], Kyung Suk Lee[f]
Ki Wha Chung[e]
Young Cheol Yoon[a]
Byung-Ok Choi[b,c,g]

[a]Departments of Radiology and [c]Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
[b]Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Seoul, Korea
[d]Department of Neurology, Kyung Hee University Hospital at Gangdong, Kyung Hee University School of Medicine, Seoul, Korea
[e]Departments of Biological Sciences and [f]Physics Education, Kongju National University, Gongju, Korea
[g]Stem Cell & Regenerative Medicine Institute, Samsung Medical Center, Seoul, Korea

Background and Purpose Mutations in the ganglioside-induced differentiation-associated protein 1 gene (*GDAP1*) are known to cause Charcot-Marie-Tooth disease (CMT). These mutations are very rare in most countries, but not in certain Mediterranean countries. The purpose of this study was to identify the clinical and neuroimaging characteristics of Korean CMT patients with *GDAP1* mutations.

Methods Gene sequencing was applied to 1,143 families in whom CMT had been diagnosed from 2005 to 2020. *PMP22* duplication was found in 344 families, and whole-exome sequencing was performed in 699 patients. Magnetic resonance imaging (MRI) were obtained using either a 1.5-T or 3.0-T MRI system.

Results We found ten patients from eight families with *GDAP1* mutations: five with autosomal dominant (AD) CMT type 2K (three families with p.R120W and two families with p.Q218E) and three with autosomal recessive (AR) intermediate CMT type A (two families with homozygous p.H256R and one family with p.P111H and p.V219G mutations). The frequency was about 1.0% exclusive of the *PMP22* duplication, which is similar to that in other Asian countries. There were clinical differences among AD *GDAP1* patients according to mutation sites. Surprisingly, fat infiltrations evident in lower-limb MRI differed between AD and AR patients. The posterior-compartment muscles in the calf were affected early and predominantly in AD patients, whereas AR patients showed fat infiltration predominantly in the anterolateral-compartment muscles.

Conclusions This is the first cohort report on Korean patients with *GDAP1* mutations. The patients with AD and AR inheritance routes exhibited different clinical and neuroimaging features in the lower extremities. We believe that these results will help to expand the knowledge of the clinical, genetic, and neuroimaging features of CMT.

Key Words Charcot-Marie-Tooth disease, *GDAP1*, autosomal dominant, autosomal recessive, CMT2K, CMTRIA.

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Correspondence
Byung-Ok Choi, MD, PhD
Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Tel +82-2-3410-1296
Fax +82-2-3410-0052
E-mail bochoi@skku.edu

Young Cheol Yoon, MD
Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Tel +82-2-3410-6454
Fax +82-2-3410-0055
E-mail youngcheol.yoon@gmail.com

*These authors contributed equally to this work.

INTRODUCTION

Charcot-Marie-Tooth disease (CMT) is the most common type of inherited peripheral neuropathy.[1] CMT can be classified based on electrophysiological findings into demyelinating type (CMT1), with reduced median motor nerve conduction velocities (MNCVs; <38 m/s); axonal type (CMT2), with preserved median MNCVs (>38 m/s); and intermediate type, with median MNCVs of 25–45 m/s.[2] The modes of CMT inheritance include autosomal dominant (AD), autosomal recessive (AR), and X-linked dominant and recessive inheritance.[3]

Mutations in the ganglioside-induced differentiation-related protein 1 gene (*GDAP1*) are known to cause the AD and AR forms of CMT. *GDAP1* patients carrying the AR form

show demyelinating, axonal, or intermediate CMT neuropathies, whereas AD mutations produce axonal CMT neuropathy.[3-6] *GDAP1* patients with the AR form exhibit an early onset and severe clinical features, while AD *GDAP1* mutations show an adult onset and mild clinical symptoms.[3,5] In addition, the genotype–phenotype correlation was reported to be weak in AD inherited mutations.

Mutations in *GDAP1* are quite rare, with prevalence rates of less than 1% in Western[7] and Asian countries,[8,9] with the exception of certain regions of Spain[10] and Italy.[11] The prevalence rate of *GDAP1* mutations is highly variable within each population due to numerous factors, including geographic distribution and racial background.[7-11] However, no previous study has investigated the mutation spectrum or prevalence rate of *GDAP1*-related Korean CMT patients.

CMT patients experience muscle weakness and atrophy because of damage to the peripheral nerves, and so magnetic resonance imaging (MRI) of the lower extremities can be used to estimate the degree of disability in a patient by examining muscle atrophy and fat infiltration.[12] The increases in the resolution of MRI over time have improved the ability to observe the severity of the muscle damage.[13] MRI examinations of the lower extremities in CMT patients are very helpful for both the patients themselves and doctors. Differences in the damage to calf muscles between CMT1 and CMT2 can be seen in lower extremity MRI, with the symptoms generally being milder for the AD than the AR type.[14-16] Although there have been studies comparing the differences in symptoms between the AD and AR types of *GDAP1*, few studies have compared the corresponding lower extremity MRI findings.[14]

The purpose of this cohort study was to describe the clinical and neuroimaging characteristics of Korean CMT patients with *GDAP1* mutations and to broaden the knowledge of genotype–phenotype correlations.

METHODS

Patients

Gene sequencing was conducted in a cohort of 1,889 patients from 1,143 unrelated families of Korean origin who had been diagnosed with CMT from April 2005 to March 2020. This cohort excluded patients with a *PMP22* deletion. There were 799 CMT families without *PMP22* duplication and 344 with *PMP22* duplication, with 699 of the latter cases analyzed using whole-exome sequencing (WES). We identified five families with AD CMT type 2K (CMT2K) (designated FC576, FC864, FC1085, FC008, and FC407) and three families with AR intermediate CMT type A (CMTRIA) (designated FC426, FC316, and FC1104) (Fig. 1A). In addition, 300 healthy controls for sequence variations were recruited from the Neurological Department after performing careful clinical and electrophysiological examinations. In accordance with the protocol approved by the Institutional Review Board of Samsung Medical Center at Sungkyunkwan University (SMC, 2014-08-057-002).

Clinical assessments

We examined motor and sensory impairment, deep tendon reflexes, and muscle atrophy. The strengths of the extensor and flexor muscles were manually assessed using the standard Medical Research Council Scale. Two measures were used to identify physical disabilities: Functional Disability Scale (FDS)[17] and CMT Neuropathy Score version 2 (CMTNS v2).[18] Disease severity was measured for each patient using a 9-point FDS. Sensory impairments were assessed in terms of the level and severity of pain, temperature, vibration, and position. The age at onset was determined by asking patients for their age at the first appearance of the symptoms (i.e., distal muscle weakness, foot deformity, or sensory change).

Electrophysiological examinations

The MNCVs and sensory nerve conduction velocities (SNCVs) in the median, ulnar, peroneal, tibial, and sural nerves were determined using standard methods with surface stimulation and recording electrodes. MNCVs of the median and ulnar nerves were determined by stimulating at the elbow and wrist while recording compound muscle action potentials (CMAPs) over the abductor pollicis brevis and adductor digiti quinti, respectively. MNCVs of peroneal and tibial nerves were determined by stimulating at the knee and ankle, while recording CMAPs over the extensor digitorum brevis and adductor hallucis, respectively. The SNCVs and sensory nerve action potentials (SNAPs) were measured over a finger–wrist segment from the median and ulnar nerves by orthodromic scoring, and were also recorded for sural nerves.

Mutation analysis

Genomic DNA was extracted from whole-blood samples of Korean CMT families using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Duplication and deletion of the 17p12 gene (*PMP22*) were first determined using hexaplex microsatellite polymerase chain reaction (PCR) and real-time PCR in all of the family samples. Mutations in *GDAP1* were screened using WES and Sanger sequencing. Exome sequencing was performed using the SureSelect Human All Exon 50M Kit (Agilent Technologies, Santa Clara, CA, USA) and the HiSeq2000 and HiSeq2500 genome analyzers (Illumina, San Diego, CA, USA). The human reference genome UCSC assembly hg19 (http://genome.ucsc.edu) was used to map sequences. All mu-

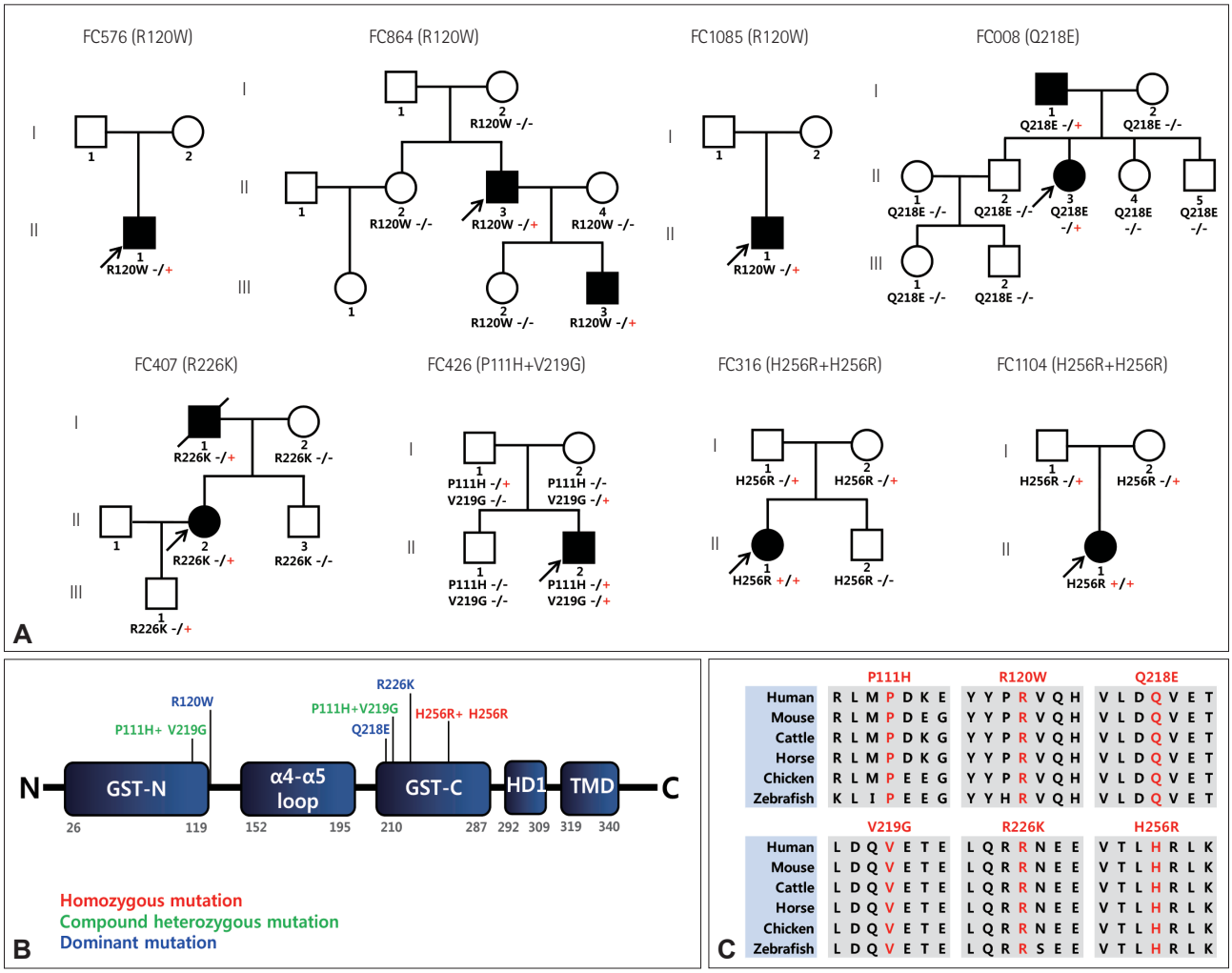

Fig. 1. The AD CMT2K and AR CMTRIA families with mutations in the *GDAP1*. A: The pedigrees and genotypes of eight CMT families with *GDAP1* mutations. FC567 (p.R120W), FC864 (p.R120W), FC1085 (p.R120W), FC008 (p.Q218E), and FC407 (p.R226K) are AD CMT2K, whereas FC426 (p.P111H and p.V219G), FC316 (p.H256R and p.H256R), and FC1104 (p.H256R and p.H256R) are AR CMTRIA. Arrows indicate the proband (□, ○: unaffected members; ■, ●: affected members). B: Locations of mutations in the *GDAP1* protein. The numbers below the *GDAP1* protein indicate the amino acid positions. AR changes are indicated in red (homozygous mutation) and green (compound heterozygous mutation), while AD changes are indicated in blue. C: Conservation of amino acid sequences at the *GDAP1* mutation sites across species. AD: autosomal dominant, AR: autosomal recessive, CMT: Charcot-Marie-Tooth disease, CMTRIA: Charcot-Marie-Tooth disease recessive intermediate A, CMT2K: Charcot-Marie-Tooth disease type 2K, GDAP1: ganglioside-induced differentiation-associated protein 1 gene.

tations identified in this study have been implicated in the pathogenesis of CMT (http://www.hgmd.cf.ac.uk/ac/index.php), and none of them were found in the control group.

Lower extremity MRI

Lower extremity axial MRI of the pelvic girdle, bilateral thigh, and lower leg was performed using either a 1.5-T or 3.0-T MRI system (Avanto or Skyra, Siemens Healthcare, Frankfurt, Germany). Follow-up MRI was performed on one CMT2K patient (FC008/II-3) and one CMTRIA patient (FC426/II-2) over a 7-year period. Axial T1-weighted MRI turbo spin-echo images of the thigh and lower leg muscles were graded for fatty infiltration based on the 5-point semiquantitative scale described by Goutallier et al.[19] The thigh muscles were evaluated bilaterally at three levels (proximal, middle, and distal), while the lower leg muscles were evaluated at two levels (proximal and distal). The levels were determined based on the following anatomical landmarks in axial T1-weighted MRI images: gluteus maximus tendon insertion (proximal thigh), just inferior to the gluteus maximus inferior margin where the muscle was no longer visible (mid-thigh), just inferior to the adductor longus inferior margin where the muscle was no longer visible (distal thigh), just inferior to the popliteus inferior margin where the muscle was no longer visible (proximal lower leg), and the uppermost part of the gastrocnemius tendon where the muscle was no longer visible (dis-

tal lower leg) (Supplementary Fig. 1 in the online-only Data Supplement).

Statistical analysis

All data are expressed as the median and interquartile range (IQR) values. The statistical significance of the presented data was evaluated in pairwise comparisons using the Mann-Whitney U test. The significance criterion was set to $p<0.05$.

RESULTS

Identification of *GDAP1* mutations in Korean CMT patients

We detected the ten patients (six males and four females) from eight unrelated Korean families with *GDAP1* variants among the CMT cohort. This study enrolled 1,143 unrelated CMT families, and excluded 344 families with *PMP22* duplication. The *GDAP1* mutation rate was 0.7% (n=8) in the 1,143 total families and 1.0% (n=8) in the 799 families without *PMP22* duplication.

We found six *GDAP1* mutations in five AD CMT2K families (p.R120W in three families, and p.Q218E and p.R226K in each of the other two families) and three AR CMTRIA families (p.P111H and p.V219G in one family, and p.H256R and p.H256R in two families) (Fig. 1A). All of these *GDAP1* mutations have previously been reported to be the underlying causes of CMT2K or CMTRIA.[6,20-22] In the AR families, the affected child inherited one mutant allele from both unaffected parents. The p.P111H and p.R120W mutations are located in the interdomain region between the glutathione S-transferase N-terminal and glutathione S-transferase C-terminal (GST-C) domains. The p.R218E, p.V219G, p.R226K, and p.H256R mutations are located in the GST-C domain (Fig. 1B). All mutation sites were well conserved between different species (Fig. 1C).

Clinical manifestations

The clinical features are summarized in Table 1. The severity scores and clinical characteristics differed between the AD and AR inheritance groups. Patients with AD mutations had mild-to-moderate neuropathy with a late onset (age: median=21.0 years, IQR=13.5−24.5 years), while those with AR had severe neuropathy with an early onset (age: median=2.0 years, IQR=1.5−2.0 years). The functional disability was significantly more severe in AR patients than in AD patients. The median FDS[17] score was 2.0 (IQR=1.5−2.5) in AD patients and 6.0 (IQR=5.0−6.0) in AR patients ($p<0.05$). The median CMTNS v2[18] was 10.0 (IQR=7.0−11.5) in AD patients and 26.0 (IQR=23.5−26.5) in AR patients ($p<0.05$). A high FDS score (6 or 7) was found only in AR patients, with members of the AD group having FDS scores lower than 5. All three AR patients were in the severe category (CMTNS v2 ≥21). Foot deformities were quite common, and four patients presented with scoliosis. However, no wheelchair dependence, vocal cord paresis, diaphragmatic weakness, or hoarseness was observed.

We also found clinical differences among CMT2K patients with three AD mutations. Patients with the p.R226K mutation had moderate early-onset neuropathy (age: median=12.5 years, IQR=11.3−13.8 years), and their functional disability was more severe than that in p.R120W patients. In the p.R120K mutant, ankle flexion weakness could be immediately detected as an ankle extension weakness, whereas the ankle extension weakness prevailed in the p.R226K mutation. The most severely affected patient (p.Q218E) had severe neuropathy, with a CMTNS v2 of 25 at 36 years old.

Electrophysiological findings

The results of the nerve conduction studies performed in ten patients are presented in Table 2. The electrophysiological findings confirmed that AR *GDAP1* patients were more severely affected than AD *GDAP1* patients. The nerve conduction velocity generally did not decrease, except in nerves with very small evoked responses. In AR patients, the reductions in CMAP and SNAP were even more pronounced, and could not even be detected in several nerves. These findings were worse in the lower extremities than the upper extremities.

Different patterns in lower extremities between AD and AR patients

The MRI findings for the lower extremity are detailed in Supplementary Tables 1 and 2 (in the online-only Data Supplement), and representative images are shown in Figs. 2, 3, and 4. Ten MRI images were obtained from eight patients: five CMT2K patients (FC864/II-3, FC1085/II-1, FC008/II-3, FC407/I-1, and FC407/II-2) and three CMTRIA patients (FC426/II-2, FC316/II-1, and FC1104/II-1). The age of the patients when MRI was performed ranged from 5 years to 48 years: 18–53 years for the CMT2K patients, and 5−13 years for the CMTRIA patients. Follow-up MRI performed in one patient (FC008/II-3) showed the progression of muscular fatty infiltration in the thigh over a 7-year period. All lower leg muscles of this patient exhibited Goutallier grade 4 fatty infiltration in the initial MRI. This patient also demonstrated Goutallier grade 3 or 4 fatty infiltration in nearly all anterior- and posterior-compartment muscles from the proximal to distal levels of the bilateral thighs on the last follow-up MRI performed at the age of 43 years. In the four other CMT2K patients, the lower leg muscles in all compartments showed fatty infiltration of variable degrees, with superficial poste-

Table 1. Clinical features in ten Korean patients from eight unrelated families with mutations in the ganglioside-induced differentiation-associated protein 1 gene (*GDAP1*)

Patients	FC576/II-1	FC864/II-3	FC1085/II-1	FC008/II-3	FC008/I-1	FC407/II-2	FC407/I-1	FC426/II-2	FC316/II-1	FC1104/II-1
Mutation	R120W	R120W	R120W	Q218E	Q218E	R226K	R226K	P111H+V219G	H256R+H256R	H256R+H256R
Mode of inheritance	AD	AD	AD	AD	AD	AD	AD	AR	AR	AR
CMT subtype	CMT2K	CMT2K	CMT2K	CMT2K	CMT2K	CMT2K	CMT2K	CMTRIA	CMTRIA	CMTRIA
Sex	M	M	M	F	M	F	M	M	F	F
Age at examination, years	39	36	48	36	68	18	53	13	11	5
Age at onset, years	31	24	21	12	25	10	15	2	2	1
Disease duration, years	8	12	27	24	43	8	38	11	9	4
FDS score	1	1	2	5	2	2	3	6	6	4
CMTNS v2	6	6	10	25	10	8	13	26	27	21
Muscle weakness										
Upper limb*	-	-	+	++	+	+	+	++	+++	+
Lower limb†	+	+	++	+++	++	++	+++	+++	+++	+++
Muscle atrophy‡	Mild (L)	Mild (L)	Mild (U<L)	Moderate (U<L)	Moderate (U<L)	Mild (U<L)	Mild (U<L)	Severe (U<L)	Severe (U<L)	Severe (U<L)
Sensory loss§	Normal	V=P	V=P	V>P	V>P	V>P	V>P	V>P	V>P	V>P
Reflexes‖										
Biceps	D	N	D	A	D	D	A	A	A	A
Knee	D	D	A	A	A	A	A	A	A	A
Foot deformity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Scoliosis	No	No	No	Yes	No	No	No	Yes	Yes	Yes

*Muscle weakness in upper limbs: +, intrinsic hand weakness of 4/5 on the MRC Scale; ++, intrinsic hand weakness of <4/5 on MRC Scale; +++, proximal weakness; –, no symptoms, †Muscle weakness in lower limbs: +, ankle dorsiflexion of 4/5 on MRC scale; ++, ankle dorsiflexion <4/5 on MRC Scale; +++, proximal weakness and wheelchair dependent, ‡Muscle atrophy: U<L, lower-limb-predominant muscle atrophy; L, only lower limb muscle atrophy, §Sensory loss: P, pain sensation; V, vibration sensation; Normal, normal sensation, ‖Deep tendon reflexes: N, normal; D, diminished; A, absent.
AD: autosomal dominant, AR: autosomal recessive, CMT: Charcot-Marie-Tooth disease, CMTRIA: Charcot-Marie-Tooth disease recessive intermediate A, CMT2K: Charcot-Marie-Tooth disease type 2K, F: female, FDS: Functional Disability Scale, M: male, MRC: Medical Research Council.

Table 2. Electrophysiological findings in ten Charcot-Marie-Tooth patients with *GDAP1* mutations

Patient	FC576/II-1		FC864/II-3		FC1085/II-1		FC008/II-3		FC008/I-1		FC407/II-2		FC407/I-1		FC426/II-2		FC316/II-1		FC1104/II-1	
Age at examination, years	39		36		48		43		68		16		46		9		11		3	
Side	Right	Left	Right	Left	Right	Left	Right	Right	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Median motor nerve																				
TL, ms	4.3	ND	3.1	3.0	3.1	3.1	4.3	4.2	3.3	3.3	2.8	2.8	3.5	3.5	3.7	3.7	A	A	3.0	ND
CMAP, mV	10.6	ND	18.9	20.5	16.0	19.1	0.8	1.0	8.8	9.4	12.8	11.0	9.5	11.6	2.2	3.7	A	A	2.0	ND
MNCV, m/s	45.9	ND	56.4	56.4	54.7	58.1	42.6	32.8	50.0	52.5	59.3	59.5	54.5	47.8	48.6	48.6	A	A	48.0	ND
Ulnar motor nerve																				
TL, ms	2.8	ND	2.5	2.2	2.7	2.9	3.2	3.2	2.9	2.7	2.4	2.3	3.0	3.2	2.8	2.4	A	A	2.2	ND
CMAP, mV	10.7	ND	19.3	18.6	15.1	14.0	1.1	4.4	9.4	11.3	17.2	14.2	10.9	11.6	2.1	0.3	A	A	4.9	ND
MNCV, m/s	56.3	ND	59.5	58.7	57.8	57.7	33.8	53.3	50.2	50.0	59.5	59.1	61.3	62.4	48.5	48.5	A	A	68.3	ND
Peroneal nerve																				
TL, ms	3.8	3.2	4.3	3.9	4.3	A	A	A	A	A	A	A	3.8	4.8	A	A	A	A	32.8	ND
CMAP, mV	2.4	2.8	3.1	5.1	3.6	A	A	A	A	A	A	A	3.8	0.8	A	A	A	A	0.1	ND
MNCV, m/s	33.9	43.9	40.9	40.5	39.5	A	A	A	A	A	A	A	37.1	35.0	A	A	A	A	A	A
Tibial nerve																				
TL, ms	4.5	5.6	5.4	6.4	4.0	5.0	A	A	A	A	4.0	3.5	3.8	5.0	A	A	A	A	ND	4.9
CMAP, mV	3.2	2.5	2.3	1.2	4.1	6.8	A	A	A	A	0.2	1.8	7.5	5.2	A	A	A	A	ND	0.3
MNCV, m/s	42.2	43.3	44.5	44.4	40.0	36.4	A	A	A	A	35.2	37.0	33.7	31.1	A	A	A	A	A	A
Median sensory nerve																				
SNAP, μV	ND	ND	1.8	1.9	7.2	4.2	5.7	5.6	6.0	3.2	6.4	4.7	10.5	3.2	A	A	1.8	0.6	ND	ND
SNCV, m/s	ND	ND	38.1	35.9	38.8	40.0	40.0	40.6	37.8	37.8	36.9	38.9	36.9	34.2	A	A	25.0	22.3	ND	ND
Ulnar sensory nerve																				
SNAP, μV	ND	ND	1.8	2.1	4.6	4.0	4.5	1.8	2.1	2.1	6.5	5.5	12.7	9.1	A	A	A	1.0	ND	ND
SNCV, m/s	ND	ND	34.5	38.1	34.7	33.3	36.3	30.3	36.4	34.9	32.9	33.8	34.9	32.1	A	A	A	22.6	ND	ND
Sural nerve																				
SNAP, μV	ND	ND	A	A	1.4	1.4	A	A	A	A	A	A	A	A	A	A	A	A	ND	ND
SNCV, m/s	ND	ND	A	A	29.2	29.2	A	A	A	A	A	A	A	A	A	A	A	A	ND	ND

Normal MNCVs: median motor nerve, ≥50.5 m/s; ulnar motor nerve, ≥51.1 m/s; tibial nerve, ≥41.1 m/s. Normal SNCVs: median sensory nerve, ≥39.3 m/s; ulnar sensory nerve, ≥37.5 m/s; sural nerve, ≥32.1 m/s. Normal CMAP amplitudes: median motor nerve, ≥6 mV; ulnar nerve, ≥8 mV; tibial nerve, ≥6 mV. Normal SNAP amplitudes: median sensory nerve, ≥8.8 μV; ulnar nerve, ≥7.9 μV; sural nerve, ≥6.0 μV.

A: absent, CMAP: compound muscle action potential, MNCV: motor nerve conduction velocity, ND: not done, SNAP: sensory nerve action potential, SNCV: motor nerve conduction velocity, TL: terminal latency.

rior-compartment muscles (soleus and gastrocnemius; Fig. 2B, white arrowhead) being the most severely and consistently affected. Fatty infiltration was more pronounced in the distal lower leg, and severe fatty infiltration (grades 3 and 4) was seen in the soleus muscles of all four patients. The severe fatty infiltration variably involved muscles in the anterior (Fig. 2B, arrow), lateral (Fig. 2B, black arrowhead), and deep posterior compartments. The thigh muscles of these patients showed fatty infiltration of grades 0–2.

MRI of the CMTRIA patients performed at pediatric ages (FC426/II-2 at 6 years and FC1104/I-1 at 5 years) showed normal (FC1104/II-1) or grade-1/2 fatty infiltration (FC426/II-2) in thigh muscles. More-severe fatty infiltration was demonstrated in the lower leg muscles, with severe fatty infiltration (grades 3 and 4) seen in FC1104/II-1 and FC426/II-2. FC426/II-2 had severe fatty infiltration in the anterior- and lateral-compartment muscles of the proximal lower leg and in all of the muscle compartments of the distal lower leg. FC1104/II-1 had severe fatty infiltration in the anterior- and lateral-compartment muscles in both the proximal and distal regions of the lower leg. Anterior-compartment muscles were the most severely affected during adolescence (FC426/II-2 at 13 years and FC316/II-1 at 11 years). In the lower legs, severe fatty infiltration was seen in all of the muscles at the proximal and distal levels. The progression of muscle degeneration over 7 years was greater in the CMTRIA patient (FC426/II-2) than in the CMT2K patient (FC008/II-3). The degeneration progressed most rapidly in the CMTRIA patient carrying p.P111H and p.V219G mutations (FC426/II-2) (Fig. 3).

DISCUSSION

This cohort study of Korean CMT patients found *GDAP1* mutations in 0.7% (n=8) of all 1,143 patients diagnosed with CMT, and in 1.0% (n=8) of 799 patients who did not

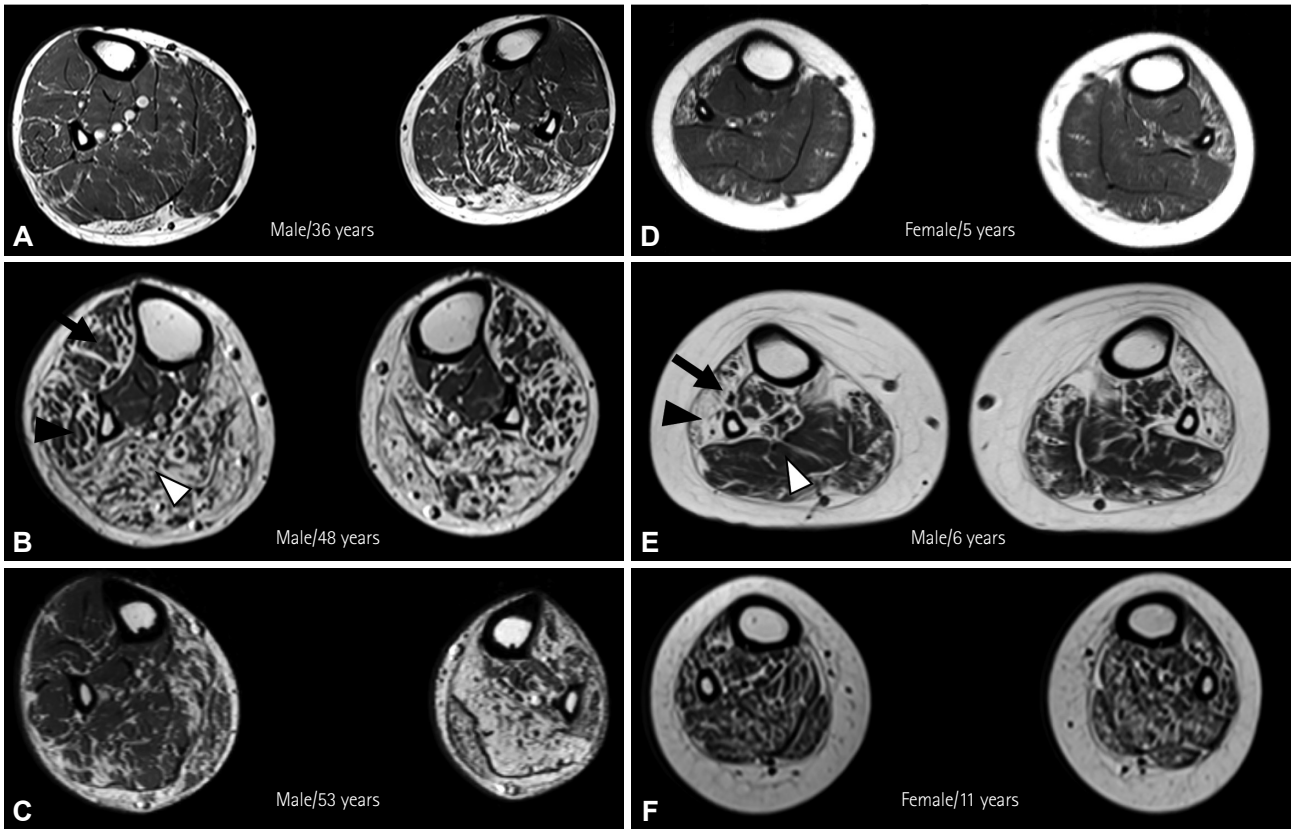

Fig. 2. Different patterns of muscle involvements in lower limb MRI between AD CMT2K (A, B, and C) and AR CMTRIA (D, E, and F) patients with *GDAP1* mutations. FC864/II-3 (A), FC1085/II-1 (B), and FC007/I-1 (C) in CMT2K patients, and FC1104/II-1 (D), FC426/II-2 (E), and FC316/II-1 (F) in CMTRIA patients. Axial T1-weighted MRI images of the calf in an AD CMT2K patient (A, B, and C) indicate severe muscular fatty infiltration most prominently in the bilateral superficial posterior-compartment muscles (white arrowhead). However, MRI images of the calf of an AR CMTRIA patient (D, E, and F) indicate that muscular fatty infiltration was seen most prominently bilaterally in muscles in the anterior (arrow) and lateral (black arrowhead) compartments. AD: autosomal dominant inheritance, AR: autosomal recessive, CMTRIA: Charcot-Marie-Tooth disease recessive intermediate A, CMT2K: Charcot-Marie-Tooth disease type 2K, GDAP1: ganglioside-induced differentiation-associated protein 1 gene, MRI: magnetic resonance imaging.

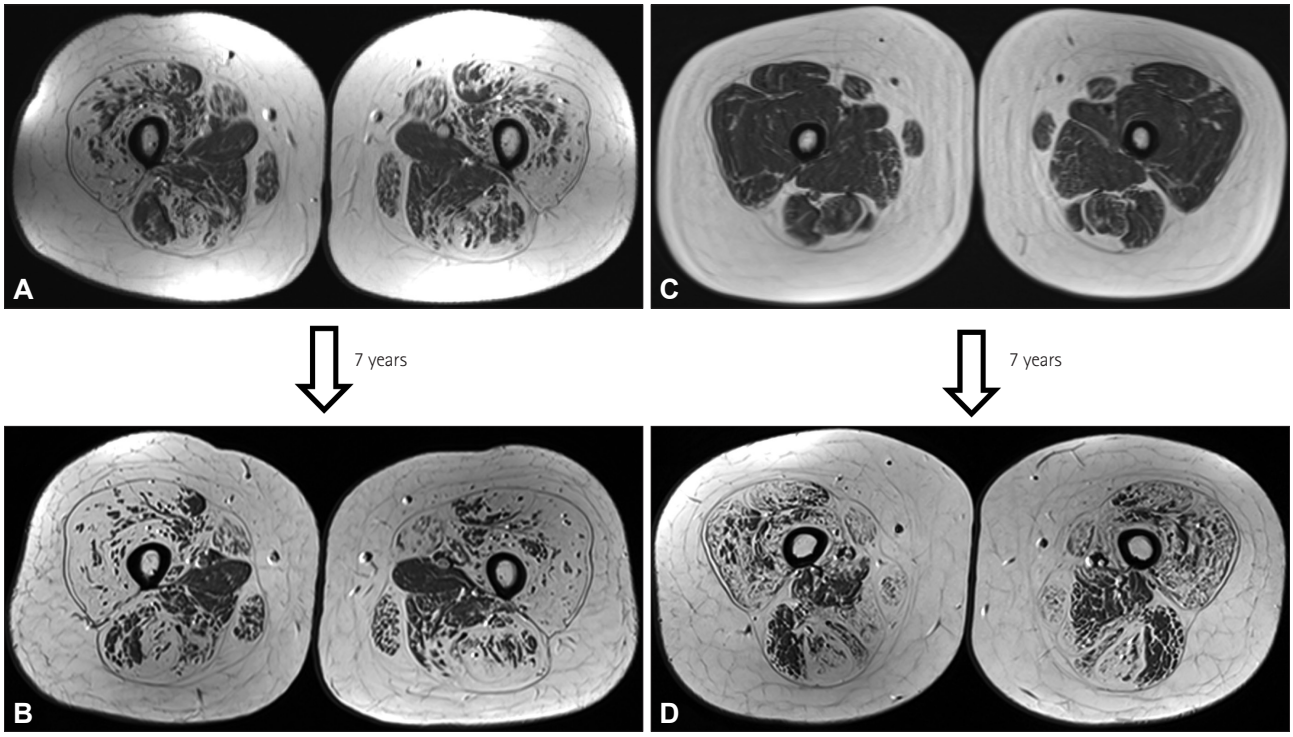

Fig. 3. Thigh MRI images of AD CMT2K (A and B) and AR CMTRIA (C and D) patients showing different degrees of muscle degeneration progression over 7 years. A and B: Axial T1-weighted MRI images at the mid-thigh level in a female CMT2K patient (FC008/II-3) obtained at 36 (A) and 43 (B) years of age, showing the progression of degeneration in some of the muscles in the anterior and posterior compartments. C and D: Thigh MRI images in a male CMTRIA patient (FC426/II-2) obtained at 6 (C) and 13 (D) years of age, showing the marked progression of muscular fatty infiltration that is strikingly more severe than the changes seen in the CMT2K patient. AD: autosomal dominant, AR: autosomal recessive, CMTRIA: Charcot-Marie-Tooth disease recessive intermediate A, CMT2K: Charcot-Marie-Tooth disease type 2K, MRI: magnetic resonance imaging.

have *PMP22* duplication. These prevalence rates are similar to those reported in most Asian and Western countries, including China, Japan, Germany, the United Kingdom, and the United States.[9-11,23,24] However, they are lower than those found in certain regions of Spain and Italy[7,8] In addition, the p.R120W mutation was reported as the most-prevalent mutation in AD *GDAP1* patients in Spain, Finland, and China,[14,25,26] and this was found three times in our patients. The present study also observed the homozygous p.H256R mutation twice in AR patients, which was similar to findings in Chinese patients.[11]

The clinical characteristics of AR and AD *GDAP1* patients in this cohort were compared. It is well known that the most important factor influencing the clinical characteristics of these patients is the genetic pattern.[7] Our AR *GDAP1* patients had earlier-onset clinical symptoms and greater disabilities than AD patients, which is consistent with previous findings.[3,5] However, the AR patients in the present study did not rely on wheelchairs, and there was no evidence of vocal cord paralysis, diaphragm weakness, or hoarseness. This apparent discrepancy might have been due to all three AR patients being younger (<14 years) than the previously reported patients.

Among the present AD *GDAP1* patients, a phenotypic variability was noticeable even within members of the same family. There was one AD patient with severe disability according to the CMTNS v2 (25 at 36 years of age), which indicated the presence of overlap with AR patients in the degree of disability. She carried the missense p.Q218E mutation and was still able to walk with crutches at the age of 43 years. This mutation was also found in her father, who had a moderate phenotype and remained ambulant with orthosis at 68 years of age (CMTNS v2=10). Clinical differences were also found between the phenotypes of the p.R120W and p.R226K mutations. Patients with the p.R120W mutation had mild neuropathy with late onset (age: median=24.0 years, IQR=22.5–27.5 years), whereas those with the p.R226K mutation had moderate neuropathy with an early onset (age: median=12.5 years, IQR=11.3–13.8 years). Functional disability was more severe in p.R226K than p.R120W patients. Ankle flexion weakness and ankle extension weakness occurred almost simultaneously in patients with the p.R120W mutation. However, in the p.R226K mutant patients, ankle extension weaknesses predominated in the neurological examinations.

This study analyzed the MRI findings of the lower extremities in eight patients with *GDAP1* mutations (five AD and three AR patients). As reported previously, the muscles in

the posterior compartment were affected earlier and more severely than those in the anterolateral compartment of the calf in AD patients (Fig. 2A).[14] However, the anterolateral-compartment muscles were affected earlier and more severely than the posterior-compartment muscles of the calf in AR patients (Fig. 2B). This pattern differs from Sivera et al.[14] reporting similar *GDAP1* MRI patterns in the lower limbs of AD and AR patients; they reported that the posterior region was totally damaged while the anterolateral region was partially damaged in one AR CMTRIA patient (p.Q163X and p.L344R).[14] In contrast, we observed that the anterolateral region was damaged earlier and more severely in three AR CMTRIA patients. Although more studies should be conducted in the future, we believe that AD and AR patients with mutations in *GDAP1* may have different MRI patterns in the lower extremities.

We also performed follow-up MRI studies of the lower extremities, which revealed differences in fat infiltration between AD and AR patients. Fat infiltration in the mid-thigh was more severe in a 13-year-old AR patient (FC426/II-2; p.P111H and p.V219G) than in a 36-year-old AD patient (FC1085/II-1, p.R120W) (Supplementary Table 1 in the online-only Data Supplement). In addition, the 7-year MRI follow-up analysis indicated that fat infiltration was more rapid in AR patients than in AD patients (Fig. 3). It is particularly interesting that an FC008/II-3 patient with p.Q218E mutation had severe symptoms and required crutches to walk,[20] with lower-limb MRI showing severe fat infiltration of the calf and thigh. In patients with the other dominant mutations (p.R120W and p.R226K), fat infiltration was mainly observed in the calf rather than the thigh muscle, and was only of mild-to-moderate severity. Therefore, we found that the clinical symptoms and MRI patterns of the p.Q218E mutation differ from those of the p.R120W and p.R226K mutations.

Fat infiltration was more severe in AR than AD patients, and the degree of fat infiltration in AD patients may differ with the mutant site. The fat infiltration was mildest in the p.R120W patients, and became more severe in p.R226K and then p.Q218E patients; this trend was consistent with that in the clinical disabilities (Fig. 4). Therefore, even in patients with the same *GDAP1* mutation, there were differences in muscle fat infiltration in MRI according to the location of the mutation. In addition, among the AR cases, fat infiltration was more severe in patients with p.P111H and p.V219G than in patients with p.H256R and p.H256R. While these findings are limited by the small number of patients, it appears that the spectrum of fat infiltration depends upon the locations of the mutations.

This study was subject to some limitations. It involved only ten patients with *GDAP1* mutations in South Korea, and so the smallness of the sample and the region being limited to South Korea may make it difficult to generalize the results. However, we have discovered differences in the lower extremity MRI findings of AR and AD *GDAP1* patients for the first time, and so more studies should be performed to verify this.

In summary, we have reported clinical and neuroimaging findings of Korean CMT patients carrying *GDAP1* mutations. The frequencies of *GDAP1* mutations in this cohort were similar to those in most previous studies, including those performed in other Asian countries, but lower than those in certain Mediterranean countries. This is the first report on differences in lower limb MRI findings between AD and AR patients with *GDAP1* mutations. We suggest that these results expand the knowledge of the clinical, genetic, and neu-

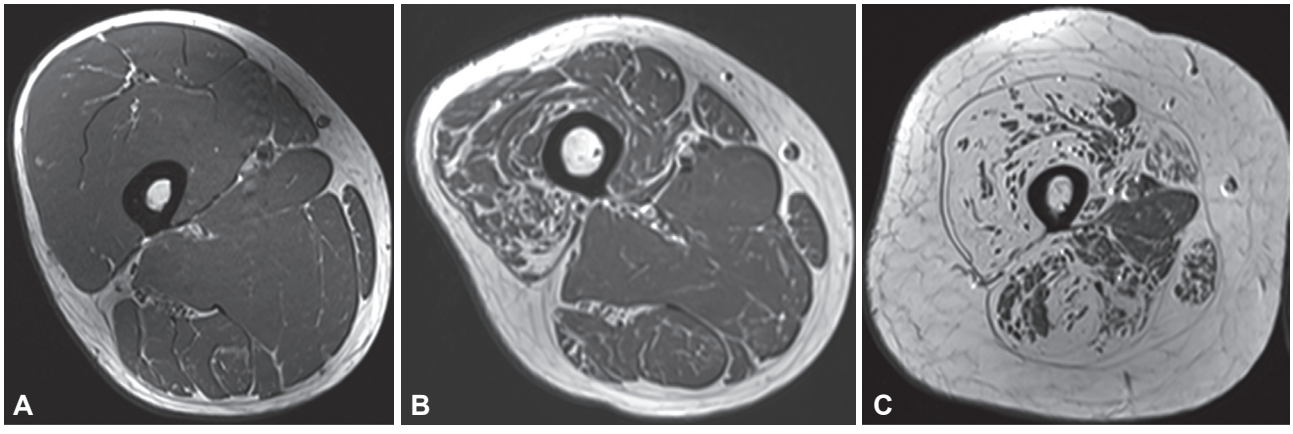

Fig. 4. Thigh MRI images of CMT2K patients with varying degrees of intramuscular fatty infiltration according to different mutation sites. A: Axial T1-weighted MRI image at the mid-thigh level of a 48-year-old male (FC1085/II-1, R120W mutation) showing nearly normal muscles with minimal fatty streaks. B: MRI image of a 53-year-old male (FC407/I-1, R226K mutation) with moderate fatty degeneration centered in the anterior-compartment muscles. C: MRI image of a 43-year-old female (FC008/II-3, Q218E mutation) showing severe fatty infiltration that is most pronounced in the anterior- and posterior-compartment muscles. CMT2K: Charcot-Marie-Tooth disease type 2K, MRI: magnetic resonance imaging.

roimaging features of CMT.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.3988/jcn.2021.17.1.52.

Author Contributions

Conceptualization: Byung-Ok Choi, Young Cheol Yoon. Data curation: Hye Jin Kim. Formal analysis: Hyun Su Kim, Hye Jin Kim, Soo Hyun Nam, Sang Beom Kim, Yu Jin Choi, Kyung Suk Lee. Funding acquisition: Byung-Ok Choi, Ki Wha Chung. Investigation: Hye Jin Kim, Soo Hyun Nam. Methodology: Hyun Su Kim, Yu Jin Choi, Kyung Suk Lee. Project administration: Byung-Ok Choi, Young Cheol Yoon. Resource: Hyun Su Kim, Hye Jin Kim, Soo Hyun Nam, Sang Beom Kim, Yu Jin Choi, Kyung Suk Lee. Software: Hyun Su Kim, Sang Beom Kim, Yu Jin Choi. Supervision: Byung-Ok Choi, Young Cheol Yoon, Ki Wha Chung. Validation: Soo Hyun Nam, Sang Beom Kim. Writing—original draft: Hyun Su Kim, Hye Jin Kim, Young Cheol Yoon, Byung-Ok Choi. Writing—review & editing: all authors.

ORCID iDs

Hyun Su Kim	https://orcid.org/0000-0002-0179-9542
Hye Jin Kim	https://orcid.org/0000-0002-7636-3639
Soo Hyun Nam	https://orcid.org/0000-0003-4421-1793
Sang Beom Kim	https://orcid.org/0000-0001-8225-3922
Yu Jin Choi	https://orcid.org/0000-0002-2437-7801
Kyung Suk Lee	https://orcid.org/0000-0003-4157-5153
Ki Wha Chung	https://orcid.org/0000-0003-0363-8432
Young Cheol Yoon	https://orcid.org/0000-0002-7822-5344
Byung-Ok Choi	https://orcid.org/0000-0001-5459-1772

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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