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BRIEF COMMUNICATION

# Compound heterozygous mutations of *SH3TC2* in Charcot–Marie–Tooth disease type 4C patients

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## Abstract

Charcot–Marie–Tooth disease type 4C (CMT4C) is an autosomal recessive neuropathy caused by *SH3TC2* mutations, characterized by spine deformities and cranial nerve involvement. This study identified four CMT4C families with compound heterozygous *SH3TC2* mutations from 504 Korean demyelinating or intermediate CMT patients. The frequency of the CMT4C was calculated as 0.79% in demyelinating and intermediate patients ($n = 504$), but it was calculated as 2.02% in patients without *PMP22* duplication ($n = 198$). The CMT4C frequency was similar to patients in Japan, but it was relatively low compared to those patients in other populations. The symptom was less severe and slowly progressed compared to the other AR-CMT. A patient harboring an intermediate neuropathy showed cranial nerve involvement but did not have scoliosis. This study will be helpful in making molecular diagnoses of demyelinating or intermediate CMT due to *SH3TC2* mutations.

**Supplementary information** The online version of this article (https://doi.org/10.1038/s10038-019-0636-y) contains supplementary material, which is available to authorized users.

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## Introduction

Mutations in *SH3TC2* cause autosomal recessive (AR) demyelinating or intermediate Charcot–Marie–Tooth disease type 4C (CMT4C) characterized by spine deformities and cranial nerve impairment [1–7]. *SH3TC2* encodes Src homology-3 (SH3) domains and tetratricopeptide repeat (TPR) motifs which are localized in the plasma membrane of the Schwann cells of peripheral nerves [8, 9].

The frequencies of CMT4C vary according to countries in AR demyelinating patients without *PMP22* duplication. CMT4C is the most frequent form of AR-CMT in several countries: >20% in Italy [4] and Czech [10]. However, CMT4C is relatively rare in Germany (5.2%) [5] and Japan (1.76%) [6]. This study examined *SH3TC2* mutations in Korean demyelinating or intermediate CMT patients.

## Materials and methods

### Subjects and clinical examinations

We collected 504 unrelated demyelinating or intermediate CMT patients from the Korean CMT cohort study group, of which 306 patients were proven to be positive for *PMP22* duplication. This study performed exome sequencing or

**Table 1** Compound heterozygous *SH3TC2* mutations in four CMT4C patients

| Family ID | Mutations | | dbSNP no | Mutant allele frequencies | | | | GERP | In silico analysis[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nucleotide[a] | Amino acid | | 1000G | ESP | ExAC | KRGDB | | PROVEAN | PP2 | MUpro | Fathmm |
| FC523, FC657 | c.929G>A | p.G310E | NR | NR | NR | 0.00001 | NR | 4.88 | −6.84* | 1.00* | −0.29* | −0.17* |
| | c.2831A>G | p.E944G | NR | NR | NR | NR | 0.00091 | 6.02 | −6.65* | 1.00* | −1.00* | −1.21* |
| FC703, FC1080 | c.929G>A | p.G310E | NR | NR | NR | 0.00001 | NR | 4.88 | −6.84* | 1.00* | −0.29* | −0.17* |
| | c.3272G>T | p.G1091V | NR | NR | NR | 0.00001 | NR | 5.76 | −8.27* | 1.00* | −1.00* | −1.73* |

*1000G* 1000 Genomes project (http://www.1000genomes.org/), *CMT4C* Charcot–Marie–Tooth disease type 4C, *ESP* exome sequencing project (http://evs.gs.washington.edu/EVS/), *ExAC* exome aggregation consortium (http://exac.broadinstitute.org/), *GERP* genomic evolutionary rate profiling score (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html), *KRGDB* Korean reference genome database (http://152.99.75.168/KRGDB/menuPages/introKor.jsp), *NR* not reported

[a]Reference DNA sequence of *SH3TC2*: NM_024577.3

[b]Scores of PROVEAN (http://provean.jcvi.org/seq_submit.php) <−2.5, PolyPhen-2 (PP2, http://genetics.bwh.harvard.edu/pph2/) ~1, MUpro (http://www.ics.uci.edu/~baldig/mutation) <0, and Fathmm (http://fathmm.biocompute.org.uk/) ≤−1.5 indicate a prediction of pathogenicity (asterisk denotes a "pathogenic" prediction)

targeted sequencing for CMT-related genes in 198 patients without *PMP22* duplication.

Physical disability was determined by the functional disability scale (FDS) and the CMT neuropathy score (CMTNS). Nerve conduction values and MRIs were obtained by previous methods [11]. Distal sural nerve was biopsied from two patients (FC657 and FC703). This study was approved by the Institutional Review Board of Sungkyunkwan University, Samsung Medical Center.

### Molecular genetic studies

Exome sequencing and targeted sequencing of the CMT-related genes were performed using the genomic DNAs isolated from blood [12, 13]. The exome was captured using the SureSelect Human All Exon 50 M Kit (Agilent Technologies, Santa Clara, CA, USA), and the subsequent sequencing was performed using the HiSeq 2000 Genome Analyzer (Illumina, San Diego, CA, USA).

## RESULTS

### Identification of *SH3TC2* compound heterozygous mutations

From the 198 CMT families without *PMP22* duplication, we identified four patients having likely pathogenic compound heterozygous mutations in *SH3TC2*: c.929G>A (p.G310E) and c.2831A>G (p.E944G) in two families (FC523 and FC667) and c.929G>A (p.G310E), and c.3272G>T (p.G1091V) in two families (FC703 and FC1080) (Table 1, Fig. 1a, b). Thus far, these compound heterozygous combinations have not been reported in either unaffected controls or CMT patients. The mutations are located at the highly conserved regions (Fig. 1c, d), and several in silico analyses suggested a pathogenic prediction of the *SH3TC2* mutations.

Several other rare heterozygous variants were found in *SH3TC2* (Supplementary Table 1). They were excluded from the genetic causes because of the deviation from the AR mode. However, if these variants exist in a homozygous or compound heterozygous state with other variants, their pathogenicity could not be ruled out.

### Clinical manifestations

The clinical features of the four patients are summarized in Table 2. Foot deformities, pes cavus, and walking difficulties were present in all the patients. No one showed lower limb proximal weakness. Areflexia was noticed in the early stages of the disease, but pathologic reflexes were not found. Mild scoliosis, cranial nerve involvement, and hearing loss were present in three patients. Disease severity according to the CMTNS varied from mild to moderate.

Nerve conduction velocities (NCVs) indicated demyelinating or intermediate type neuropathies. Median motor NCVs in the FC657 patient were 36.2–38.0 m/s, and the sural nerve biopsy findings revealed both axonal and demyelinating neuropathies, which indicate the intermediate type. Compound muscle action potentials of the lower limb muscles were considerably decreased compared to the upper limb muscles.

T1-weighted coronal MRIs of a patient (FC703) showed a fatty infiltration and atrophy in the distal leg muscles (Fig. 1e–j). Although the axial MRIs showed normal images at the hip and thigh levels (Fig. 1g, h), whole compartment muscles in the calf revealed fatty replacement (Fig. 1e, f). The T1 signal intensity was increased in the anterior muscle compartment of the lower leg which was accompanied by fatty infiltration. Hypertrophy was observed in both sciatic nerves, and these findings are appropriate for

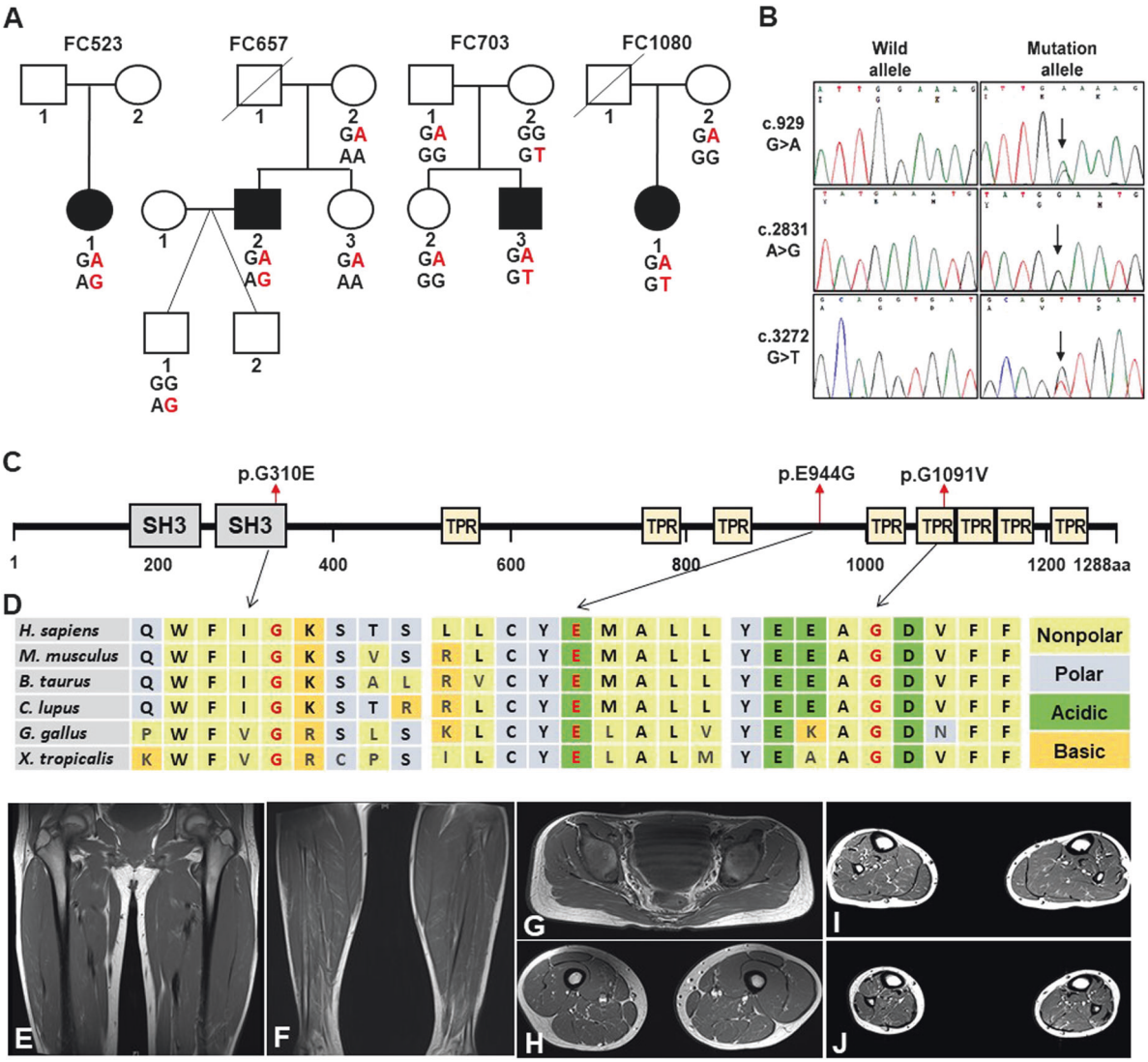


**Fig. 1** Charcot–Marie–Tooth disease type 4C (CMT4C) families with *SH3TC2* mutations. **a** Four Pedigrees of CMT4C families. Genotypes of the *SH3TC2* mutations are indicated at the bottom of all the examined family members. Filled and open symbols represent affected and unaffected individuals, respectively. **b** Sequencing chromatograms of the *SH3TC2* mutations. **c** Domain structure of the SH3TC2 protein. The three observed mutations are indicated by arrows (SH3: Src homology-3 domain, TPR: tetratricopeptide repeat motif). **d** Conservation of the amino acids at the mutation sites among several vertebrate species (*H. sapiens*: NP_078853.2, *M. musculus*: NP_766216.2, and *B. taurus*: XP_015327803.1, *C. lupus*: XP_022273537.1, *G. gallus*: XP_025010675.1, and *X. topicalis*: XP_002939154.2). **e**–**j** Hip, thigh, and calf MRIs of the FC703 patient (16 years old). **e**, **f** Coronal images of the thigh (**e**) and calf muscles (**f**). T1-weighted coronal leg MRIs of the lower extremities showed fatty replacement and muscle atrophies in the calf. Axial images of the hip (**g**) and thigh (**h**) muscles revealed no fatty replacements. Axial images at the upper third (**i**) and lower third calf (**j**) showed fatty streaks which were involved in the whole compartment calf muscles. Those findings were consistent with the patterns of muscle involvements in length dependent axonal degeneration

neuromuscular disease which may be related to neuromuscular junction changes in the CMT4C mouse model [14]. Although they had cranial nerve involvement, the brain MRI did not reveal any abnormalities in all four patients (data not shown). Semithin transverse sections from the distal sural nerve biopsy (FC703, 16 years) showed rather characteristic myelin abnormalities, with the loss of large myelinated fibers, and there was frequent evidence of onion bulb.

## Discussion

We identified two combinations of likely pathogenic compound heterozygous *SH3TC2* mutations in four CMT4C patients. The CMT4C frequency was calculated as 2.02% in the demyelinating or intermediate patients without *PMP22* duplication ($n = 198$), which is similar or slightly lower than patients in Japan (1.76%) [6] and Germany (5.2%) [5]. The frequencies were 0.39% in the total Korean CMT

**Table 2** Clinical characterization of the four patients with compound heterozygous *SH3TC2* mutations

| Item/Patient ID | FC523 | FC657 | FC703 | FC1080 |
|---|---|---|---|---|
| Mutation | G310E, E944G | G310E, E944G | G310E, G1091V | G310E, G1091V |
| Sex | Female | Male | Male | Female |
| Age at onset (years) | 30 | 38 | 10 | 8 |
| Disease duration (years) | 23 | 14 | 6 | 17 |
| Age at exam (years) | 53 | 52 | 16 | 25 |
| Muscle weakness[a] | | | | |
| Upper limb | + | + | + | + |
| Lower limb | ++ | + | + | ++ |
| Muscle atrophy[b] | U < L | L | L | U < L |
| Sensory loss | V > P | V > P | V = P | V > P |
| DTR | Absent | Absent | Absent | Absent |
| Scoliosis | Yes | No | Yes | Yes |
| Cranial nerve involvements | No | Deafness, diplopia, facial palsy | Deafness | Deafness, diplopia |
| Foot deformities | Yes | Yes | Yes | Yes |
| Wheel-chair bound | No | No | No | No |
| FDS/CMTNS | 2/15 | 2/11 | 2/11 | 2/12 |
| Brain MRI | Normal | Normal | Normal | Normal |
| Lower limb MRI | ND | ND | Fatty replacement | Fatty replacement |
| Sural nerve biopsy | ND | Mixed type[c] | Demyelinating type | ND |
| Nerve conduction study (Right/Left)[d] | | | | |
| Median nerve | | | | |
| CMAP (mV) | 7.2/7.4 | 10.3/11.3 | 12.1/15.5 | 11.3/18.4 |
| MNCV (m/s) | 30.0/30.1 | 38.0/36.2 | 27.0/27.8 | 36.9/34.9 |
| Peroneal nerve | | | | |
| CMAP (mV) | A/A | A/A | 0.2/2.1 | 2.6/1.8 |
| MNCV (m/s) | A/A | A/A | 13.8/17.5 | 28.1/30.6 |
| Sural nerve | | | | |
| SNAP (μV) | 2.9/1.6 | 7.0/A | 2.1/A | 7.1/7.1 |
| SNCV (m/s) | 35.6/31.4 | 32.6/A | 28.0/A | 33.3/35.0 |

*A* absent potential, *CMAP* compound muscle action potential, *CMTNS* CMT neuropathy score, *DTR* deep tendon reflexes, *FDS* functional disability scale, *MNCV* motor nerve conduction velocity, *MRI* magnetic resonance imaging, *ND* not done, *P* pain sense, *SNAP* sensory nerve action potential, *SNCV* sensory nerve conduction velocity, *V* vibration sense

[a]Upper limb: + indicates intrinsic hand weakness 4/5 on medical research council (MRC) scale. Lower limb: ++ indicates ankle dorsiflexion <4/5 on MRC scale

[b]U < L indicates lower limb predominant muscle atrophy, L indicates lower limb muscle atrophy alone

[c]Mixed type: both axonal and demyelinating neuropathies

[d]Normal nerve conduction velocities: median nerve ≥ 50.5 m/s, peroneal nerve ≥ 41.2 m/s, sural nerve ≥ 32.1 m/s; normal amplitude values: median nerve ≥ 6 mV, peroneal nerve ≥ 1.6 mV, sural nerve ≥ 6.0 μV

cohort ($n = 1035$), and 0.79% in the demyelinating and intermediate patients ($n = 504$). The CMT4C frequency in the Korean cohort was relatively lower than other groups: 0.8% in USA [15], 1.7% in Germany [5], 0.47% in Japan [6], and 0.56% in United Kingdom [16]. Moreover, no homozygous mutation was found in the Korean patients, which may be partly due to a strict legal prohibition of consanguineous marriage.

Onset ages in the two patients with p.G310E and p.G1091V were 8–10 years; however, the other two patients with p.G310E and p.E944G had quite a late onset (30 and 38 years, respectively). Spine deformities appeared comparably early, similarly to previous reports [1, 3, 4, 6]. Cranial nerve involvement was evident in three patients; however, it occurred independently from the disease duration and neuropathy severity. Although some studies have

suggested an early proximal involvement [1, 3], we could not find any proximal weakness. Based on the motor and sensory amplitudes, the lower limb nerves were definitively more compromised than the upper limb nerves, and the sensory nerves appeared to be more affected than the motor nerves. We found no correlation between nerve conduction slowing and disease duration which was also reported by other studies [1, 7, 16]. Interestingly, the FC703 patient showed electrophysiological and pathological evidence of intermediate type with variable degrees of axonal loss.

This study is the first report of Korean CMT4C families, and it will be helpful for molecular diagnoses of demyelinating and intermediate AR-CMT due to *SH3TC2* mutations.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.



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