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Case Report

A novel single-point mutation of NEFH and biallelic SACS mutation presenting as intermediate form Charcot-Marie-Tooth: A case report in Vietnam

Anh Tuan Truong[1], Anh Thi Lan Luong[2], Linh Hai Nguyen[3], Huong Van Nguyen[3], Diep Ngoc Nguyen[4], Ngoc Thi Minh Nguyen[2]

[1]Department of Clinical Medicine, Nam Dinh University of Nursing, Nam Dinh, [2]Department of Medical Biology and Genetics, Hanoi Medical University, [3]Department of Neurology, Hanoi Medical University, Hanoi, [4]Institute of Theoretical and Applied Research (ITAR), School of Medicine and Pharmacy, Duy Tan University, Da Nang, Vietnam.

E-mail: Anh Tuan Truong - anhtt@ndun.edu.vn; Anh Thi Lan Luong - luongthilananh@hmu.edu.vn; *Linh Hai Nguyen - linhnh95@gmail.com; Huong Van Nguyen - nguyenvanhuong@hmu.edu.vn; Diep Ngoc Nguyen - nguyenngocdiep7@duytan.edu.vn; Ngoc Thi Minh Nguyen - minhngoc1591@gmail.com

***Corresponding author:**
Linh Hai Nguyen,
Department of Neurology,
Hanoi Medical University,
Hanoi, Vietnam.

linhnh95@gmail.com

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ABSTRACT

Background: Charcot-Marie-Tooth disease (CMT) is among the most common group of inherited neuromuscular diseases. *SACS* mutations were demonstrated to cause autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). However, there have been few case reports regarding to *NEFH* and *SACS* gene mutation to CMT in Vietnamese patients, and the diagnosis of *CMT* and *ARSACS* in the clinical setting still overlapped.

Case Description: We report two patients presenting with sensorimotor neuropathy without cerebellar ataxia, spasticity and other neurological features, being diagnosed with intermediate form CMT by electrophysiological and clinical examination and neuroimaging. By whole-exome sequencing panel of two affected members, and PCR Sanger on *NEFH* and *SACS* genes to confirm the presence of selected variants on their parents, we identified a novel missense variant *NEFH c.1925C>T* (inherited from the mother) in an autosomal dominant heterozygous state, and two recessive *SACS* variants (*SACS c.13174C>T*, causing missense variant, and *SACS c.11343del*, causing frameshift variant) (inherited one from the mother and another from the father) in these two patients. Clinical and electrophysiological findings on these patients did not match classical ARSACS. To the best of our knowledge, this is the first case report of two affected siblings diagnosed with CMT carrying both a novel *NEFH* variant and biallelic *SACS* variants.

Conclusion: We concluded that this novel *NEFH* variant is likely benign, and biallelic *SACS* mutation (*c.13174C>T* and *c.11343del*) is likely pathogenic for intermediate form CMT. This study is also expected to emphasize the current knowledge of intermediate form CMT, ARSACS, and the phenotypic spectrum of *NEFH*-related and *SACS*-related disorders. We expect to give a new understanding of CMT; however, further research should be conducted to provide a more thorough knowledge of the pathogenesis of CMT in the future.

Keywords: Autosomal recessive spastic ataxia of Charlevoix-Saguenay, Intermediate Charcot-Marie-Tooth disease, NEFH, Novel variant, SACS

INTRODUCTION

Hereditary motor and sensory neuropathies, classically known as Charcot-Marie-Tooth disease (CMT), are among the most common group of inherited neuromuscular diseases. These

disorders are often characterized by progressive distal muscle atrophy and weakness, mild-to-moderate distal sensory loss, and foot deformities.[20] The pathogenesis was still unknown, and a variety of molecular genetics have been discovered to be pathogenic to this disease. In molecular genetics, these pathogenic genes play an important role in maintaining the structural integrity and functional integrity of neuronal elements. At present, no fewer than 80 genes have been reported to cause CMT and related disorders.[26] CMT was classified into demyelinating form (CMT1) and axonal form (CMT2) based on motor nerve conduction velocity (MCV), amplitudes, and sensory nerve action potentials. In which, CMT1 was defined as significantly reduced MCV (<38 m/s) with evidence of extensive segments on nerve biopsy; whereas, CMT2 was defined as normal or slightly reduced MCV, and significantly reduced amplitudes of motor and sensory nerve action potentials with evidence of axonal damage and/or slight demyelination on nerve biopsy.[10] In addition, intermediate CMT form was between those of typical CMT1 and CMT2, with MCV ranging from 35 to 45 m/s, and nerve biopsy showed the evidence of both demyelination and axonal degeneration. Intermediate form CMT was described in the literature as axonal CMT, autosomal dominant peripheral neuropathy, which mainly affects lower limbs (LLs), resulting in progressive muscle weakness, muscle atrophy and gait ataxia, distal sensory impairment, and less severe involvement of the upper limbs (ULs).[25] However, while searching international and national research databases, we found no epidemiological study reporting the prevalence of CMT in the Vietnamese population. The most relevant CMT report in a South-east Asian cohort of CMT patients in China was reported in 2019 by Chen *et al*, whose most prominent causative gene mutations among 138 CMT patients was located on *PMP22*, followed by *GJB1*, *MFN2*, and *MPZ*.[7]

Neurofilaments (NF) were the major scaffolding protein, with a diameter of 10 mm, almost exclusively expressed in the neuronal skeleton, classified into heavy (NEFH), medium (NEFM), and light (NEFL) chains based on the molecular weight. The NFs were formed in the neuronal cell body, and transported to the axons, forming the neuronal network structures to maintain the structural, functional, and electrical integrity of axons. Of those, *NEFL* gene mutation was confirmed to cause CMT1F and CMT2 with impaired axonal transport.[26] NEFM was responsible for the elongation of neuronal structures.[23] Recently, *in vivo* and rabbits, *NEFM* variants were associated with the susceptibility of Parkinson's disease and the occurrence of Alzheimer's disease.[11] Meanwhile, NEFH was responsible for the primary structural role in determining the type of axonal calibers. The literature demonstrated that NF accumulation, either alone or partly, plays a role in the pathogenesis of neurodegenerative central nervous system diseases (Amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease) and neurodegenerative peripheral nervous system diseases.

Furthermore, single nucleotide polymorphisms and mutation variants may explain the structural and functional changes associated with the increased risk of these diseases. Several reports showed the relation between *NEFH* as a biomarker of neuronal damage related to intermediate form CMT and ALS;[16] however, in this section, we mainly mention intermediate form CMT. The first case report of two families with intermediate form CMT, identified causative variant *NEFH c.3010_3011delGA* and *c.3017_3020dup*.[25] Since then, there were five reports on NEFH variants to be pathogenic for intermediate form CMT, located in the 3 kb and subsequent regions, which affected the 3'UTR area.[3,6,12,13,19] In addition, substitution variant on *NEFH* gene was reported to cause intermediate form CMT by Yan *et al*. in 2021 (*NEFH c.2215C>T* [p.Pro739Ser]).[29] These mutations could act as potential mechanisms for neurotoxicity, and axonal damage, accompanied by pyramidal signs overlapping with those of motor neuron diseases.

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is one rare neurodegenerative disorder which belongs to spastic ataxia syndrome. Spastic ataxia is characterized by the combination of cerebellar ataxia with spasticity and other pyramidal features. ARSACS (MIM: 270550) is characterized by early-onset spastic ataxia and sensorimotor neuropathy.[24] First discovered in a Quebec population, this type of ataxia was also reported in other non-Quebec populations worldwide, with no relation to the French origin, and becoming one of the most frequent causes of spastic ataxia.[4,14,27] ARSACS is caused by pathogenic mutations in *SACS* gene.

Sacsin (*SACS*) gene is located on chromosome 13, comprising 10 exons, encoding a large multidomain protein called sacsin. Sacsin is expressed in different tissues, notably in the central nervous system. In the brain, sacsin is most expressed in the motor system, including the granular cerebellar system and Purkinje cells.[22] SACS domain was shown to regulate intermediate filaments assembly; in animal models, SACS knockout mice exhibit early ataxia and abnormal bundling of NF in neuronal populations.[15]

Recently, the phenotypical spectrum of *SACS* variants has been expanded thanks to revolutions in genetic testing. Diverse clinical features are not only framed in the classic triad of ARSACS described in the literature (cerebellar ataxia, spasticity, and peripheral neuropathy), atypical features such as deafness, seizures, and mental retardation have been reported.[14,27] Atypical cases presenting with adult onset ataxia, CMT-like phenotype, or isolated spasticity were also reported.[27]

In the literature, inherited peripheral neuropathies (in which the most common entity is CMT) were described as the

disorder between Schwann cells and axons in peripheral nerves, resulting in abnormalities in myelin assembly and axonal transport, causing peripheral demyelination and peripheral axonopathy.[18] In 2015, Larivière *et al*. established a hypothesis to explain the pathogenesis of ARSACS on Sacs-knockout mice. Null-sacs models showed abnormal accumulation of non-phosphorylated NF bundles in vulnerable neuronal populations, first cerebellar Purkinje cells, then spinal motor neuron cells, and secondary peripheral neuropathy.[15] Therefore, the most significant difference in distinguishing CMT versus ARSACS is the pathogenic involvement of the central nervous system. However, it remains a challenge because of the unavailability of brain tissue from the affected patients. To date, cells and mouse models have been developed with ARSACS to research disease mechanisms in human conditions; however, the differences between humans and mice still show limitations in the ideal human simulation.

Concerning the overlap between ARSACS and CMT in the clinical setting to diagnose a patient presenting with hereditary sensorimotor neuropathy, herein, we report a case of intermediate form CMT regarding a highly pathogenic cause of biallelic SACS mutations and a likely benign novel NEFH variant and address several limitations during diagnosis due to restriction of condition in a low- and middle-income country like Vietnam. Thus, a genetic panel to aid in diagnosing CMT and neurological diseases, particularly in the clinical setting of developing countries, should be optimized.

CASE DESCRIPTION

Two patients (one female and one male) were two non-consanguineous siblings in one family with normal neuropsychological development, examined by two authors. Their family history, history, and history of exposure were unremarkable. Deceased or alive family members within three generations who had the same symptoms were not reported by the patients. Nerve conduction studies (NCS) and electromyography (EMG) were performed using standard techniques.

Definition

Definite conduction blocks were defined as a decrease of more than 50% in distal compound muscle action potential (CMAP) amplitude. Then, probable conduction blocks were defined as a decrease of 30–50% in distal CMAP amplitude.

DNA samples were extracted from peripheral blood, and whole-exome sequencing (WES) neuropathy multigene panel was performed on two affected members. Due to restriction of finance, we only utilized PCR Sanger sequencing to confirm the presence of preselected variants (NEFH variants and two SACS variants identified in the WES panel of two affected members) on their parents, who were in normal-phenotype.

A database of clinical variant alleles was utilized to search for the variants' allele frequencies and interpretation.

Written informed consent forms were obtained from the proband's family for participation in this case report, in agreement with local ethic committees of Hanoi Medical University Hospital, Vietnam, and with the 1964 Helsinki Declaration and its later amendments.

Clinical and neuroimaging findings

The pedigree in this paper is summarized in Figure 1. Clinical findings of the family members are displayed in Table 1.

Patient I2

The proband, a 24-year-old male, presented to our hospital with walking difficulties due to LL weakness. He reported no significant symptoms until he was 15; he first recognized slight LL weakness and progressive foot deformities, affecting his daily life and schooling unremarkably, so he did not go to the hospital for examination. At the age of 20, he developed weakness of predominantly distal LLs, weak bilateral high-arched feet, and unable to dorsiflex. At the last examination, he could walk and run slowly without aid. Neurological examination revealed distal muscle atrophy predominantly in the LLs; pes cavus, and hammer toes; distal muscle weakness of four limbs, predominantly LLs (UL: 4/5 at distal and 5/5 at proximal; whereas LL: 3/5 at distal and 4/5 at proximal); distal hypoesthesia in a stocking-and-glove

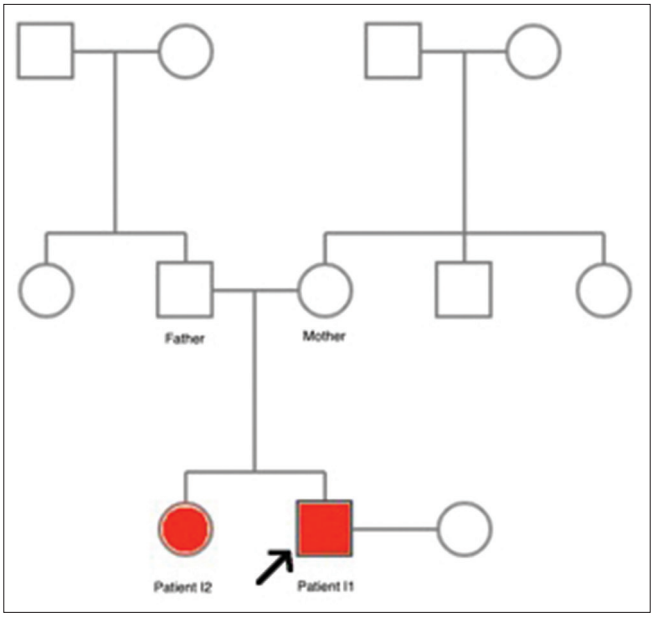

Figure 1: Three-generation pedigree of the family. The proband is indicated with black arrow. Squares indicate males, circles indicate females. Red for affected individuals, white for normal individuals.

Table 1: Summary of clinical findings and genetic findings in patients with *NEFH* and *SACS* variants.

Patient	Mother	Father	Elder sister	Proband
Gender	F	M	F	M
Age at onset (yr)	–	–	10	15
Age at examination and testing (yr)	61	63	41	24
Initial symptoms	Normal	Normal	Walking difficulties	Walking difficulties
Cognitive symptoms	–	–	–	–
Muscle wasting				
UL	–	–	–	–
LL	–	–	–/+	–
Muscle weakness				
Proximal UL	5/5	5/5	5/5	5/5
Distal UL	5/5	5/5	4/5	4/5
Proximal LL	5/5	5/5	3/5	4/5
Distal LL	5/5	5/5	2/5	3/5
Distal muscle atrophy				
UL	–	–	+	–
LL	–	–	+ (peroneal atrophy)	+ (peroneal atrophy)
Sensory loss (hypoesthesia) – pin, touch, vibration				
UL	+ (carpal tunnel syndrome)	–	+	+
LL	–	–	+	+
Deep tendon reflex				
UL	2+	2+	2+	2+
LL	2+	2+	1+	1+
Plantar reflex	–	–	–	–
Scioliosis	–	–	+	–
Pyramid signs				
UL	–	–	–	–
LL	–	–	–	–
Ataxia	–	–	–	–
Spasticity	–	–	–	–
Hearing loss	–	–	–	–
Pes cavus	–	–	+	+
Hammer toes	–	–	+	+
Neuro-opthalmological findings	–	–	–	–
Sensorineural hearing loss	–	–	–	–
Functional disability scale	0	0	4	2
Genetic testing				
NEFH c. 1925C>T	+	–	+	+
SACS c. 13174C>T	–	+	+	+
SACS c. 11343del	+	–	+	+

F: Female, M: Male, –: Absent, +: Present, yr: year, UL: Upper limb, LL: Lower limb. Functional Disability Scale from 0 to 8 (0 = normal, 1 = normal, but with cramp or fatigability, 2 = inability to run, 3 = walking is difficult but still possible unaided, 4 = able to walk with a cane, 5 = able to walk with crutches, 6 = able to walk with a walker, 7 = wheel chair bound, 8 = bedridden)

distribution; reduced deep tendon reflexes, no Babinski sign, no spasticity, and no cerebellar and gait ataxia [Figure 2]. Laboratory tests (CK, B12, HbA1c, metabolic disorders, and cerebrospinal fluid analysis) remained within normal range. Brain MRI revealed slight atrophy of superior cerebellar vermis and bilateral linear hypodensities on the T2-weighted sequence [Figure 3].

Patient I1

Until after the proband's first presentation to the hospital, we consulted him to bring all of his family members for a comprehensive neurological consultation. The proband's elder sister, a 41-year-old unmarried female, presented to the hospital with severe weakness in four limbs. She was unsure about the onset and dropped out of schooling at 16 because of financial burden. Neurological examination revealed atrophy of thenar eminence on both hands, pes cavus, hammer toes, Achilles contractures, and distal muscle weakness of four limbs. Laboratory tests remained within normal range. She presented with similar findings but more severe than her brother's. Their clinical examination images are also shown in [Figure 2].

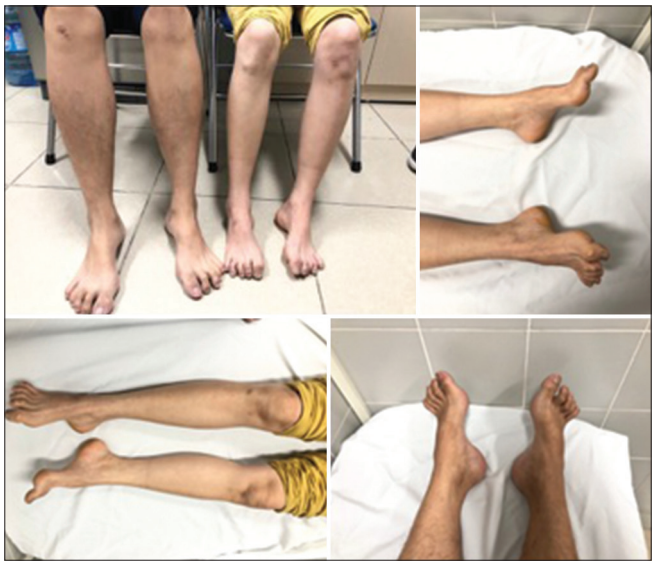

Figure 2: Clinical examination in both patients disclosed "inverted champagne bottle" appearance, pes cavus, and hammer toes, characterizing for Charcot-Marie-Tooth disease.

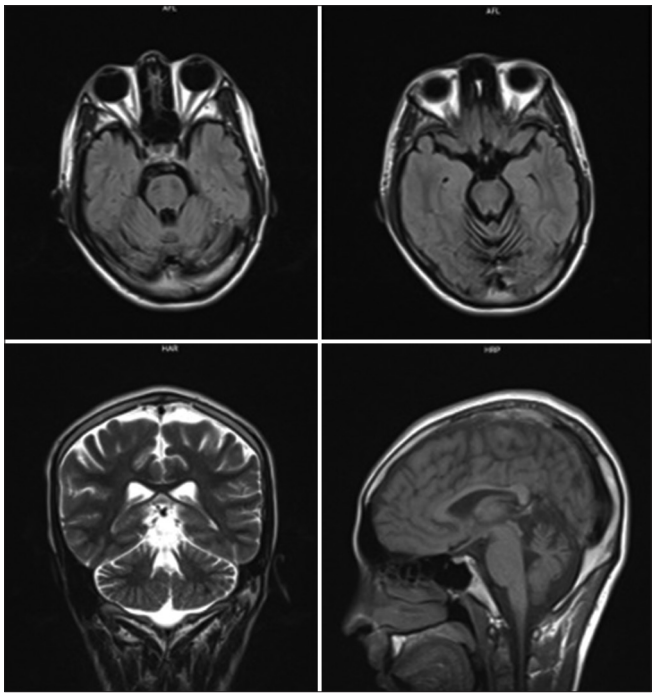

Figure 3: The proband's brain MRI revealed mild atrophy of superior cerebellar vermis, upper pons, and bilateral linear hypointensities in the pons on T2-weighted sequence.

Electrophysiological findings

A standardized electrophysiological study was performed on the entire family, showing unremarkable in both parents. The results of sensory and motor NCS and EMG in the affected members are displayed in [Table 2]. In motor NCS findings, both affected members showed moderately reduced median conduction velocities (38–43 m/s) and reduced amplitudes of CMAP in the median, ulnar, peroneal, and tibial nerves, predominantly in the LLs. EMG showed chronic neuropathogenic changes with polyphasic wide unit motor potentials, fibrillations, and positive sharp waves with reduced recruitment pattern.

Table 2: Neurophysiological findings of two patients.

Patient	I1 (Proband)	I2 (Sister)
MNC (DL/Amp/CV)		
L Median nerve	5.73/6.3/42	5.10/5.5/39
R Median nerve	5.96/6.2/39	5.21/5.4/38
L Ulnar nerve	3.85/4.6/43	3.69/6.6/44
R Ulnar nerve	3.95/4.5/41	3.75/6.7/42
L Peroneal nerve	NO	4.16/1.6/34
R Peroneal nerve	NO	3.95/1.5/35
L Tibial nerve	6.61/0.9/252	NO
R Tibial nerve	NO	7.92/1.0/27
SNC (DL/Amp)		
L Median nerve	NO	NO
R Median nerve	NO	NO
L Ulnar nerve	NO	NO
R Ulnar nerve	NO	NO
L Superficial peroneal nerve	NO	NO
R Superficial peroneal nerve	NO	NO
L Sural nerve	NO	NO
R Sural nerve	NO	NO
Needle EMG pattern	Reduced recruitment	Reduced recruitment

DL: Distal latency, CV: Conduction velocities, Amp: Amplitude, CMAP: Compound muscle action potential, MNC: Motor nerve conduction, SNC: Sensory nerve conduction, NO: Not obtained

Sural nerve biopsy

We could not perform nerve biopsy on these affected patients because this invasive diagnostic technique was not authorized in Vietnam. We are in the process of demanding permission to conduct this technique soon at our medical center, and we expect it to happen quickly.

WES

We carried out WES on the DNA of the proband and his elder sister, prioritizing variants of genes known to be responsible for CMT and neuromuscular disorders [Table 3]. The sequencing analysis was performed at the Department of Medical Genetics Laboratory, Hanoi Medical University (Hanoi, Vietnam). Each WES costs 300 USD. After this genetic analysis, the result showed that the proband and his elder sister had a novel heterozygous missense variant *NEFH c.1925C>T*, which indicates an autosomal dominant inheritance pattern, resulting in protein changes p.Thr642Met in exon 4.

Table 3: Targeted gene panel of neuromuscular disorders used in this study. (Method: Next-generation sequencing Nextseq, Illumina, USA).

Genes related to CMT diseases	Genes related to myopathy and muscular dystrophy
AARS, AIFM1, AMACR, ARHGEF10, ATL1, ATP7A, BAG3, BSCL2, CCT5, COX10, CTDP1, DCTN1, DHTKD1, DNM2, DNMT1, DST, DYNC1H1, EGR2, FAM134B, FBLN5, FGD4, FIG4, FXN, GAN, GARS, GDAP1, GJB1, GNE, HADHB, HARS, HINT1, HK1, HSPB1, HSPB8, IGHMBP2, IKBKAP, INF2, KARS, KIF1A, KIF1B, KIF5A, LDB3, LITAF, LMNA, LRSAM1, MED25, MFN2, MPZ, MTMR2, MYOT, NDRG1, NEFH, NEFL, NGF, NTRK1, PLEKHG5, PMP22, POLG, PRPS1, PRX, RAB7A, REEP1, SACS, SBF2, SCN9A, SETX, SH3TC2, SLC12A6, SMAD3, SPG11, SPTLC1, SPTLC2, SURF1, TFG, TRPV4, TTR, TYMP, VCP, WNK1, YARS, ZFYVE26.	*ACTA1, ANO5, ATP2A1, B3GALNT2, BAG3, CAPN3, CAV3, CFL2, COL4A1, COL4A2, COL6A1, COL6A2, COL6A3, CRYAB, DES, DMD, DNAJB6, DYSF, EMD, FHL1, FKRP, FKTN, FLNC, GAA, ISPD, ITGA7, KBTBD13, LAMA2, LARGE, LDB3, LMNA, MEGF10, MTM1, MYH7, MYOT, NEB, PNPLA2, POLG, POMT1, SGCA, SGCB, SGCD, SGCG, SYNE1, TCAP, TMEM43, TNNT1, TPM2, TPM3, TRIM32, TTN, VMA21, VPS13A*

CMT: Charcot-Marie-Tooth disease

- cDNA level: NM_021076.4: c.1925C>T
- gDNA level: Chr22 (29489565)
- Protein level: p.Thr642Met

We also identified two heterozygous SACS mutation (a missense mutation *SACS c.13174C>T* in exon 2, and a frame shift mutation *SACS c.11343del* in exon 2) from the probands's and his elder sister's SACS gene, both of which indicate the autosomal recessive inheritance pattern.

- cDNA level: NM_001278055.2: c.13174C>T
- gDNA level: Chr13 (23330261)
- Protein level: p.Pro4392Ser

And: cDNA level: NM_001278055.2: c.11343del

- gDNA level: Chr13 (23332092)
- Protein level: p.His3782IlefsTer15

Initially, we did not expect biallelic SACS mutations to be pathogenic due to the absence of clinical and electrophysiological findings supporting the diagnosis of spastic ataxia in these two patients. PCR Sanger on preselected *NEFH* and *SACS* variants for the proband's parents to confirm the presence of these variants, concluding that *SACS c.13174C>T* variant inherited from the father, and *NEFH c.1925C>T* and *SACS c.11343del* inherited from the mother [Figure 4].

We noticed that the affected patients carried *NEFH* variant and biallelic *SACS* variants while the normal-phenotype persons did not. Based on using bioinformatics variant analysis tools: Mutation Taster https://www.mutationtaster.org/, Polyphen2 http://genetics.bwh.harvard.edu/pph2/, PROVEAN, and SIFT http://provean.jcvi.org/seq_submit.php, we defined *NEFH* variant as likely benign. This *NEFH* mutation was not reported in ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), thus being considered as a novel variant. In addition, the report showed changes in *SACS* genes with two variants: *SACS c.13174C>T* (missense mutation) and *SACS c.11343del* (frameshift mutation) in biallelic state, suggesting pathogenic in this study.

SACS mRA and sacsin quantification

We notice that *SACS* mRA and sacsin quantification are unavailable in our laboratory.

DISCUSSION

Mutations in NF genes are recently reported to be the likely causative mutant of neurodegenerative diseases, peripheral neuropathies, anterior horn cell diseases, and Parkinson's disease.[1] Recent studies have revealed the relationship between NF and CMT. Recently, intermediate form CMT, whose causative mutation was identified as *NEFH* gene mutation in 2016, attracted more and more concern about its relationship and CMT pathogenesis. However, the *NEFH*-related clinical spectrums are still unclear – the similar attraction to *SACS*-related disorders. To date, there was no clear genotype-phenotype of neither *NEFH* mutations nor *SACS* mutations.[5]

In this case report, two affected members met the diagnostic criteria of intermediate form CMT, including: (1) regarding clinical features, symmetrical, distal motor and sensory involvement, predominantly in the LLs, absence of ataxia, absence of the upper motor neuron signs, absence of spasticity, and age of onset ranging from 3 to 43 years; (2) regarding electrophysiological findings, needle EMG and NCS consistent with both axonal and demyelinating lesion with distal motor and sensory axonal neuropathy, and moderately reduced MCV ranging from 38 to 42 m/s. These findings were consistent with the previous reports of intermediate form CMT.[2,3,12,13,19,21] (3) Vermeer *et al*. also reported that patients presented with SACS gene mutations showed a strikingly uniform and recognizable clinical phenotype of early-onset cerebellar ataxia (before the age of 13), LL spasticity, sensorimotor peripheral neuropathy, and/or cerebellar vermis atrophy on MRI scan.[28] (4) According to Synofzik *et al*., each clinical feature in ARSACS could be missing but characteristic MRI findings of ARSACS (extending to supratentorial regions and even to

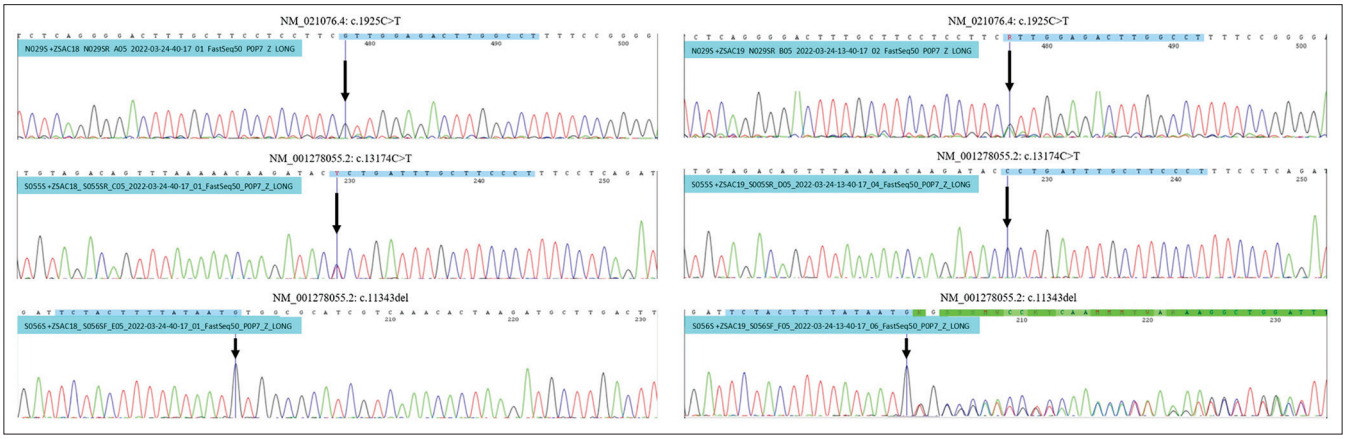

Figure 4: Analysis of PCR Sanger to confirm the presence of these variants on their parents revealed, both patients were inherited *NEFH c.1925C>T* variant in autosomal recessive trait, and *SACS c.11343del* variant from the mother. *SACS c.13174C>T* variant was inherited from the father. Left PCR belongs to the father, right PCR belongs to the mother.

the cerebral cortex) are still present.[27] Thus, we diagnosed these patients with intermediate form CMT on clinical and electrophysiological examination and neuroimaging.

After genetic testing of the family members, we also combine several bioinformatics tools to aid in diagnosis. We found that NEFH c.1925C>T in the proband and his elder sister was inherited from the normal phenotype mother, suggesting that this novel variant was likely benign. SACS variants in the proband and his elder sister were inherited in an autosomal recessive pattern. These affected members possessed biallelic SACS mutation. ClinVar database predicted this novel NEFH c.1925C>T variant was "benign," and the other SACS variants were "likely pathogenic." Hence, we safely conclude that the NEFH c.1925C>T variant identified in the proband's family was considered as "benign to CMT" and autosomal dominant trait, and biallelic SACS mutation was considered as "pathogenic to CMT" and autosomal recessive trait.

Most recent studies on CMT report the insertion or deletion fragments at 3 kb, which caused a frameshift and may affect the 3'UTR region on *NEFH* gene. One report by Yan *et al*. showed a single-point *NEFH* mutation causing CMT, suggesting the hypothesis that nucleotides encoded by *NEFH* near 2 kb may still alter final protein structures, resulting in neurological disorders.[29] However, *NEFH* single-point mutation identified in our report was likely benign and did not support this hypothesis.

In addition, several genetic mutations are shown to be prone to muscular damage, for example, *NEFL* mutations whose CK activity increases with axonal damage and correlates with muscle tissue changes. It was concluded that increased CK activity was often present with axonal phenotypes.[8,9] However, the data depicting the association between CK activity and specific mutations are still limited. In our study, the level of CK activity remained within normal values, suggesting that *NEFH* and *SACS*-associated clinical phenotypes may correlate with less axonal damage and muscle tissue damage than *NEFL*-associated clinical spectrums.

Concerning the definitive diagnosis of CMT or ARSACS with pure sensorimotor neuropathy (CMT-like ARSACS), ARSACS classically represented ataxia and spastic paraplegia. However, one report by Souza *et al.* reported two cases of unrelated patients with CMT carrying homozygous *SACS* mutation *c.11542_11544 del* [*p.Ile3848del*], and questioned that *SACS* gene mutation may cause early-onset pure axonal neuropathy, with classical neuroimaging findings, but without other neurological features, hence emphasizing the importance of neuroimaging in the definitive diagnosis of ARSACS versus CMT. Few studies reported ARSACS with pure axonal neuropathy and the role of *SACS* gene mutations in correlation with phenotype. In our case study, the affected patients also carried a novel *NEFH* variant. We were concerned whether the novel *NEFH c.1925C>T* may be genetic epistasis with biallelic *SACS c.13174C>T* and *SACS c.11343del* to CMT-like ARSACS is still unclear; thus, the further role of each variant should be investigated carefully.

A recent study in 2021 by Longo *et al.* has demonstrated that sacsin is absent in patients with ARSACS, and SACS mRNA is reduced in fibroblasts carrying variants. The authors proposed that the evaluation of sacsin levels should be included in clinical genetic practice to establish a definite ARSACS diagnosis. However, this quantification technique is still unavailable in our country and remains a challenge for our diagnosis.[17]

Due to the restriction of financial support for the study, only the proband and his elder sister underwent a WES panel of neuromuscular disorders (which cost 300 USD for each), the parents underwent *NEFH c.1925C>T* and

SACS c.13174C>T and *SACS c.11343del* site verification by PCR Sanger (which cost at a lower fee of 60 USD for each; however, the Department of Medical Genetics has sponsored these performances). Moreover, only two members in one generation within three generations were affected, and we still have to wait for the clinical phenotype of the proband's child (because his elder sister decided not to marry and give birth). Therefore, the genetic information was insufficient, and whether the above three variants were family-inherited or distinct cannot be determined. The relevant clinical evidence cannot support the variants' pathogenicity to intermediate form CMT. We will follow-up with the proband's family for predicted intermediate form CMT on his child, practice medical genetic consultation on the proband's couple, and screen for other diseases which may sprout in the future.

For the drawbacks of our study, because the variant is rare, and only two among four family members were reported with CMT, the sample size was too small to implicate the variant pathogenic effect from a genetic point of view. Second, we could not analyze the proband's parents to discover further variants other than three pointed mutations from the proband and his elder sister. Third, the study did not implement a sural nerve biopsy due to current unavailability and authorization in Vietnam. Forth, the diagnosis of CMT and ARSACS in a clinical setting remains a challenge. Although analysis of several reports on the association of *NEFH* mutations and intermediate CMT was reviewed above to suggest that biallelic *SACS* mutation may play a role in the pathogenesis of CMT, it is still challenging to explain the pathogenesis of CMT and related disorders.

CONCLUSION

Our study was the first report in Vietnam associated with intermediate form CMT, which emphasizes the importance of genetic testing and neuroimaging to aid in the diagnosis of CMT versus ARSACS in patients presenting with pure sensorimotor neuropathy, and expects to expand the current understanding about NEFH and SACS-related clinical spectrums. It is also the first report of two patients with CMT presenting with a novel NEFH variant with biallelic SACS mutations, raising concern about genetic epistasis in the pathophysiological mechanism of CMT. Further studies should be conducted to diagnose and reveal the exact pathogenesis of these diseases.

Ethical standards

This study was approved by the Institutional Review Board of Hanoi Medical University and informed consents were obtained from all participating members.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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