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# Distal Myopathy with Rimmed Vacuoles Confirmed by Whole Exome Sequencing

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Distal myopathy with rimmed vacuoles (DMRV) or hereditary inclusion body myopathy 2 is an autosomal recessive muscular disorder characterized by early adult-onset weakness of distal muscles and rimmed vacuoles in muscle biopsy. Mutations in the *UDP-N-acetylglucosamine 2-epimerase/N-ace-tylmannosamine kinase* (*GNE*) gene are associated with the development of DMRV. In this study, whole exome sequencing (WES) revealed compound heterozygous *GNE* mutations of p.Asp176Val and p.Val572Leu in a patient with distal limb weakness. Three hundred healthy controls did not show these mutations. All other variants found in distal myopathy-relevant genes were polymorphic. These findings confirmed that the patient had DMRV. This work underscores the usefulness of WES in improving the molecular diagnosis of myopathy.

**Key words** : Distal myopathy with rimmed vacuoles (DMRV), molecular diagnosis, UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE), whole exome sequencing (WES)

## Introduction

Distal myopathies are hereditary muscle disorders defined by onset of muscle weakness and atrophy in hands or feet [28]. They include a wide variety of diseases, such as Welander distal myopathy, tibial muscular dystrophy, myofibrillar myopathy, Miyoshi myopathy, and distal myopathy with rimmed vacuoles (DMRV). For the diagnosis of distal myopathies, a serial approach is generally used. First, patients are classified according to age of onset, inheritance pattern, and clinical course. Second, histopathological analysis of muscle biopsy, especially immunohistochemistry, is used.

Prior to the advance of next generation sequencing, Sanger sequencing for the coding exons of genes reported to cause the respective phenotypes was usually done to screen for the exact causative mutation. However, the overlapping phenotypes or clinical-genetical heterogeneities make this screening of possible candidate genes elusive [3, 25]. For instance, genes like dysferlin and myotillin are associated with both distal myopathies and limb girdle muscular dystrophies [7, 22, 23]. Recently, next-generation sequencing has made it possible to cost-effectively and rapidly sequence the protein-coding exons of the genome, by a process termed 'whole exome sequencing (WES)'. The use of WES has identified the causative genetic defect in many monogenic diseases including Welander distal myopathy [2, 4, 12].

The *UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase* (*GNE)* gene on chromosome 9p13.3 encodes the bifunctional rate limiting enzyme for the sialic acid biosynthetic pathway by initiating and regulating the biosynthesis of N-acetlyneuraminic acid (NeuAc) a precursor of sialic acid [8, 10]. The *GNE* gene is ubiquitously expressed and has two functional domains: the epimerase and the kinase domains located in the N-terminus encoding the N-actylglucosamine 2 epimerase and the C-terminus encoding the N-acetylmannosamine kinase, respectively [14, 19]. Mutations in *GNE* have been linked to not only DMRV (MIM 605820) [27, 29] but also sialuria (MIM 269921) [13, 24]. In particular, many mutations of *GNE* have been reported to be the underlying causes of DMRV [6, 9, 11, 14, 15, 18, 29].

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In the present study, we report the clinical and genetic diagnosis of a Korean patient with undetermined distal myopathy type using WES, which revealed a pair of compound heterozygous mutations in the *GNE* gene and the myopathy type.

## Materials and Methods

### Subjects

We enrolled a Korean family with one patient with distal myopathy and two healthy individuals (family ID: FC532, Fig. 1A). Korean healthy controls with no familial history of neuromuscular disorders (n=300) were also recruited. Paternity was confirmed by genotyping of 15 microsatellites using the PowerPlex 16 System (Promega, Madison, WI, USA). Written informed consent was obtained from all participants according to the protocol approved by the Institutional Review Board for Ewha Womans University, Mokdong Hospital.

### Clinical and electrophysiological assessments

The patient was examined for mental function, cranial nerve dysfunction, motor and sensory impairments, presence of contractures, deep tendon reflexes, and muscle atrophy. The strength of flexor and extensor muscles were assessed manually using the Medical Research Council (MRC) scale. Serum creatine kinase (CK) levels were measured. Nerve conduction studies (NCS) and needle electromyographies (EMG) were performed by standard methods [21].

### Exome sequencing and identification of causative mutation

The exome for the patient (II-1) was captured using the Human SeqCap EZ Human Exome Library v3.0 (Roche/NimbleGen, Madison, WI, USA). Captured DNA was sequenced on the HiSeq 2000 Genome Analyzer (Illumina, San Diego, CA, USA). Sequences were mapped/aligned to the reference human genome (GRCh37, UCSC hg19) using BWA (http://bio- bwa.sourceforge.net/) via a pileup file from the BAM file. Variant calling was performed using the SAMtools (http://samtools.sourceforge.net/) and GATK programs (http://www.broadinstitute.org/gatk/). Variants were submitted to ANNOVAR (http://www.openbioinformatics.org/annovar/) for functional annotation. Single nucleotide polymorphisms (SNPs) with a quality value >20 were considered a true variant call.

Registered, novel, or uncommon variants (minor allele frequency$\leq$0.01) in dbSNP138 (http://www.ncbi.nlm.nih.gov), the 1000 Genomes project database (http://www.1000genomes.org/), and Exome Variant Server (http://evs.gs.washington.edu/EVS/) were examined. All variants present in reported myopathy genes were sorted. Candidate variants considered as causative were confirmed by Sanger's sequencing method using an ABI 3100XL automatic sequencer (Applied Biosystems, Foster City, CA, USA). Mutations were considered to be an underlying cause when they were detected only in the affected member of the family and not detected in more than 300 healthy controls.

### *In silico* analysis

The affection of protein function due to amino acid substitution were assessed using SIFT (http://sift.jcvi.org/) and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/); and protein stability by MUpro (http://mupro.proteomics.ics.uci.edu/). The conservation pattern of the amino acid positions were done by multiple sequence alignment of protein sequences with MEGA5 software (http://www.megasoftware.net/). The genomic evolutionary rate profiling (GERP) scores (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html) of the nucleotide positions were also assessed.

## Results

### Clinical manifestations and electrophysiological features

The proband (II-1) was a 38-year-old woman who presented with slowly progressive distal muscle weakness. At the age of 35 years, she experienced frequent falling and noticed muscle weakness and atrophy of the distal lower limbs. Within one year, she noticed muscle weakness of bilateral hands. She denied any other medical diseases. Family history was unremarkable (Fig. 1A). When we examined her at age 45, distal muscles of upper and lower limbs were more severely affected than proximal muscles. Deep tendon reflexes are reduced. Pain and vibration sense was intact. Serum CK level was 350 IU/L (normal range: <170/L). NCS and EMG showed a generalized myogenic process with distal accentuation. Based on clinical, laboratory, and electrophysiological features, she was diagnosed with distal myopathy. However, we did not determine candidate genes

Table 1. Whole exome sequencing analysis

| Items | |
|---|---|
| Total yields (Gbp) | 7.95 |
| Mappable reads (%) | 99.4 |
| Target coverage (≥10X, %) | 89.7 |
| Total SNP number | 101,743 |
| Coding SNP number | 22,084 |
| Total indel number | 8,410 |
| Coding indel number | 317 |
| Myopathy gene variants[a] | 49 |

[a]Functionally significant variants include nonsynonymous, splicing site, frameshift, stop gain, stop loss, and coding indels.

for mutational analysis due to small-sized pedigree and non-specific clinical presentation. Therefore, we performed WES.

### Identification of a compound heterozygous missense mutation in *GNE* gene

The summary of whole exome sequencing data is outlined in Table 1. From the exome data, 49 variants were found in known myopathy genes (24 genes). Within these variants, capillary sequencing analysis of the extended family members detected a pair of compound heterozygous mutations in *GNE* (NM_005476.5), c.527A>T (p.Asp176Val, paternal origin), and c.1714G>C (p.Val572Leu, maternal origin) that perfectly co-segregated within the family in a recessive pattern (Fig. 1A, Fig. 1B). The c.527A>T (p.Asp176Val) and c.1714G>C (p.Val572Leu) lie in the highly conserved sites of the epimerase and kinase domains of GNE protein, respectively. These mutations have been previously reported to cause DMRV, and are the most common mutations in Japanese patients [2, 22]. None of the 300 healthy controls harbored these mutations. Both mutations were reported in the dbSNP137 but not in 1000 Genome Database and Exome Variant Server (EVS). All *in silico* predictions (SIFT, PolyPhen2, MUpro, and GERP) yielded commendable results (Table 2) and the amino acid positions were well conserved throughout different vertebrate species (Fig. 1C).

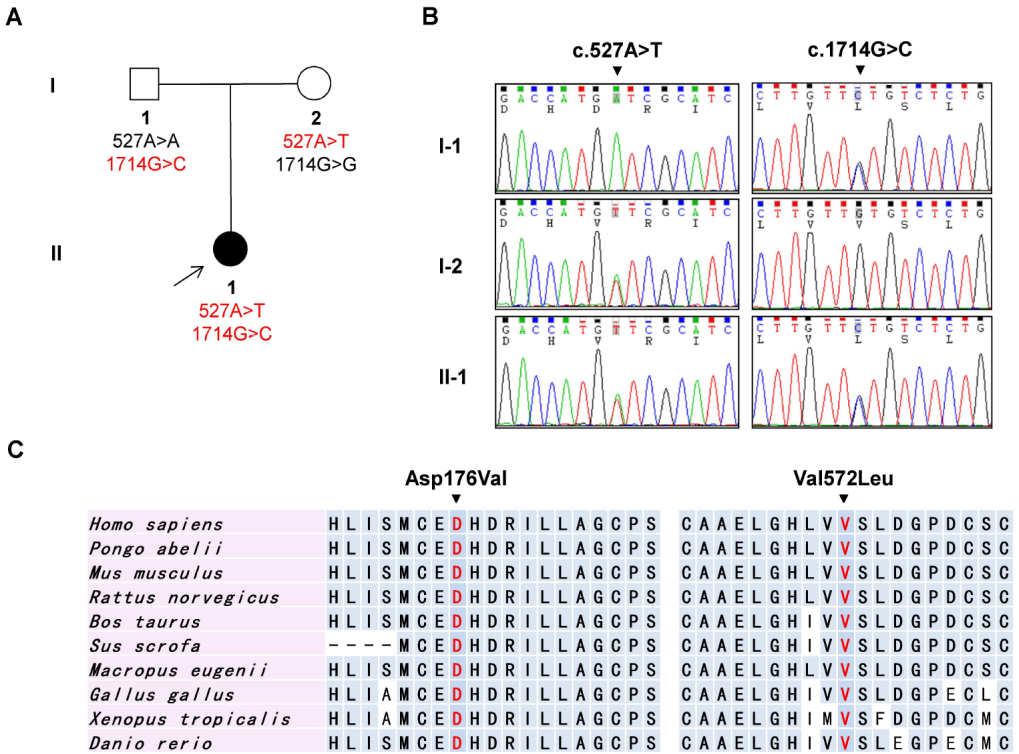


Fig. 1. Pedigree, sequencing chromatograms, and conservation profile. (A) Pedigree of the FC532 DMRV family. The proband is indicated by an arrow. Filled symbol indicates affection and open symbols indicate unaffected members. Genotypes of two *GNE* mutations are denoted at bottom of the each family member. (B) Sequencing chromatograms of *GNE* mutations c.527A>T (p.Asp176Val) and c.1714G>C (p.Val572Leu). The patient reveals both mutations, whereas the proband's parents have only a mutation each (I-1: c.1714G>C and I-2: c.527A>T). (C) Conservation analysis results. The mutation sites were well conserved among the subset of species studied and lay in the UDP-N-acetylglucosamine 2-epimerase domain and N-acetylmannosamine kinase domain, respectively.

Table 2. Mutations of *GNE* gene in the distal myopathy patient

| Gene | Mutation | | Domain | GERP[a] | *In silico* analysis[b] | | |
|---|---|---|---|---|---|---|---|
| | Nucleotide | Amino acid | | | SIFT | Polyphen2 | MUpro |
| *GNE* | c.527A>T | D176V | GT1-UDP-GlcNAc 2-epimerase domain | 5.67 | 0.07 | 1.00* | -0.11* |
| | c.1714G>C | V572L | Kinase domain | 5.75 | 0.00* | 0.92* | -0.356* |

[a]Genomic evolutionary rate profiling score
[b]SIFT score ≤0.05, PolyPhen2 score ~1, and MUpro scores <0 indicate a prediction of pathogenicity
*Denotes a "pathogenic" prediction

Thus, p.Asp176Val and p.Val572Leu mutations in *GNE* were determined as the underlying cause of our patient.

In addition to the two causative *GNE* mutations, many polymorphic or rare nonsynonymous variants were identified in a large number of myopathy-related genes from the exome data of the proband (Table 3). However, they were not considered as underlying causes because they met at least one of the following conditions: 1) noncosegregation with affected individuals within pedigrees, 2) same variant was found in controls, or 3) inconsistency in the inheritance manner for corresponding genes.

## Discussion

By WES analysis, we identified a set of compound heterozygous mutations at c.527A>T and c.1714G>C in the *GNE* gene in a patient with undetermined distal myopathy. These mutations lie in both the highly conserved bifunctional domains of the UDP-N-acetylglucosamine 2 epimerase/N-acetylmannosamine kinase enzyme: c.527A>T (p.Asp176Val) in the epimerase domain and c.1714G>C (p.Val572Leu) in the kinase domain. The co-segregation, absence of the same mutations in control samples, *in silico* predictions, and well conserved patterns leads us to affirm that the two compound heterozygous *GNE* mutations are the underlying cause of DMRV in this patient.

DMRV is also known as Nonaka myopathy, GNE myopathy, or hereditary inclusion body myopathy. It is an autosomal recessive distal myopathy caused by the alterations in the *GNE* gene [5]. This disease generally develops in early adulthood and is clinically characterized by preferential involvement of ankle dorsiflexors [1, 20]. In addition, muscle pathology typically reveals muscle fiber atrophy with rimmed vacuoles and intracellular congophilic deposits [19]. These characteristic clinical and pathologic findings are important for the initial suspicion of DMRV. However, the diagnosis of DMRV is not always easy. Expansion of mutational analysis in *GNE* gene has indicated that DMRV patients often have an atypical clinical presentation that includes proximal muscle weakness [16, 26]. Muscle biopsy is necessary for histopathological evaluation, but is a very invasive method. In addition, there have been several instances where patients clinically and pathologically compatible with DMRV were subsequently genetically diagnosed with other myopathies [25]. Our patient showed no preferential involvement of ankle dorsiflexors and refused muscle biopsy. Therefore, we did not suspect her case as a DMRV before the WES analysis.

WES is an effective strategy for discovering the underlying genetic defect in monogenic disorders because more than 90% of the pathogenic mutations of monogenic disorders are found in exons [2]. This technology currently has several limitations. These include shorter read lengths compared to the Sanger method, ambiguity in alignment, assembly in repetitive nucleotide regions, and large volume of data [17]. Despite these limitations, WES is an attractive strategy to diagnose genetic disease by a minimally invasive method.

In conclusion, we were able to find the exact genetic cause and designate the myopathy type of an undetermined distal myopathy patient using WES. Although no novel mutations were found, we were able to give a mutualistic clinical- genetic diagnosis without the involvement of muscle biopsy, which is otherwise an invasive method. This work underscores the usefulness of WES for the diagnosis of myopathy.

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Table 3. Nonsynonymous variants in distal myopathy-related genes from exome sequencing

| Gene | Chr:position | Mutation | | dbSNP137 | 1000G | Description[a] |
|---|---|---|---|---|---|---|
| | | Nucleotide | Amino acid | | | |
| *SEPN1* | 1:26131654 | c.425G>A | C142Y | rs7349185 | 0.83 | poly |
| | 1:26140573 | c.1506C>A | N502K | rs2294228 | 0.71 | poly |
| *NEB* | 2:152346965 | c.25216_25218del | 8406_8406del | . | . | NS |
| | 2:152352843 | c.G24433C | A8145P | rs7575451 | 0.68 | poly |
| | 2:152422076 | c.G18305C | R6102T | rs2288210 | 0.7 | poly |
| | 2:152436012 | c.A16544C | K5515T | rs62174690 | . | poly |
| | 2:152490458 | c.T9124C | C3042R | rs6710212 | 0.98 | poly |
| | 2:152496526 | c.T8734C | S2912P | rs6713162 | 0.42 | poly |
| | 2:152527608 | c.G4435A | V1479I | rs34577613 | 0.32 | poly |
| *TTN* | 2:179397561 | c.G76586A | R25529H | rs3829747 | 0.19 | poly |
| | 2:179406191 | c.G70418A | R23473H | rs3731749 | 0.19 | poly |
| | 2:179421694 | c.T60992C | I20331T | rs9808377 | 0.45 | poly |
| | 2:179427536 | c.A56128G | I18710V | rs3829746 | 0.45 | poly |
| | 2:179430997 | c.C52667T | T17556M | rs3731746 | 0.32 | poly |
| | 2:179434160 | c.G49504T | V16502F | rs3813244 | 0 | poly |
| | 2:179436020 | c.C47644T | R15882C | rs744426 | 0.19 | poly |
| | 2:179444768 | c.G40051C | A13351P | rs4145333 | 0.99 | poly |
| | 2:179444939 | c.G39880A | V13294I | rs2303838 | 0.33 | poly |
| | 2:179451420 | c.C37013T | T12338I | rs2042996 | 0.45 | poly |
| | 2:179457147 | c.C32390T | P10797L | rs16866406 | 0.19 | poly |
| | 2:179458591 | c.G31241A | R10414H | rs2288569 | 0.19 | poly |
| | 2:179464527 | c.A28906G | N9636D | rs1001238 | 0.46 | poly |
| | 2:179558366 | c.A27832G | I9278V | rs2042995 | 0.42 | poly |
| | 2:179579093 | c.A22676G | N7559S | rs12693164 | 0.23 | poly |
| | 2:179581835 | c.G21894T | Q7298H | rs2562832 | 0.07 | poly |
| | 2:179582327 | c.G21542A | S7181N | rs13390491 | 0.23 | poly |
| | 2:179583496 | c.A20699C | E6900A | rs16866465 | 0.23 | poly |
| | 2:179600648 | c.G10793A | R3598K | rs2742347 | 0.14 | poly |
| | 2:179604160 | c.A12711C | L4237F | rs1883085 | 0.09 | poly |
| | 2:179604366 | c.A12505C | T4169P | rs2562829 | 0.09 | poly |
| | 2:179606538 | c.C10333T | P3445S | rs2627037 | 0.22 | poly |
| | 2:179611711 | c.G15416T | R5139M | rs66677602 | 0.09 | poly |
| | 2:179615654 | c.G11473T | G3825C | rs138440219 | 0 | poly |
| | 2:179615887 | c.A11240G | D3747G | rs922984 | 0.78 | poly |
| | 2:179615931 | c.G11196C | L3732F | rs922985 | 0.98 | poly |
| | 2:179620951 | c.G10739A | G3580D | rs7585334 | 0.84 | poly |
| | 2:179621477 | c.G10213A | A3405T | rs6433728 | 1 | poly |
| | 2:179623758 | c.G10118A | S3373N | rs2291310 | 0.84 | poly |
| | 2:179629461 | c.G9643A | V3215M | rs2291311 | 0.84 | poly |
| | 2:179634421 | c.A8749C | T2917P | rs200875815 | . | poly |
| | 2:179637861 | c.G7692C | M2564I | rs56142888 | 0.09 | poly |
| | 2:179644035 | c.C3746T | S1249L | rs1552280 | 0.93 | poly |
| | 2:179659681 | c.G1213A | A405T | rs112266780 | . | poly |
| | 2:179659912 | c.C982T | R328C | rs16866538 | 0.23 | poly |
| *MYOT* | 5:137206560 | c.A220C | K74Q | rs41431944 | 0.99 | poly |
| *ANO5* | 11:22271870 | c.A963T | L321F | rs7481951 | 0.35 | poly |
| *COL6A1* | 21:47423040 | c.A2441G | K814R | rs11553518 | 0.04 | poly |

[a]Pol: polymorphic; NS: nonsegregated with affected individual

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## 초록 : Rimmed vacuole을 가진 원위부 근육병증의 전체 엑솜 서열분석을 이용한 유전적 원인 규명

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Rimed vacuole을 가진 원위 근육병(distal myopathy with rimmed vacuoles, DMRV)은 제2형 유전성 봉입체 근육병으로도 불리며 초기 성인기에 발병하여 원위부의 근력약화를 보이는 임상양상과 rimmed vacuole의 근육병리소견을 특징으로 하는 상염색체 열성의 근육병이다. 이러한 DMRV의 원인은 *UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase* (*GNE*) 유전자의 돌연변이임이 밝혀져 있다. 저자들은 원위부 근력약화를 호소하는 환자에서 전체 엑솜 염기서열분석을 이용하여 *GNE* 유전자의 복합 이형접합성 돌연변이(p.Asp176Val 및 p.Val572Leu)를 확인하여 DMRV를 진단할 수 있었다. 본 연구는 근육병의 정확한 분자진단에 있어서 전체 엑솜 염기서열분석의 유용성을 보여주었기에 이를 보고하는 바이다.