ISSN (Online) 2287-3406
Journal of Life Science **2020** Vol. 30. No. 8. 672~679 DOI : https://doi.org/10.5352/JLS.2020.30.8.672

A Novel PHKA1 Mutation in a Patient with Glycogen Storage Disease Type IXD

Hye Jin Kim[1], Soo Hyun Nam[2,3], Sang Beom Kim[4], Ki Wha Chung[5]* and Byung-Ok Choi[1,2,3]*

[1]*Department of Health Sciences and Technology, Samsung Advanced Institute for Health Science & Tech., Sungkyunkwan University, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea*
[2]*Stem Cell & Regenerative Medicine Institute, Samsung Medical Center, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea*
[3]*Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea*
[4]*Department of Neurology, Kyung Hee University Hospital at Gangdong, Kyung Hee University School of Medicine, Seoul 05278, Korea*
[5]*Department of Biological Science, Kongju National University, Gongju 32588, Korea*

Received April 22, 2020 / Revised June 29, 2020 / Accepted August 4, 2020

Distal myopathy is a clinically and genetically heterogeneous group of degenerative diseases of the distal muscle. Glycogen storage disease type IXD (GSD9D) is a metabolic distal myopathy characterized by muscle deficiency of phosphorylase kinase, a key regulatory enzyme in glycogen metabolism. Affected individuals may develop muscle weakness, degeneration, and cramps, as well as abnormal muscle pain and stiffness after exercise. It has been reported that mutations in the PHKA1 gene which encodes the alpha subunit of muscle phosphorylase kinase cause GSD9D. In this study, we examined a Korean GSD9D family with a c.3314T>C (p.I1105T) mutation in the PHKA1 gene. This mutation has not been previously reported in any mutation database nor was it found in 500 healthy controls. The mutation region is well conserved in various other species, and in silico analysis predicts that it is likely to be pathogenic. To date, only seven mutations in the PHKA1 gene have been documented, and this is the first report of Korean GSD9D patients. This study also describes and compares the clinical symptoms and pathological conditions of previously reported cases and these Korean patients. We believe that our findings will be useful for the molecular diagnosis of GSD9D.

Key words : Distal myopathy, Glycogen storage disease type 9D, MRI, mutation, *PHKA1*

Introduction

Distal myopathy is a degenerative disease of the distal muscle and is a clinically and genetically heterogeneous group [7]. Distal myopathy is usually classified as Nonaka, Miyoshi, Laing, Welander, Udd and Markesbery-Griggs distal myopathy [7]. Metabolic myopathy is a myopathy that causes distal weakness [14]. Glycogen storage disease type 9D (GSD9D, OMIM 300599) is one of the metabolic myopathies caused by the lack of decomposition of glycogen due to the deficiency of Phosphorylase kinase (PhK), so that sufficient energy for muscle contraction cannot be obtained [18, 20]. GSD9D belongs to a mild metabolic disorder, and is characterized by progressive muscle weakness, exercise intolerance and cramps, and extensive death in muscle tissue [1, 17, 20, 21]. Most patients develop symptoms as adults and mainly show weakness in the distal muscles and muscle atrophy [1, 17, 20, 21].

Phosphorylase kinase is composed of four homotetramers: α (PHKA1, PHKA2), β (PHKB), γ (PHKG1, PHKG2), and δ (CALM1) [2, 4, 10, 11-13, 16, 19]. The γ subunit acts as a catalyst and is regulated by the α and β subunits, and δ is calmodulin, which gives the enzyme Ca^{2+} sensitivity [2]. There are two types of α subunits, liver-specific PHKA2 and muscle-specific PHKA1, and both genes exist on the X chromosome [11, 20]. To date, over 100 PHKA2 mutations in HGMD (http://www.hgmd.cf.ac.uk/ac/index.php) have been reported to cause GSD9A. It has also been reported in Korea [5]. However, only 7 mutations in *PHKA1* have been reported to cause GSD9D and have not been reported in Korea [1, 3, 8, 10, 15, 18, 20, 21].

We investigated patients with GSD9D who visited the hospital suspected of distal hereditary motor neuronopathies (dHMN) in another hospital. dHMN does not show any damage to the sensory nerves, and only the motor nerves have abnormalities, so it shows symptoms of weakness in

***Corresponding authors**
Tel : +82-2-2148-9496, Fax : 82-2-3410-0052
E-mail : bochoi@skku.edu (Byung-Ok Choi)
kwchung@kongju.ac.kr (Ki Wha Chung)

the distal muscles [9]. Distal myopathy also indicates weakness in the distal muscles and muscle atrophy, making dHMN difficult to distinguish clinically from distal myopathy. Therefore, it is very necessary to distinguish dHMN from distal myopathy when the patient visits with distal muscular weakness and gait disorder. Because dHMN and distal myopathy are caused by mutations in different genes, genetic testing is a good way to distinguish these two diseases.

Therefore, we performed genetic analysis in Korean GSD9D patients and found *PHKA1* mutation. In addition, the patient's clinical symptoms, pathological features, and MRI results were described. Accordingly, we attempted to compare the mutations and clinical patterns of patients enrolled in this study and several previously reported GSD9D patients.

Materials and Methods

Patients

This study enrolled a X-linked recessive Korean family with GSD9D (FC975, Fig. 1A). This study also included 500 healthy controls who had no clinical features or family history of distal myopathy, which was confirmed after careful clinical and electrophysiological examinations. Written informed consent was obtained from all participants according to the protocol approved by the institutional review board for Sungkyunkwan University, Samsung Medical Center.

Exome sequencing and filtering of variants

Exome sequencing was performed with the Human SeqCap EZ Human Exome Library v3.0 (Roche/NimbleGen, Madison, WI), and the HiSeq2500 Genome Analyzer (Illumina, San Diego, CA) for 1 sample from the proband of FC975. The University of California, Santa Cruz assembly hg19 was the reference sequence. We selected functionally significant variants (missense, nonsense, exonic indel, and splicing site variants) from the whole exome sequencing data, and then variants registered as novel or uncommon variants (minor allele frequencies ≤ 0.01) in dbSNP150 (http://www.ncbi.

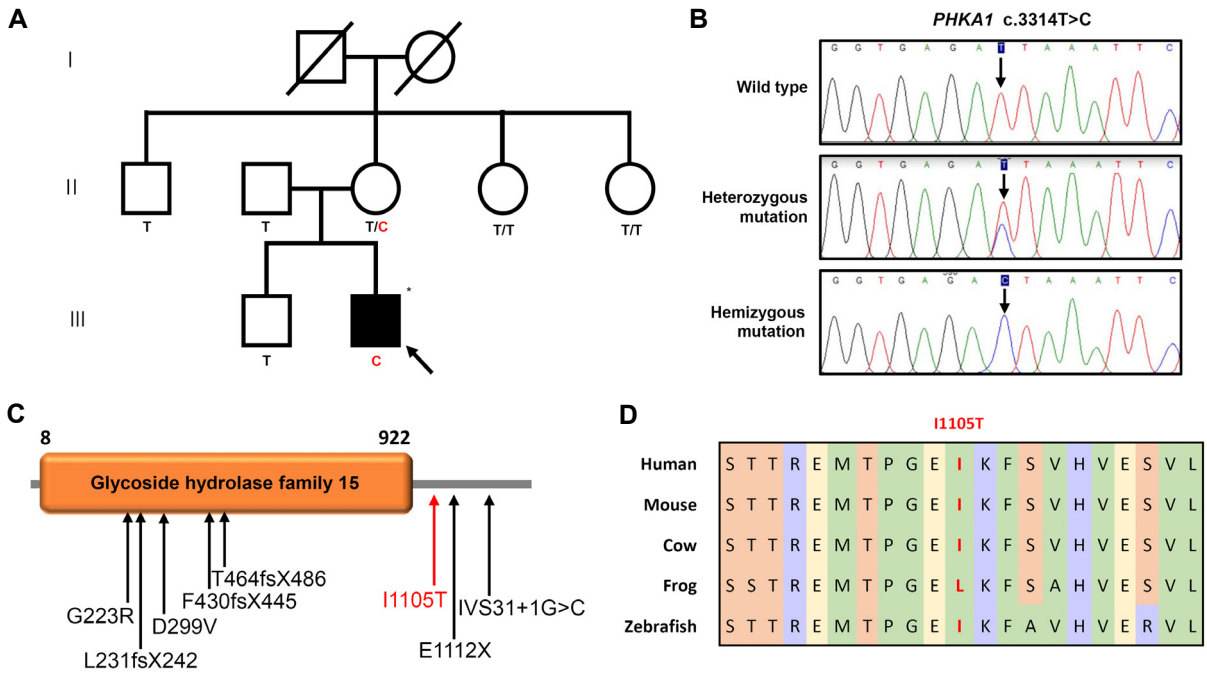

Fig. 1. X-linked recessive metabolic myopathy family with novel hemizygous mutation in *PHKA1*. (A) Pedigrees of FC975 family. Arrows indicate probands whose DNA were used for the WES (□, ○ : unaffected; ■ : affected). (B) Chromatograms of the mutation sites by capillary sequencing method. The mutation of c.3314T>C (p.I1105T) in *PHKA1* is clearly shown in the mutant alleles (arrows). (C) PHKA1 protein structure and causative mutations. The present five mutations as well as previously reported mutations are indicated below the diagram. (D) Conservation of amino acid sequences in the mutation site. Multiple protein sequence alignment revealed strong conservation of amino acid sequences at the p.I1105T mutation site among different vertebrate species (Human: NP_002628.2, Mouse: NP_032858.2, Cow: XP_002700055.1, Frog: NP_001121510.1, Zebrafish: XP_005166576.1).

nlm.nih.gov), the 1,000 Genomes project database (http://www.1000genomes.org/) were further filtered.

In silico analysis

The Sanger sequencing method confirmed the variant using the genetic analyzer ABI3130XL (Life Technologies, Foster City, CA). The genomic evolutionary rate profiling (GERP) scores were determined by the GERP program (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html). We performed conservation analysis of the protein sequences using MEGA5, version 6.06 (http://www.megasoftware.net/). In silico analyses were done with the prediction algorithms SIFT (http://sift.jcvi.org), MUpro (http://www.ics.uci.edu/~baldig/mutation), and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/). The SMART program predicted domains for the PHKA1 protein (http://smart.embl.de/).

Clinical and electrophysiological examinations

Clinical information included assessments of age at onset, muscle impairments, sensory loss, deep tendon reflexes, and muscle atrophy. The muscle strength of the flexor and extensor muscles was assessed manually with the standard Medical Research Council scale. The age at onset was determined by asking patients for their ages when symptoms, including distal muscle weakness, first appeared. Neurophysiological studies were done on proband. Motor and sensory conduction studies of the median, ulnar, peroneal, tibial, and sural nerves were tested, and needle electromyography was performed in the bilateral upper and lower limb muscles. In a patient, serum CK levels were measured.

Muscle biopsy and histological examination

Histopathological analyses including immunohistochemistry of the left vastus lateralis muscles were done in the proband (Ⅲ-2). Frozen 10 mm sections were examined after staining with hematoxylin and eosin (H&E), modified Gomori trichrome (GT), nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-tr), and myosin adenosine triphosphatase (ATPase) preincubated at pH4.3, 4.6, and 9.4. Staining with Congo red, acid phosphatase, and periodic acid-Schiff (PAS) was performed on a frozen muscle specimen of Patient.

Lower limb MRI

The patient was examined by lower limb magnetic resonance imaging (MRI) of the hip, thigh, and calf muscles at 32 years. MRI was undertaken by a 1.5T system (Siemens Vision, Erlan-gen, Germany). The imaging was done in the axial (field of view [FOV]524 – 32cm, slice thickness510mm, slice gap50.5 – 1.0mm) and coronal planes (FOV538 – 40cm, slice thickness54 – 5mm, slice gap50.5 – 1.0mm). The following protocol was used for all patients: T1-weighted spin-echo (SE) (repetition time [TR]5570 – 650 milliseconds, echo time [TE]514 – 20 milliseconds, 512 matrixes), T2-weighted SE (TR52,800 – 4,000 milliseconds, TE596 – 99 milliseconds, 512 matrixes), and fat-suppressed T2-weighted SE (TR53,090 – 4,900 milliseconds, TE585 – 99 milliseconds, 512 matrixes)

Results

Identification of novel mutation in *PHKA1*

Whole exome sequencing (WES) was performed to find the causative mutation of the patient. The patient was screened for mutations in the distal myopathy and metabolic myopathy genes and peripheral neuropathy genes. As a result of the screening, we did not find any causative candidates within the distal myopathy and peripheral neuropathy genes. However, within the metabolic myopathy gene, a c.3314T> C (p.I1105T) missense mutation was found in exon 31 of the *PHKA1* gene (Table 3). Other mutations were frequently found in several public mutation databases (dbSNP 153, 1000 Genomes project and Exome Variant Server) and 500 healthy controls. The *PHKA1* p.I1105T mutation was previously not reported in any mutation database and was not found in 500 healty controls. This mutation was located below the glycosidase family 15 domain (Fig. 1C) and this region was well conserved within various other species (Fig. 1D). The GERP score of the mutation was significantly high at 5.12, and three in silico analyzes predicted that the mutation is likely to be pathogenic. (SIFT: 0.02, Damaging; Poly Phen-2: 0.975, Probably damaging; MUpro: -0.997, Decrease the stability of protein structure).

Clinical manifestations and electrophysiologic feature

Table 1 shows the clinical symptoms of the patients included in this study and the 7 previously reported cases. The patient found no abnormalities in the developmental process and found it difficult to run at age 22. Along with this, he noticed that it was difficult to walk long. At age 27, he noticed that both toes weren't moving, and he noticed that his leg muscles were weakening. When he was 32, he visited an outside hospital with symptoms of both his toes

Table 1. Clinical features in patients with mutation in the *PHKA1* gene

References	Mutation	Sex	Age at onset, yr	Age at examination, yr	Distal weakness	Distal muscle atrophy	Exercise intolerance	Muscle cramps	Sensory loss	Creatine kinase, IU/l[a]
This patient	I1105T	Male	22	32	Yes	Yes	Yes	No	No	577
Ørngreen MC et al. (2008)	G223R	Male	Childhood	50	Yes	ND	Yes	Yes	ND	400
Wuyts W et al. (2005)	L231fsX242	Male	43	43	Yes	Yes	ND	Yes	No	1,458
Preisler N et al. (2012)	L231fsX242	Male	64	69	ND	ND	No	No	ND	1,000
Clemens PR et al. (1990)	D299V	Male	18	35	No	Yes	Yes	Yes	ND	>120 fold
Preisler N et al. (2012)	F430fsX445	Male	32	39	ND	ND	No	No	ND	332
Echaniz-Laguna A et al. (2008)	T464fsX486	Male	17	17	No	No	No	No	ND	1,000
Clemens PR et al. (1990)	E1112X	Male	46	28	Yes	Yes	Yes	No	ND	> 2 fold
Wilkinson DA et al. (1994)	IVS31+1G>C	Male	Childhood	15	Yes	ND	Yes	No	ND	> 3 fold

ND Not done
[a]Creatine kinase: normal range in our laboratory < 185IU/l

not moving. At the time, the neuroscientist's opinion was suspected of dHMN, and he came to the hospital.

However, when examined in the neurology department of our hospital, there was no loss of sensory nerves and only impaired motor function was observed. As a result of the nerve conduction test, sensory nerves showed normal SMAP and NCS in both upper and lower extremities. Normal CMAP and NCS were found in the median, ulnar nerve, and fibula nerves of the motor nerve. However, CMAP was not measured in the left and right tibial nerves of the motor nerve (Table 2). Electromyography showed reasonable results for myopathy. The CK concentration in the blood was 577 IU / l, showing a nearly three-fold increase compared to normal subjects.

The patient's type did not show any neurological abnormalities and neither father nor mother (Fig. 1A). In addition, the parents and siblings of the patient's mother showed no abnormality based on the history examination.

Histopathological findings

Muscle histological examination revealed that angulated muscle fibers and subsarcolemmal vacuoles were observed in H&E staining, and atrophy and abnormal hypertrophy of muscle fibers were also observed (Fig. 2A). In addition, PAS positive muscle fibers were observed. These pathological features were similar to those of previously reported GSD9D patients [20]. Electron microscopy revealed glycogen granules of muscle fibers and thickened Z-band and mitochondrial swelling and degradation were also observed (Fig. 2B).

Fatty infiltration of the calf muscle

A patient's lower extremity MRI study was performed at

Table 2. Electrophysiological features of the patient with *PHKA1* mutation

Patient			Normal value
Side	Right	Left	
Motor nerve studies			
Median motor nerve			
TL (ms)	3.6	3.6	<3.9
CMAP (mV)	27.8	29.2	>6.0
MNCV (m/s)	52.2	54.5	>50.5
Ulnar motor nerve			
TL (ms)	2.8	2.9	<3.0
CMAP (mV)	18.7	13.4	>8.0
MNCV (m/s)	52.9	50.2	>51.1
Peroneal nerve			
TL (ms)	5.0	5.5	<5.3
CMAP (mV)	2.6	2.1	>1.6
MNCV (m/s)	43.8	43.0	>41.2
Tibial nerve			
TL (ms)	A	A	<5.4
CMAP (mV)	A	A	>6.0
MNCV (m/s)	A	A	>41.1
Sensory nerve studies			
Median sensory nerve			
SNAP (μV)	51.1	47.0	>8.8
SNCV (m/s)	42.7	41.3	>39.3
Ulnar sensory nerve			
SNAP (μV)	33.0	33.0	>7.9
SNCV (m/s)	42.9	39.3	>37.5
Sural nerve			
SNAP (μV)	19.1	26.1	>6.0
SNCV (m/s)	40.0	41.2	>32.1

Abbreviations: NP: no potential, CMAP: compound muscle action potential, MNCV: motor nerve conduction Velocity, SNAP: sensory nerve action potential, SNCV: sensory nerve conduction velocity.

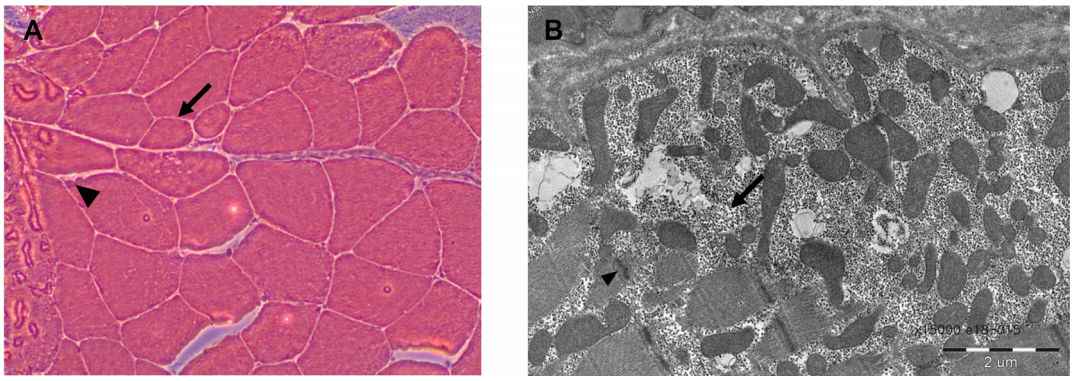

Fig. 2. Histopathologic observations of vastus lateralis muscle biopsies. (A) H&E showing angulated small size of myofibers with atrophy (arrow) and myofibers (arrowhead). (B) Electron micrograph showing a lot of glycogen (arrow) and and Z-band thickening (arrowhead).

age 33 (Fig. 3A - Fig. 3E). In the patient, no damage was observed in the hip and thigh muscles, and only moderate damage was observed in the calf. Severe gastrocnemius muscle damage was found in the right calf muscle, and soleus and gastrocnemius muscles were equally damaged in the left muscle. In the calf, the rear compartments soleus and gastrocnemius showed severe muscle atrophy and fat infiltration, while the anterior and lateral compartment muscles were not damaged.

Discussion

This study describes the genetic and MRI data and clinical characteristics of Korean GSD9D patients with *PHKA1* mutations compared to previously reported cases. A total of seven *PHKA1* mutations have been reported to cause GSD9D to date [1, 3, 8, 10, 15, 18, 20, 21]. Table 1 compares the clinical symptoms of these patients. Patients enrolled in this study had similar symptoms, such as late onset age, distal weakness, and intolerance to exercise, with previously reported GSD9D patients. However, no muscle cramp was found in our patients, which was also not found in some of the previously reported patients. The onset age of GSD9D was reported to be 17-64 years (average age of 34.6 years), excluding 2 children onset, and this patient was also 22 years old.

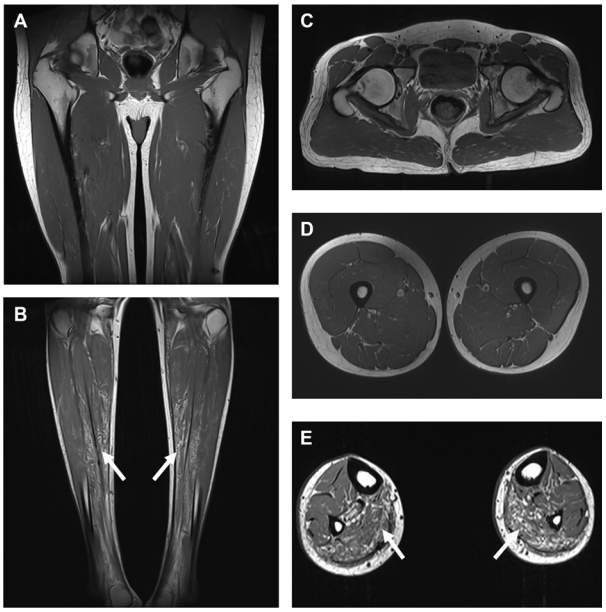

Fig. 3. T1-weighted magnetic resonance imaging (MRI) of patient. Coronal images of thigh (A) and calf (B). Axial image of hip (C) and thigh (D), calf (E).

The *PHKA1* mutation was inherited from the patient's mother. Mothers showed little clinical symptoms because they had mutations as heterozygotes. The mutation was not found in the patient's older brother and the father's and mother's siblings. It was also not found in 500 healthy controls and was also not reported in the dbSNP153 and 1,000 genome databases. Therefore, this *PHKA1* mutation is considered a novel mutation. Previously reported mutations were mainly located in the glycoside hydrolase family 15 domain, but our patient mutations were located under the domain with p.E1112X and IVS31+1G>C. There were no symptom differences between the mutations in the domain and the mutations below.

In addition, this study performed MRI examination of the lower limbs for the first time in patients with GSD9D. As a result of MRI, fat infiltration was not observed in the hip and thigh, and fat infiltration was observed only in the calf, distal to the lower extremity. In the calf muscle, no damage was observed in the anterior and lateral compartments, whereas atrophy and severe fat infiltration were observed

Table 3. Functionally significant variants found in 44 metabolic myopathy genes

Chr	Gene	Nt change[a]	AA change	dbSNP153	1,000 G	Inheritance[b]	In-house Freq (n=500)	Description
chrX	*PHKA1*	c.3314T>C (hem)	I1105T	·	·	XR	0	Pathogenic
chr01	*AGL*	splicing site mutation		rs2307130	0.43	AR	0.83	Polymorphic
		c.1109G>A (het)	R370Q	rs17121464	0.06		0.4	Polymorphic
chr03	*GBE1*	c.1000A>G (hom)	I334V	rs2172397	0.99	AR	1	Polymorphic
		c.568A>G (hom)	R190G	rs2229519	0.33		0.71	Polymorphic
chr04	*HADH*	c.257T>C (hom)	L86P	rs4956145	0.89	AR	1	Polymorphic
chr04	*ETFDH*	c.92C>T (hom)	T31I	rs11559290	0.72	AR	1	Polymorphic
chr12	*PFKM*	c.5A>T (het)	H2L	rs11609399	0.35	AR	0.8	Polymorphic
chr12	*ISCU*	c.19T>G (hom)	F7V	rs10778647	0.88	AR	0.38	Polymorphic
		c.20T>G (hom)	F7C	rs10778648	0.88		0.37	Polymorphic
		c.35C>T (hom)	A12V	rs2287555	0.53		0.1	Polymorphic
chr16	*PHKB*	c.2653G>A (het)	G885R	rs149983469	.	AR	0	Polymorphic
chr17	*ENO3*	c.212A>G (het)	N71S	rs238238	0.6	AR	0.66	Polymorphic
		c.254T>C (het)	V85A	rs238239	0.35		0.2	Polymorphic
chr17	*GAA*	c.596A>G (hom)	H199R	rs1042393	0.62	AR	0.84	Polymorphic
		c.668G>A (hom)	R223H	rs1042395	0.61		0.85	Polymorphic
		c.1726G>A (het)	G576S	rs1800307	0.05		0.36	Polymorphic
		c.2065G>A (het)	E689K	rs1800309	0.09		0.53	Polymorphic
		c.2338G>A (hom)	V780I	rs1126690	0.69		0.92	Polymorphic
chr19	*GYS1*	c.1246A>G (het)	M416V	rs5447	0.03	AR	0.25	Polymorphic
chr19	*ETFB*	c.461C>T (het)	T154M	rs1130426	0.46	AR	0.54	Polymorphic

[a]het: heterozygous, hom: homozygous and hem: hemizygous.
[b]AD: autosomal dominant, AR: autosomal recessive and XR: X-linked recessive

in the anterior compartment soleus and gastrocnemius muscles. This phenomenon is thought to be a singularity, so the patient showed disagreement with distal myopathy.

In summary, we wanted to present clinical features to patients with new *PHKA1* mutations. We report clinically typical GSD9D symptoms and detect and report distal muscle damage by MRI. Since there are no reported mutations in Korea among the 7 cases, this study is the first to report a case of 9D type Glycogen patients in Korea. This study will expand the clinical spectrum of GSD9D with the *PHKA1* mutation and suggest that it will be useful for molecular diagnosis of heterogeneous metabolic myopathy.

Acknowledgements

This work was supported by grants from the National Research Foundation (2016R1A5A2007009, 2018R1A4A1024506 and 2019R1A2C1087547) and the Korean Health Technology R&D Project, Ministry of Health & Welfare (HI14C3484 and HI16C0426), Republic of Korea.

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Bruno, C., Manfredi, G., Andreu, A. L., Shanske, S., Krishna, S., Ilse, W. K. and DiMauro, S. 1998. A splice junction mutation in the alpha(M) gene of phosphorylase kinase in a patient with myopathy. *Biochem. Biophys. Res. Commun.* **249**, 648-651.
2. Brushia, R. J. and Walsh, D. A. 1999. Phosphorylase kinase: the complexity of its regulation is reflected in the complexity of its structure. *Front. Biosci.* **4**, D618-641.
3. Burwinkel, B, Hu, B., Schroers, A., Clemens, P. R., Moses, S. W., Shin, Y. S., Pongratz, D., Vorgerd, M. and Kilimann, M. W. 2003. Muscle glycogenosis with low phosphorylase kinase activity: mutations in PHKA1, PHKG1 or six other candidate genes explain only a minority of cases. *Eur. J. Hum. Genet.* **11**, 516-526.
4. Burwinkel, B., Maichele, A. J, Aagenaes, O., Bakker, H. D., Lerner, A., Shin, Y. S., Strachan, J. A. and Kilimann, M. W. 1997. Autosomal glycogenosis of liver and muscle due

to phosphorylase kinase deficiency is caused by mutations in the phosphorylase kinase beta subunit (PHKB). *Hum. Mol. Genet.* **6**, 1109-1115.
5. Choi, R., Park, H. D., Kang, B., Choi, S. Y., Ki, C. S., Lee, S. Y., Kim, J. W., Song, J. and Choe, Y. H. 2016. PHKA2 mutation spectrum in Korean patients with glycogen storage disease type IX: prevalence of deletion mutations. *BMC. Med. Genet.* **17,** 33.
6. Clemens, P. R., Yamamoto, M. and Engel, A. G. 1990. Adult phosphorylase b kinase deficiency. *Ann. Neurol.* **28,** 529-538.
7. Dimachkie, M. M. and Barohn, R. J. 2014. Distal myopathies. *Neurol. Clin.* **32,** 817-842.
8. Echaniz-Laguna, A., Akman, H. O., Mohr, M., Tranchant, C., Talmant-Verbist, V., Rolland, M. O. and Dimauro, S. 2010. Muscle phosphorylase B kinase deficiency revisited. *Neuromuscul. Disord.* **20,** 125-127.
9. Harding, A. E. and Thomas, P. K. 1980. The clinical features of hereditary motor and sensory neuropathy types I and II. *Brain* **103,** 259-280.
10. Harmann, B., Zander, N. F. and Kilimann, M. W. 1991. Isoform diversity of phosphorylase kinase alpha and beta subunits generated by alternative RNA splicing. *J. Biol. Chem.* **266,** 15631-15637.
11. Hendrickx, J., Coucke, P., Dams, E., Lee, P., Odièvre, M., Corbeel, L., Fernandes, J. F. and Willems, P. J. 1995. Mutations in the phosphorylase kinase gene PHKA2 are responsible for X-linked liver glycogen storage disease. *Hum. Mol. Genet.* **4,** 77-83.
12. Koller, M., Schnyder, B. and Strehler, E. E. 1990. Structural organization of the human CaMIII calmodulin gene. *Biochim. Biophys. Acta.* **1087,** 180-189.
13. Maichele, A. J., Burwinkel, B., Maire, I., Søvik, O. and Kilimann, M. W. 1996. Mutations in the testis/liver isoform of the phosphorylase kinase γ subunit (PHKG2) cause autosomal liver glycogenosis in the gsd rat and in humans. *Nat. Genet.* **14,** 337-340.
14. Mastaglia, F. L. and Laing, N. G. 1999. Distal myopathies: clinical and molecular diagnosis and classification. *J. Neurol. Neurosurg. Psychiatry* **67,** 703-707.
15. Ørngreen, M. C., Schelhaas, H. J., Jeppesen, T. D., Akman, H. O., Wevers, R. A., Andersen, S. T., ter Laak, H. J., van Diggelen, O. P., DiMauro, S. and Vissing, J. 2008. Is muscle glycogenolysis impaired in X-linked phosphorylase B kinase deficiency? *Neurology* **70,** 1876-1882.
16. Pegues, J. C. and Friedberg, F. 1990. Multiple mRNAs encoding human calmodulin. *Biochem. Biophys. Res. Commun.* **172,** 1145-1149.
17. Preisler, N., Orngreen, M. C., Echaniz-Laguna, A., Laforet, P., Lonsdorfer-Wolf, E., Doutreleau, S., Geny, B., Akman, H. O., Dimauro, S. and Vissing, J. 2012. Muscle phosphorylase kinase deficiency: a neutral metabolic variant or a disease? *Neurology* **78,** 265-268.
18. Schneider, A., Davidson, J. J., Wüllrich, A. and Kilimann, M. W. 1993. Phosphorylase kinase deficiency in I-strain mice is associated with a frameshift mutation in the alpha subunit muscle isoform. *Nat. Genet.* **5,** 381-385.
19. Wehner, M. and Kilimann, M. W. 1995. Human cDNA encoding the muscle isoform of the phosphorylase kinase γ subunit (PHKG1). *Hum. Genet.* **96,** 616-618.
20. Wehner, M., Clemens, P. R., Engel, A. G. and Kilimann, M. W. 1994. Human muscle glycogenosis due to phosphorylase kinase deficiency associated with a nonsense mutation in the muscle isoform of the alpha subunit. *Hum. Mol. Genet.* **3,** 1983-1987.
21. Wuyts, W., Reyniers, E., Ceuterick, C., Storm, K., de Barsy, T. and Martin, J. J. 2005. Myopathy and phosphorylase kinase deficiency caused by a mutation in the PHKA1 gene. *Am. J. Med. Genet. A.* **133A,** 82-84.

초록 : 당원 축적병 9D (GSD9D) 환자의 신규 PHKA1 돌연변이

김혜진[1] · 남수현[2,3] · 김상범[4] · 정기화[5*] · 최병옥[1,2,3*]

([1]성균관대학교 삼성융합의과학원 융합의과학과, [2]삼성서울병원 줄기세포 재생의학 연구소, [3]성균관대학교 의과대학 신경과학교실, [4]강동경희대학교 의과대학 신경과학교실, [5]공주대학교 생명과학과)

원위 근병증은 원위 근육의 퇴행성 질환이며 임상적, 유전적으로 이질적인 그룹이다. 당원 축적병 9D (GSD9D)는 원위 근병증 중 하나이며, 근육의 포스포릴라아제키나아제(phosphorylase kinase) 결핍을 특징으로 하는 대사 근병증이다. GSD9D 환자는 운동 후 근육 약화, 근육 변성, 경련과 비정상적인 근육통 및 근육 경직이 발생될 수 있다. GSD9D는 글리코겐 대사의 주요 조절 효소 인 근육 포스포릴라아제키나아제의 알파 소단위를 암호화하는 *PHKA1* 유전자의 돌연변이로 유발된다. 이 연구에서 우리는 한국인 GSD9D 가족에 대해 *PHKA1* 유전자에서 c.3314T> C (p.I1105T) 돌연변이를 동정하였다. 이 돌연변이는 이전에 어떠한 돌연변이 데이터베이스에서도 보고되지 않았으며 500명의 건강한 대조군에서도 발견되지 않았다. 이 돌연변이 영역은 다양한 다른 종 내에서 잘 보존되었으며 *in silico* 분석에서 돌연변이가 병원성일 가능성이 있다고 예측했다. 현재까지 *PHKA1* 유전자에는 보고된 병원성 돌연변이가 7개뿐이며 한국에서는 보고된 사례가 없다. 따라서 이 연구는 한국 GSD9D 환자의 첫 번째 사례이다. 또한 이 연구는 이전에 보고된 환자와 한국 환자의 임상 증상과 병리 상태를 비교하고 설명하고자 하였다. 아울러 우리는 본 연구가 GSD9D의 분자 진단에 유용하게 활용될 것으로 기대한다.