



# Molecular Genetic Diagnosis of a Bethlem Myopathy Family with an Autosomal-Dominant *COL6A1* Mutation, as Evidenced by Exome Sequencing

Hyung Jun Park<sup>a\*</sup>  
 Young-Chul Choi<sup>b\*</sup>  
 Seung Min Kim<sup>b</sup>  
 Se Hoon Kim<sup>c</sup>  
 Young Bin Hong<sup>d</sup>  
 Bo Ram Yoon<sup>e</sup>  
 Ki Wha Chung<sup>e</sup>  
 Byung-Ok Choi<sup>d</sup>

<sup>a</sup>Department of Neurology,  
 Mokdong Hospital, Ewha Womans  
 University School of Medicine, Seoul,  
 Korea

<sup>b</sup>Departments of Neurology and  
<sup>c</sup>Pathology, Yonsei University  
 College of Medicine, Seoul, Korea  
<sup>d</sup>Department of Neurology,  
 Samsung Medical Center,  
 Sungkyunkwan University  
 School of Medicine, Seoul, Korea  
<sup>e</sup>Department of Biological Science,  
 Kongju National University, Gongju,  
 Korea

**Received** May 16, 2013  
**Revised** September 3, 2013  
**Accepted** September 6, 2013

## Correspondence

Byung-Ok Choi, MD  
 Department of Neurology,  
 Samsung Medical Center,  
 Sungkyunkwan University  
 School of Medicine,  
 81 Irwon-ro, Gangnam-gu,  
 Seoul 135-710, Korea  
**Tel** +82-2-3410-1296  
**Fax** +82-2-3410-0052  
**E-mail** bochoi@skku.edu

Ki Wha Chung, PhD  
 Department of Biological Science,  
 Kongju National University,  
 56 Gongjudaehak-ro,  
 Gongju 314-701, Korea  
**Tel** +82-41-850-8506  
**Fax** +82-41-850-0957  
**E-mail** kwchung@kongju.ac.kr

\*Hyung Jun Park and Young-Chul Choi  
 contributed equally to this work.

**Background** We describe herein the application of whole exome sequencing (WES) for the molecular genetic diagnosis of a large Korean family with dominantly inherited myopathy.

**Case Report** The affected individuals presented with slowly progressive proximal weakness and ankle contracture. They were initially diagnosed with limb-girdle muscular dystrophy (LGMD) based on clinical and pathologic features. However, WES and subsequent capillary sequencing identified a pathogenic splicing-site mutation (c.1056+1G>A) in *COL6A1*, which was previously reported to be an underlying cause of Bethlem myopathy. After identification of the genetic cause of the disease, careful neurologic examination revealed subtle contracture of the interphalangeal joint in the affected members, which is a characteristic sign of Bethlem myopathy. Therefore, we revised the original diagnosis from LGMD to Bethlem myopathy.

**Conclusions** This is the first report of identification of *COL6A1*-mediated Bethlem myopathy in Korea, and indicates the utility of WES for the diagnosis of muscular dystrophy.

**Key Words** Bethlem myopathy, collagen type VI alpha 1 (*COL6A1*), whole exome sequencing.

## INTRODUCTION

Muscular dystrophy is a clinically and genetically heterogeneous inherited disorder characterized by progressive muscle weakness and wasting. A step-by-step approach with assessment of medical history, clinical examination, laboratory evaluation, muscle pathology, muscle immunoanalysis, and mutational analysis is typically used for the diagnosis of muscular dystrophy.<sup>1,2</sup> However, this serial approach often fails to identify causative mutations due to high phenotypic and pathologic variability, small pedigrees, and the limited power of traditional linkage analyses.<sup>3</sup> Recent advances in next-generation sequencing has made it possible to selectively sequence only the protein-coding exons of the genome, a process termed 'whole exome sequencing' (WES). The application of WES not only saves time but is also cost-effective for the identification of causative genes in Mendelian diseases.<sup>4</sup> Therefore, WES is being increasingly adopted for the identification of causative genes in muscular dystrophy research.<sup>5-8</sup>

Herein we report a mutation in the gene encoding collagen type VI  $\alpha 1$  (*COL6A1*) in a large Korean family with autosomal-dominant Bethlem myopathy that was detected using WES.

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

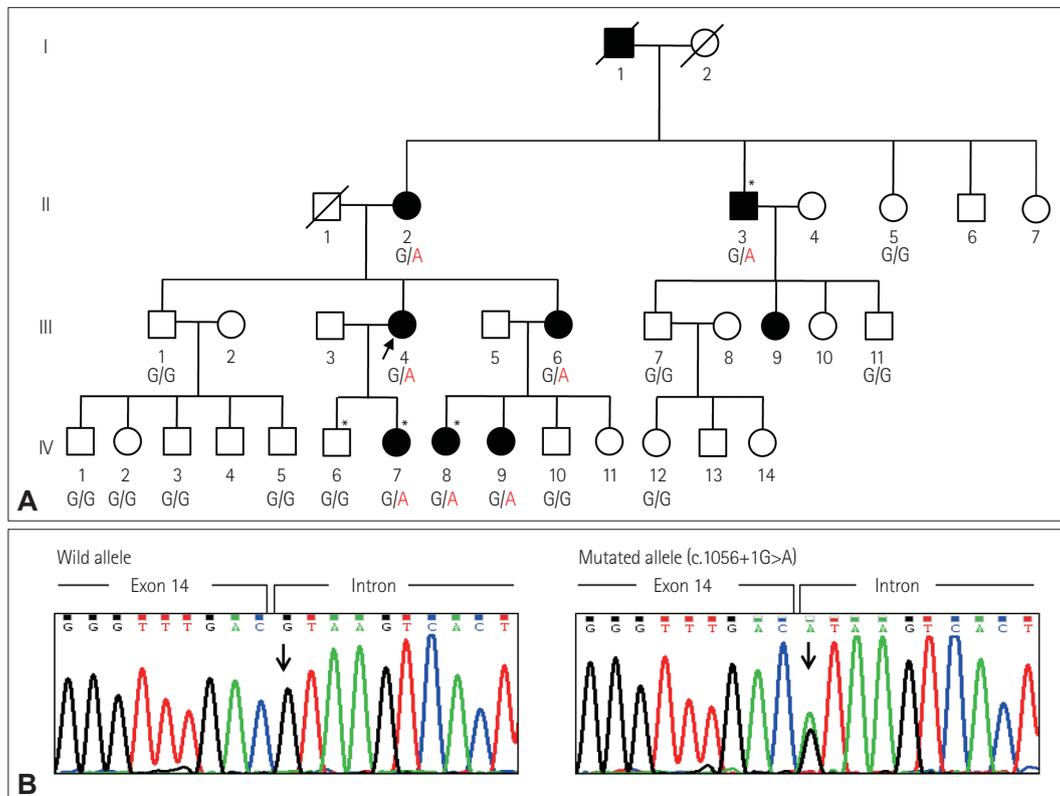
CASE REPORT

Eighteen members of a large Korean family with dominantly inherited myopathy (7 affected and 11 unaffected) were enrolled (Fig. 1A). Written informed consent to participate was obtained from all participants and from the parents of participants younger than 18 years, according to a protocol approved by the Institutional Review Board for Ewha Womans University Mokdong Hospital, Seoul, Korea.

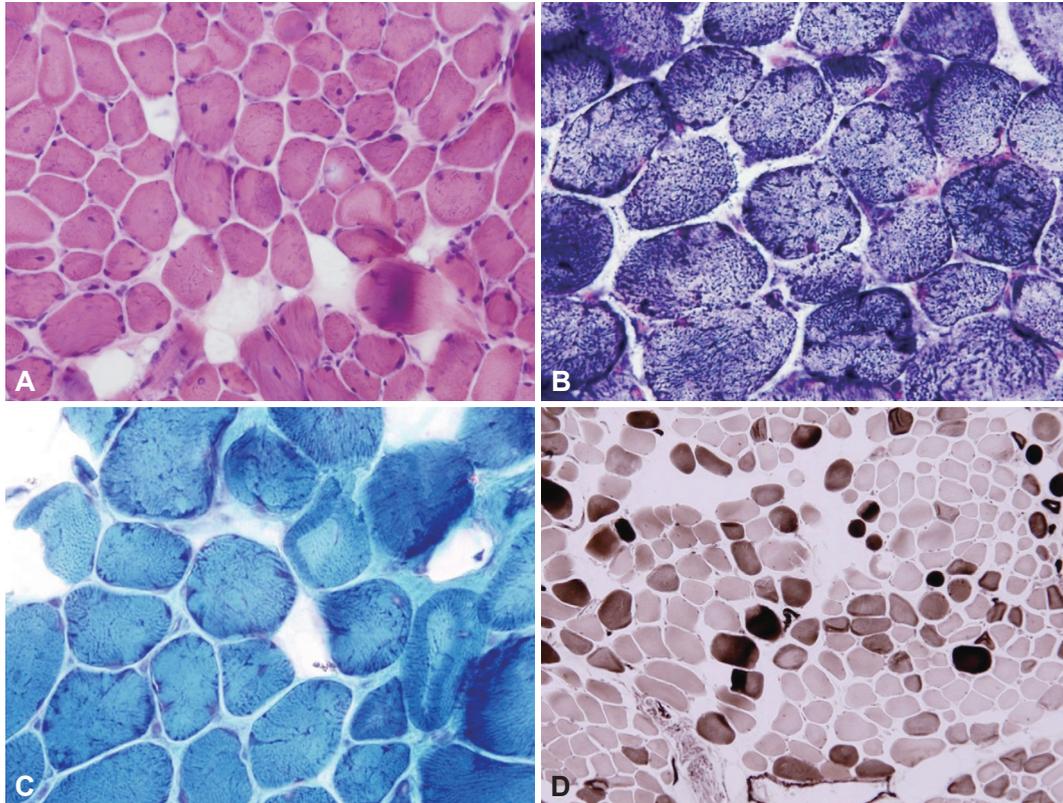
Patients

The proband, a 38-year-old woman (III-4), presented with progressive proximal weakness and ankle contractures (Supplementary Table 1 in the online-only Data Supplementary). Her initial development after birth was reportedly normal, but she did not begin walking until she was 16 months old. She recalled that she had always been weaker than her peers. Her motor function remained relatively stable until her mid-20s. However, she experienced slowly progressive muscle weakness after the delivery of her first child at an age of 27 years. Her neck flexors [Medical Research Council (MRC)

grade 3] appeared to be more damaged than her neck extensor (MRC grade 4+). The proximal muscles of her upper and lower limbs were more severely involved than the distal muscles, and her ankle joints were affected by contracture. In addition, she appeared to exhibit mild facial weakness and absent tendon reflexes in the upper and lower limbs. However, sensory examination revealed no abnormalities. Laboratory studies revealed a serum creatine kinase level of 66 IU/L (normal, <135 IU/L), and her vital capacity was 3,120 mL. Electrocardiography and echocardiography findings were normal. Nerve conduction studies and needle electromyography revealed active generalized myopathy. A muscle biopsy sample obtained from the left biceps brachii revealed nonspecific muscular dystrophic changes. Hematoxylin and eosin staining revealed variation in muscle fiber size (Fig. 2A), and modified Gomori trichrome staining revealed a few ragged red fibers (Fig. 2B). Architectural changes of disorganized intermyofibrillar networks, such as lobulated fibers, were accentuated in staining with nicotinamide adenine dinucleotide tetrazolium reductase (Fig. 2C). In addition, adenosine triphosphatase (pH 9.4) staining demonstrat-



**Fig. 1.** A: Pedigree of an autosomal-dominant Bethlem myopathy family. Asterisks (\*) indicate individuals whose DNA was used for exome sequencing. Genotypes of COL6A1 (c.1056+1G>A) are indicated under each subject (arrow, proband; square, male; circle, female; filled, affected; not filled, unaffected; diagonal bar across symbol, deceased). B: Sequencing chromatograms of the c.1056+1G>A splicing-site mutation in COL6A1. Arrow indicates the polymorphic site. The COL6A1 mutations detected by exome sequencing were confirmed by capillary sequencing. The heterozygous c.1056+1G>A mutation was completely cosegregated with the affected individuals within this family, and was not found in a sample of 200 healthy controls.



**Fig. 2.** Histopathologic observations of biceps brachii muscle samples taken from the proband (III-4). A: Hematoxylin and eosin staining revealed variations in muscle fiber size and some fibers with internal nuclei ( $\times 200$ ). B: Modified Gomori trichrome staining revealed a few ragged red fibers ( $\times 200$ ). C: Staining with nicotinamide adenine dinucleotide tetrazolium reductase revealed architectural changes of disorganized intermyofibrillar networks, such as lobulated fibers ( $\times 400$ ). D: Adenosine triphosphatase (pH 9.4) staining demonstrated a 69% predominance of type I fibers ( $\times 100$ ).

ed a 69% predominance of type I fibers (Fig. 2D). Electron microscopy revealed many mitochondria, and immunohistochemical analyses of the muscle specimens revealed normal staining patterns for the C-terminal of dystrophin, rod domain of dystrophin, N-terminal of dystrophin, dysferlin,  $\alpha$ -sarcoglycan,  $\beta$ -sarcoglycan,  $\gamma$ -sarcoglycan,  $\delta$ -sarcoglycan,  $\alpha$ -dystroglycan, and caveolin.

The other affected members of the family had similar clinical presentations (Supplementary Table 1 in the online-only Data Supplementary). They experienced very slow progressive muscle weakness and lived without significant disability until old age. Subject II-2 required aid for ambulation after the age of 50 years, while subject II-3 (61 years old) was able to walk independently. The ankle joints were affected by contracture in all seven affected patients, while the elbow joints were involved only in subject II-2. Based on the clinical and pathologic features, the family was initially diagnosed with autosomal-dominant limb-girdle muscular dystrophy.

After identification of the causative gene, all affected members underwent a second neurologic examination. This identified contracture of the interphalangeal joint—which is a

characteristic sign of Bethlem myopathy—in five family members (II-2, II-3, III-4, III-6, and IV-7). These contractures were very subtle and were not found on routine neurologic examination; they were only apparent when the wrist and fingers were extended passively.

### Genetic analysis

Whole exome sequencing was performed for five members of the family, including four affected members (II-3, III-4, IV-7, and IV-8) and one unaffected member (IV-6) to identify the genetic causes of the disease, following the method described by Choi et al.<sup>9</sup> The exome sequencing data are summarized in Supplementary Table 2 (in the online-only Data Supplementary). The mean total sequencing yield was 9.3 Gbp/sample, and the coverage rate of the targeted exon regions ( $\geq 10\times$ ) was 93.56%. The average read depth of the target regions was 69.3 reads, and the average number of observed variants per sample was 92,174 SNPs and 9,321 indels. By comparing the exome data between 4 affected and 1 unaffected family members, we found that 15 functionally significant cosegregated variants (Supplementary Table 3 in the online-only Data Supplementary). Subsequent capillary

sequencing analysis of control samples and other family members who were not included in the exome sequencing excluded most variants as the underlying cause of myopathy. However, a c.1056+1G>A splicing-site mutation in *COL6A1* completely cosegregated with affected status within the family (Fig. 1B), and was not found in 200 healthy controls. This mutation has been reported to be the underlying cause of Bethlem myopathy.<sup>10-13</sup> Thus, we determined that the c.1056+1G>A mutation in *COL6A1* was the underlying cause of the disease in this family.

## DISCUSSION

Whole exome sequencing of five members from a single family identified a splice donor site mutation at c.1056+1G>A of *COL6A1*. This mutation causes the formation of abnormal collagen VI protein by skipping of exon 14 and consequent in-frame deletion of amino acids from the triple helical domain of the  $\alpha 1$  chain.<sup>12</sup>

Bethlem myopathy is a dominantly inherited myopathy caused by mutations in one of three genes encoding collagen type VI alpha (*COL6A1*, *COL6A2*, and *COL6A3*).<sup>14</sup> The phenotype is characterized by slowly progressive proximal weakness and multiple contractures. Prominent contracture in the early stages of the disease is one of the most important clinical features in Bethlem myopathy, Emery-Dreifuss muscular dystrophy, and Ullrich congenital muscular dystrophy. Among these conditions, Bethlem myopathy demonstrates the most benign clinical course and mildest contractures.

Bethlem myopathy is often difficult to diagnose and its frequency may be underestimated for several reasons. First, mild contractures often lead to confusion in the diagnosis.<sup>15,16</sup> In the present family, even though ankle contracture was initially detected, this is a common nonspecific finding in many other neuromuscular diseases. Contracture of the interphalangeal joint is a hallmark of Bethlem myopathy, but is often so subtle that it goes unrecognized. Second, muscle biopsy is not typically used for confirmatory diagnosis of Bethlem myopathy due to nonspecific myopathic changes and lack of detected abnormalities of collagen VI, even in immunohistochemical analyses. Both Ullrich congenital muscular dystrophy and Bethlem myopathy are collagen-IV-related myopathies. Immunohistochemistry in Ullrich congenital muscular dystrophy exhibits complete absence or unequivocal reduction of collagen VI compared to normal; it can thus be used for diagnostic purposes. However, in Bethlem myopathy, immunostaining of muscle biopsy with various collagen VI antibodies is usually normal.<sup>14</sup> Third, identification of the causative genes by general sequencing is costly and time-consuming because it is necessary to screen all 107

exons in all 3 genes for molecular genetic diagnosis. For these reasons, careful clinical assessment and cost-effective, time-saving strategies for genetic analysis are important for the diagnosis of Bethlem myopathy.

Whole exome sequencing is a well-justified strategy for discovering the causative genes of muscular dystrophy. WES is based on next-generation sequencing, which reduces the cost and time relative to Sanger sequencing.<sup>17</sup> In addition, WES focuses only on protein-coding regions, but it is still an effective diagnostic tool because more than 90% of the pathogenic mutations for Mendelian disorders are found in exons.<sup>4</sup>

In conclusion, we identified a *COL6A1* mutation in a Korean family with Bethlem myopathy; this is the first such report in Korea. Even though the causative mutation identified in the present study has been reported previously, this work underscores the usefulness of WES for the diagnosis of muscular dystrophy.

## Supplementary Materials

The online-only Data Supplement is available with this article at <http://dx.doi.org/10.3988/jcn.2015.11.2.183>.

## Conflicts of Interest

The authors have no financial conflicts of interest.

## Acknowledgements

This study was supported by the Korean Health Technology R&D Project (A120182), Ministry of Health & Welfare, Republic of Korea.

## REFERENCES

- Norwood F, de Visser M, Eymard B, Lochmüller H, Bushby K; EFNS Guideline Task Force. EFNS guideline on diagnosis and management of limb girdle muscular dystrophies. *Eur J Neurol* 2007;14:1305-1312.
- Bushby K. Diagnosis and management of the limb girdle muscular dystrophies. *Pract Neurol* 2009;9:314-323.
- Ku CS, Naidoo N, Pawitan Y. Revisiting Mendelian disorders through exome sequencing. *Hum Genet* 2011;129:351-370.
- Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, Nickerson DA, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet* 2011;12:745-755.
- Davidson AE, Siddiqui FM, Lopez MA, Lunt P, Carlson HA, Moore BE, et al. Novel deletion of lysine 7 expands the clinical, histopathological and genetic spectrum of TPM2-related myopathies. *Brain* 2013;136(Pt 2):508-521.
- Klar J, Sobol M, Melberg A, Mäbert K, Ameer A, Johansson AC, et al. Welander distal myopathy caused by an ancient founder mutation in TIA1 associated with perturbed splicing. *Hum Mutat* 2013;34:572-577.
- Ronchi D, Di Fonzo A, Lin W, Bordoni A, Liu C, Fassone E, et al. Mutations in DNA2 link progressive myopathy to mitochondrial DNA instability. *Am J Hum Genet* 2013;92:293-300.
- Weterman MA, Barth PG, van Spaendonck-Zwarts KY, Aronica E, Poll-The BT, Brouwer OE, et al. Recessive MYL2 mutations cause infantile type I muscle fibre disease and cardiomyopathy. *Brain* 2013;136(Pt 1):282-293.
- Choi BO, Koo SK, Park MH, Rhee H, Yang SJ, Choi KG, et al. Exome

- sequencing is an efficient tool for genetic screening of Charcot-Marie-Tooth disease. *Hum Mutat* 2012;33:1610-1615.
10. Pan TC, Zhang RZ, Sudano DG, Marie SK, Bönnemann CG, Chu ML. New molecular mechanism for Ullrich congenital muscular dystrophy: a heterozygous in-frame deletion in the COL6A1 gene causes a severe phenotype. *Am J Hum Genet* 2003;73:355-369.
  11. Luciola S, Giusti B, Mercuri E, Vanegas OC, Lucarini L, Pietroni V, et al. Detection of common and private mutations in the COL6A1 gene of patients with Bethlem myopathy. *Neurology* 2005;64:1931-1937.
  12. Baker NL, Mörgelin M, Pace RA, Peat RA, Adams NE, Gardner RJ, et al. Molecular consequences of dominant Bethlem myopathy collagen VI mutations. *Ann Neurol* 2007;62:390-405.
  13. Lamandé SR, Shields KA, Kornberg AJ, Shield LK, Bateman JF. Bethlem myopathy and engineered collagen VI triple helical deletions prevent intracellular multimer assembly and protein secretion. *J Biol Chem* 1999;274:21817-21822.
  14. Lampe AK, Bushby KM. Collagen VI related muscle disorders. *J Med Genet* 2005;42:673-685.
  15. Scacheri PC, Gillanders EM, Subramony SH, Vedanarayanan V, Crowe CA, Thakore N, et al. Novel mutations in collagen VI genes: expansion of the Bethlem myopathy phenotype. *Neurology* 2002;58:593-602.
  16. Merlini L, Morandi L, Granata C, Ballestrazzi A. Bethlem myopathy: early-onset benign autosomal dominant myopathy with contractures. Description of two new families. *Neuromuscul Disord* 1994;4:503-511.
  17. Metzker ML. Sequencing technologies - the next generation. *Nat Rev Genet* 2010;11:31-46.