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Genetic Therapies for Duchenne Muscular Dystrophy and Beyond

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Progressive weakness of skeletal muscle is the hallmark of muscular dystrophies. It is often accompanied by cardiomyopathy and respiratory insufficiency. It has generally been perceived as incurable diseases, while the advent of genetic therapy is changing the paradigm. Most research and achievements have been for the treatment of Duchenne muscular dystrophy, while it is promising to hope for therapies for other myopathies. Drugs for nonsense read-through and exon skipping are already approved for clinical use in Europe and the United States, respectively. Gene therapy using adeno-associated virus is in early phase of clinical trial. In this review, most promising genetic therapies will be briefly described.

Key words: Duchenne muscular dystrophy, Gene therapy, Exon skipping, Antisense oligo, Read-through

REVIEW ARTICLE

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INTRODUCTION

The initial description of muscular dystrophy dates back to the mid-19th century. It causes progressive skeletal muscle weakness, accompanied by heart failure or respiratory insufficiency depending on the types. In case of Duchenne muscular dystrophy (MIM#310200), the most frequent childhood form, the first symptom of hip girdle weakness develops before 5 years of age. The weakness aggravates slowly but relentlessly, to make the child limited to a wheelchair in late childhood or early teen. It is usual to have fatal complications from respiratory insufficiency or cardiomyopathy in their late teens or early twenties without active intervention. Use of corticosteroid and ventilator support in concert with the other supportive management have greatly improved the length and quality of life with Duchenne muscular dystrophy, but it is still perceived as an incurable disease.

However, the effort in medical research has been as unyielding as the nature of the disease. Dystrophin (DMD, MIM*300377), the causative gene of Duchenne muscular dystrophy, was cloned in 1986. It was the first pathogenic gene identified by positional cloning, which opened a new era of human molecular genetics. One after another, pathogenic genes have been characterized to differentiate each myopathy by the gene involved. Animal models were established, and the research in molecular mechanism followed. Now, two of the innovative therapies have been approved for clinical use, and many of them are under development with promising results.

READ-THROUGH OF NONSENSE MUTATION

The aminoglycosides antibiotics bind to the aminoacyl site of ribosomal RNA

and lead to misreading of the genetic code and inhibition of translation (Fig. 1). This error-prone protein synthesis by aminoglycoside brings broad-spectrum bactericidal activity. It also induces error in termination of protein elongation on stop codon, which can be exploited to promote read-through of nonsense mutation. Approximately 15% of Duchenne muscular dystrophy patients harbor nonsense point mutation.

Gentamicin has been tested if it can achieve desired read-through effect in Duchenne muscular dystrophy patients with nonsense mutation. It could successfully reduce creatine kinase level and restore dystrophin expression at sarcolemma of Duchenne muscular dystrophy patients with nonsense mutation, unlike those with frameshift mutation [1]. Nevertheless, the toxicity of aminoglycoside and need for intravenous injection discourage long-term use of gentamicin in these patients.

Ataluren is a low-molecular-weight compound screened from orally available non-toxic chemical library. It makes ribosome less sensitive to premature stop codon to facilitate read-through of nonsense mutation, though the exact mechanism of action is still unknown [2]. European Medical Agency conditionally approved ataluren for clinical use in Duchenne muscular dystrophy with nonsense mutation in 2014. A phase 3 study revealed significant benefit in Duchenne patients with moderate disability over 48 weeks with excellent safety profile [3].

Theoretically, ataluren should be effective in any disease by nonsense mutation. It is actively being tested experimentally and clinically in several diseases including dysferlin-deficient muscular dystrophy, aniridia [4], metabolic diseases, and ion

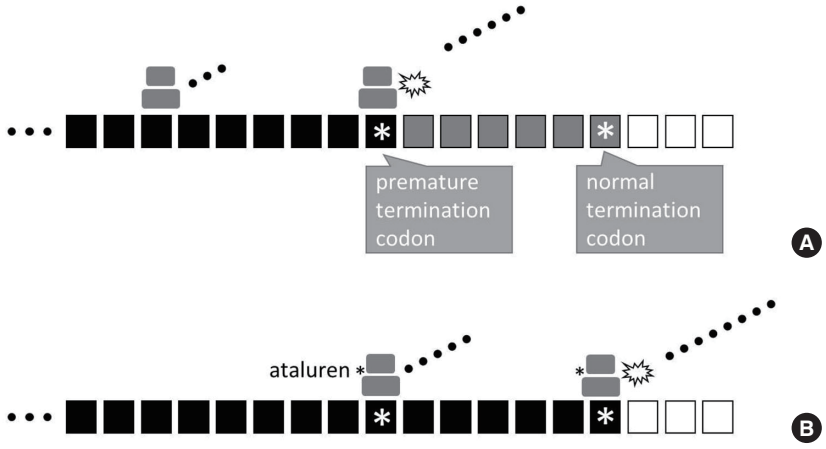


Fig. 1. Read-through of nonsense mutation. (A) Nonsense mutation is a premature termination codon that truncates the translation process of protein synthesis. Shorter than expected size of protein is produced. (B) The affects the translation machinery to ignore premature stop codon. Full-length protein is produced and released at the normal termination codon.

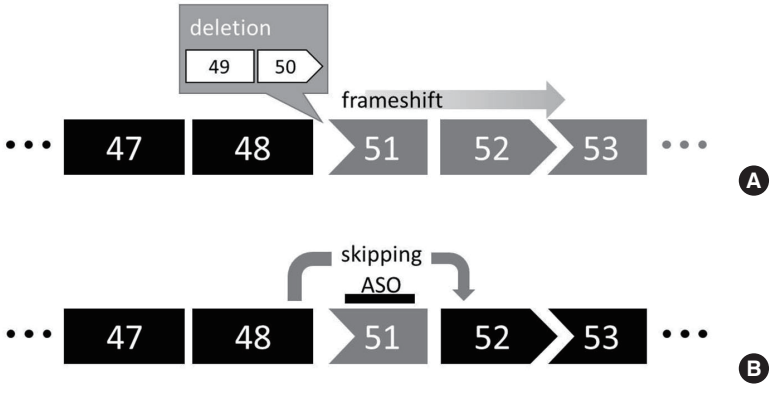


Fig. 2. Skipping of dystrophin exon 51. (A) In patients with deletion of dystrophin exon 49 & 50, shift in reading frame occurs from exon 51 and downstream. It results in the loss of functionally critical region of dystrophin. (B) Antisense oligonucleotide binds to splicing signal sequence of exon 51. Splicing machinery fails to recognize exon 51 and splices exon 48 to exon 52, skipping exon 51. This restores reading frame from exon 52 and downstream, while information of exon 51 is omitted. ASO, antisense oligonucleotide.

channel diseases. But contrary to the expectation, its effect on cystic fibrosis was not superior to the inhaled tobramycin [5].

Exon skipping

Duchenne muscular dystrophy is peculiar in that majority of cases are due to large deletions involving one or more exons. Some exons are in-frame containing multiple of 3 nucleotides; deletion of those exons causes loss of information corresponding to the deletion, more likely to cause less severe phenotype, Becker muscular dystrophy (MIM#300376). Other exons are naturally composed of nucleotides not in line with codon. Deletions of those exons disrupt reading frame in the codons of following exons, leading to classic phenotype of Duchenne muscular dystrophy.

It is possible to restore reading frame by covering another out-of-frame exon and resulting in the skipping of the exon (Fig. 2). Anti-sense oligonucleotide, actually a synthetic nucleotide analogue, is used to promote this exon skipping. The anti-sense oligonucleotide is designed to bind to a specific region of pre-mRNA critical to the splicing. Deletions of DMD gene in Duchenne patients are concentrated between exon 45 to 55. It is estimated that exon 51 skipping can be applied to approximately 13% of Duchenne patients. Another 8% of patients will be amenable to skipping of exon 53 and exon 45 respectively [6].

Eteplirsen for exon 51 skipping is approved in 2016 by the US Food and Drug Administration. Clinical trials for exon 53 skipping and for exon 45 skipping are ongoing (Table 1). As exon skipping depends on the specific exon structure, the design of antisense oligonucleotide should be customized according to the deletions. Potentially, exon skipping strategy can be applied to more than 70% of Duchenne muscular dystrophy cases with large deletion, while clinical trials for minor mutations may face difficulty in recruiting subjects as well as funding.

Antisense oligonucleotides with next-generation chemistry are under development to achieve higher expression of dystrophin, ideally also in heart. Myopathies other than Duchenne muscular dystrophy may benefit from exon skipping [7, 8], though it is not actively under clinical trial.

Gene replacement therapy

Replacement of defective gene is the most intuitive way to cure genetic disease. Gene replacement therapy holds an advantage over other strategies in that it can be effective regardless of mutation type. The history of gene therapy development proves it is not as easy as its concept. Sustained high-level expression of exogenous gene is often not without immunologic rejection.

Adeno-associated virus (AAV) is a vector closest to this goal. It was first isolated as a contaminant of adenovirus preparation. It is not pathogenic in nature and dependent on other viruses for replication. It infects non-dividing cells in high energy tissues including muscle, heart, liver, and nervous system, and mostly stay unintegrated to the genome as an episome. Recombinant AAV is devoid of any wild type viral gene except for the short strip of inverted terminal repeats. AAV is also actively under investigation for the treatment of diseases other than muscular dystrophy [9-11].

Small packaging capacity is the only downside of AAV. Coding sequence for dystrophin spans up to 11 kb, while the recombinant AAV can deliver less than 4.9 kb in a single capsid (Fig. 3). Micro-dystrophin with minimal functional component of dystrophin just fits in AAV capsid. Experiments in mice [12] and dogs [13] ensured functional improvement with long-term expression [14]. Early phases of clinical trials is ongoing to show safety and exploratory clinical efficacy. Success in gene therapy for Duchenne muscular dystrophy will accelerate clinical trials for other muscle gene therapy [15,16].

Gene editing

Clustered regularly interspaced short palindromic repeats

Table 1. Dystrophin deletions amenable to skipping of exon 51, 53, and 45

Exon 51 skip-amenable	Exon 53 skip-amenable	Exon 45 skip-amenable
3-50, 4-50, 5-50, 6-50, 9-50, 10-50, 11-50, 13-50, 14-50, 15-50, 16-50, 17-50, 19-50, 21-50, 23-50, 24-50, 25-50, 26-50, 27-50, 28-50, 29-50, 30-50, 31-50, 32-50, 33-50, 34-50, 35-50, 36-50, 37-50, 38-50, 39-50, 40-50, 41-50, 42-50, 43-50, 45-50, 47-50, 48-50, 49-50, 50-52, 52-58, 52-61, 52-63, 52-64, 52-66, 52-76, 52-77	3-52, 4-52, 5-52, 6-52, 9-52, 10-52, 11-52, 13-52, 14-52, 15-52, 16-52, 17-52, 19-5,2 21-52, 23-52, 24-52, 25-52, 26-52, 27-52, 28-52, 29-52, 30-52, 31-52, 32-52, 33-52, 34-52, 35-52, 36-52, 37-52, 38-52, 39-52, 40-52, 41-52, 42-52, 43-52, 45-52, 47-52, 48-52, 49-52, 50-52, 52, 54-58, 54-61, 54-63, 54-64, 54-66, 54-76, 54-77	7-44, 12-44, 18-44, 44-46, 46-47, 46-48, 46-49, 46-51, 46-53, 46-55, 46-57, 46-59, 46-60, 46-67, 46-69, 46-75, 46-78

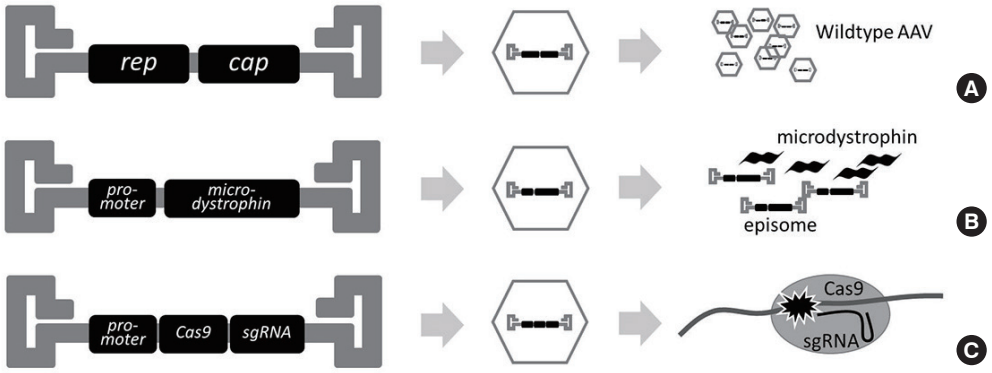


Fig. 3. AAV genome and application. (A) Wildtype AAV genome contains rep and cap protein. With the help of other virus like adenovirus, it can replicate to complete life cycle. (B) Recombinant AAV genome containing micro-dystrophin is devoid of wildtype AAV gene except for inverted terminal repeats at the ends. It can be produced without any use of wiltype virus. Once transduced into muscle cells, it sustains long-term expression of microdystrophin as episome. (C) Cas9 system can be packaged into recombinant AAV. It can be efficiently delivered into target cells with guide RNA to make a break at the target sequence (AAV, adeno-associated virus; sgRNA, guide RNA).

(CRISPR) and CRISPR-associated proteins (Cas) technology enabled revolutionized gene editing method. It makes breaks in the specific sequence of the genome guided by RNA sequence. The CRISPR-Cas system was trimmed to fit into the AAV vector, that it can be efficiently delivered to muscle. Experiments in mice with dystrophin mutation could successfully restore dystrophin expression by cutting the mutant exon out [17].

Gene editing is expected to be the most versatile tool for autosomal dominant muscle diseases [18]. However, prolonged enzymatic activity and potential off-target effects should further be investigated before it can be used in clinical trials. Base editing is a technology in an even earlier stage of development than gene editing. It can correct a missense mutation unlike gene editing cutting a few nucleotides out of the target sequence [19].

CONCLUSIONS

The enzyme replacement therapy of Pompe disease [20], approved in 2006, was the first specific therapy for muscle disease. It is now established as the standard treatment, and modifications to enhance the efficacy is under active investigation. Genetic therapies are expanding the list of treatable muscle diseases, starting from Duchenne muscular dystrophy. These therapies are harnessing cutting-edge sciences leading the medical research in inherited disorders. Other approaches specifically targeting biochemical defects are upcoming, for example, nucleoside supplementation for myopathic mitochondrial DNA depletion syndrome [21] and sialic acid supplementation for GNE myopathy [22].

Hope for new therapies for muscular dystrophy is more promising than ever. This warrants special emphasis for the patients and the doctors to stick to the standard care. It includes corticosteroid in Duchenne muscular dystrophy and the multidisciplinary support [23]. Early diagnosis and meticulous genotype-phenotype correlation is an essential step to be extended to national/international registry and patient support group, which will feed forward to promote the development of new therapies in this field.

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Novel variants of *IDS* gene, c.1224_1225insC, and recombinant variant of *IDS* gene, c.418+495_1006+1304del, in Two Families with Mucopolysaccharidosis type II

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In this report, the phenotypes of three patients from two families with mucopolysaccharidosis type II (MPS II) are compared: a novel variant and recombinant variant of *IDS* gene. The results of urine in patients showed a pronounced increase in glycosaminoglycan excretion with decreased iduronate-2-sulfatase enzyme activity in leukocyte, leading to a diagnosis of MPS II. A patient has a novel variant with 1 bp small insertion, c.1224_1225insC in exon 9, which caused frameshifts with a premature stop codon, and two patients have a recombination variant, c.418+495_1006+1304del, leading to the loss of exons 4, 5, 6, and 7 in genomic DNA, which is relatively common in Korean patients. They had different phenotypes even in the same mutation. The patients have now been enzyme replacement therapy with a significant decrease in glycosaminoglycan excretion. Further study on residual enzyme activity, as well as experience with more cases, may shed light on the relationship between phenotypes in MPS II and gene mutations.

Key words: Mucopolysaccharidosis type II, Iduronate-2-sulfatase, Enzyme replacement therapy

CASE REPORT

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INTRODUCTION

Mucopolysaccharidosis type II (MPS II) is a rare inborn error of metabolism that affects every organ of the body [1]. It is caused by a deficiency of the lysosomal enzyme, iduronate-2-sulfatase (IDS), which catalyses the degradations of the glycosaminoglycan (GAG) dermatan sulfate and heparan sulfate [1, 2]. As a result, lysosomal accumulation of upstream metabolites affects a variety of organ systems, including the visceral organs, connective tissue, skeleton, and the central nervous system [3, 4]. The incidence of MPS II is 1 per 162,000 births [5]. Its common phenotypes are: coarse facial features, upper airway obstruction, cardiac valve regurgitation, restrictive lung disease, hepatosplenomegaly, hernias, joint contractures, and reduced quality of life [6]. Variation of mutations in *IDS* gene results in differences in clinical symptoms among patients [7]. In this study, two families with MPS II which have variants of small insertion or recombination mutation in *IDS* gene were reported: c.1224_1225insC or c.418+495_1006+1304del.

PATIENTS REPORT

Patient 1 presented with stunted growth at 9 years of age. The boy was born at 40 weeks of gestation, with a birth weight of 3,700 g. He is the second child of healthy, non-consanguineous Korean parents. His neonatal period was unremark-

able. He had episodes of recurrent acute otitis media after 3 years. At 4 years of age he underwent adenoid-tonsillectomy. His height was between the 10th and 25th percentiles. Coarse facial features, including macrocephaly were noted. He had hepatosplenomegaly and dysostosis multiplex. In addition, he had high tone sensorineural hearing loss, reduced pulmonary function, and carpal tunnel syndrome. He showed high intelligence (IQ score 140). He showed elevated urinary GAG level (220.06 mcg/ml; reference range (RR), <36 mcg/ml) and decreased IDS enzyme activity (1.03 nmol/4 hr/mg protein; RR, 18-57 nmol/4hr/mg protein) in leukocyte. He has now been on enzyme replacement therapy (ERT) for 24 months, resulting in improvement of urinary GAG, liver/spleen volume, 6-Min Walk Test, passive joint range of motion, and growth velocity.

Patient 2 complained of intellectual disability (second grade level) at 3 years of age. He had hepatosplenomegaly, dysostosis multiplex and Mongolian spots. His height was normal (75-90th percentile). He had undergone an umbilical hernia operation at two years of age, and had markedly elevated urinary GAG level (535.0 mcg/ml; RR, <36 mcg/ml) and reduced IDS enzyme activity (0.1 nmol/4hr/mg protein; RR, 18-57 nmol/4 hr/mg protein) in leukocyte. During the treatment with ERT in patient 2, his older brother (patient 3) was admitted to evaluate growth and development. He complained of intellectual disability (first grade level) at 3 years of age. Physical examination showed he had a profound short stature (<3th percentile), hepatosplenomegaly, and dysostosis multiplex. He underwent a meningomyelocele operation at age three years, and showed a highly elevated urinary GAG level (927.0 mcg/ml; RR, <36 mcg/ml) and reduced IDS enzyme activity (0.03 nmol/4 hr/mg protein; RR, 18-57 nmol/4 hr/mg protein). He showed severe cognitive impairment (IQ score 40) at the age of 5 year

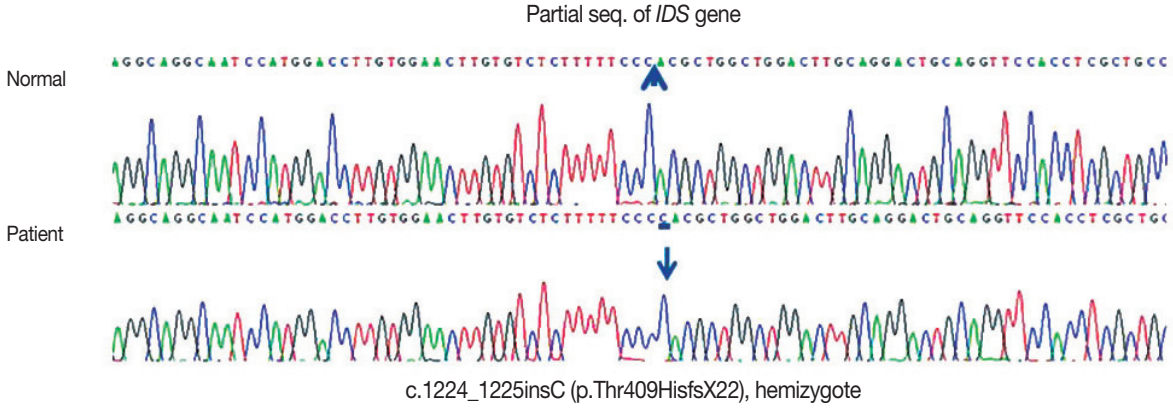


Fig. 1. Novel c.1224_1225insC (p.T409Hfs*22) mutation in exon 9 of *IDS* gene.

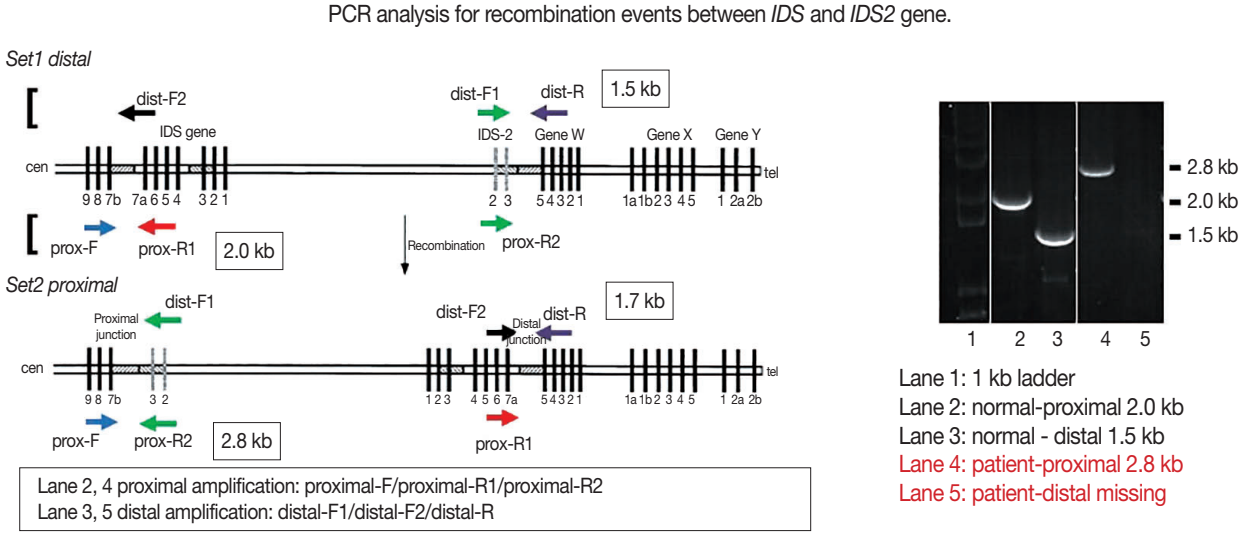


Fig. 2. PCR analysis for recombination events between IDS and *IDS2* gene.

old. The sibling patients have been on ERT for 24 months, resulting in improvement of urinary GAG, liver/spleen volume, passive joint range of motion, and growth velocity without experiencing further aggravation of neurological deficit.

IDS Genetic Analysis

Analysis of the *IDS* gene was performed for definitive analysis of the type of MPS in these patients. By direct sequencing, patient 1 revealed a variant with 1 bp insertion in exon 9 (NM_000202.4), c.1224_1225insC (p.T409Hfs*22), which caused frameshifts starting from codon 409 with a premature stop codon (Fig. 1). This hemizygous mutation was derived from his mother and has not been reported previously. His older brother showed symptom and had no mutation of *IDS* gene. According to 2015 ACMG guideline, this variant was regarded as pathogenic. The PCR-based recombination detection method of patient 2 and 3 was shown an *IDS-IDS2* recombination mutation, c.418+495_1006+1304del in intron 3 and 7, leading to the loss of exons 4, 5, 6, and 7 in genomic DNA (Fig. 2). Informed consent was obtained from patients and their family.

DISCUSSION

In these cases of MPS II, the T409Hfs*22 mutation in exon 9 was identified which has never previously been reported, and recombination mutation of *IDS* and *IDS2*, which is relatively common in Korean patients. The T409Hfs*22 mutation leads to the introduction of a premature termination codon and might cause subsequent non-sense mediated mRNA decay of mutant *IDS* mRNA. The patient 1 in present study presented with stunted growth. The laboratory results of urine and blood analysis suggested that this case was a metabolic disease of MPS II, and the patient was treated with ERT. Before the start of ERT, the urine GAG level was 220.06 mcg/ml, as compared with 66.3 mcg/mL after treatment. Two sibling patients (patient 2 and patient 3) also showed significant decrement in urine GAG level (535.0 mcg/mL vs. 136.3 mcg/mL and 927.0 mcg/mL vs. 114.2 mcg/mL, respectively) after ERT. Of interest, sibling patients with the same mutation had different clinical manifestation. Patient 3 had a profound short stature and underwent a meningomyelocele surgery. However, patient 2 had a normal stature and MRI scan of the brain was normal. This result suggested the clinical heterogeneity of MPS II even in the same genotype. Although the phenotype of MPS II depends on the mutation types at the *IDS* gene [8, 9] as well as residual enzyme activity, no strict relationship between genotype and clinical phenotype has been established [10]. In MPS II patients, more than 400 different genotypic variations have been documented in the *IDS* gene, which is approximately 24 kb with 9 exons [7]. The patients with the *IDS-IDS2* recombination mutation in the present study had severe MPS II phenotypes, which were most frequently observed in Korean patients [11]. The recombination mutation identified in our patients involves homologous recombination between intron 3, 7 of the *IDS* gene and the homologous region of its closely related pseudogene (*IDS2*), and leads to the loss of exons 4, 5, 6, and 7 in genomic DNA. The deletion spanned a region of 13,807 bp that comprised the half of the region of *IDS* gene. Ultimately, *IDS-IDS2* recombination was identified by a PCR-based recombination deletion method, resulting in a definitive diagnosis of MPS II. The limitations of this study is that functional study about a novel variant detected was not conducted.

Taken together, urine glycosaminoglycan and enzyme activity study of specific lysosomal storage disease can help diagnosis. A high index of suspicion is the most important factor for diagnosing lysosomal storage disease, and if the symptoms suggest a specific diagnosis, a genetic test is helpful to take genetic counseling for families at risk and prenatal diagnosis. Further experience with more cases may shed light on the relationship between gene mutations and phenotypes in MPS II.

DISCLOSURE

The authors have no conflicts of interest to disclose.

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First Korean Case of 16p11.2 Duplication Syndrome Diagnosed by Chromosomal Microarray Analysis

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A 10-year and 5 month-old girl with developmental delay, intellectual disability, attention deficit hyperactivity disorder, poor weight gain, and microcephaly was transferred to our pediatric clinic for genetic evaluation. Her height was within the 5-10th percentile, and her weight was under the 3rd percentile. On the social maturity scale, her developmental status was scored as 3 years 9 months for social age, and the social quotient was 35.98. A chromosomal microarray analysis was performed and the microduplication at chromosome 16p was observed: arr[GRCh37] 16p11.2 (29580020_30190029)×3. Currently, the patient is diagnosed with Grade 2 intellectual disability and is attending a computerized cognitive rehabilitation class twice weekly. In addition, nutritional support and growth follow up are also ensured in the Pediatric Gastrointestinal and Endocrinology clinic.

Key words: 16p11.2 microduplication, 16p11.2 copy number variations, Chromosomal microarray

CASE REPORT

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INTRODUCTION

Both deletion and duplication of chromosome 16p11.2 have been associated with developmental delay, intellectual disability, and several psychiatric disorders [1]. Thus, early genetic diagnosis and intervention in these patients are important. However, no case of copy number variations (CNVs) in 16p11.2 has been reported from Korea yet. Here, we report the first Korean case of 16p11.2 duplication syndrome having classical characteristic features as confirmed by chromosomal microarray analysis (CMA).

CASE DESCRIPTION

A 10-year and 5 month-old-girl was transferred to our pediatric clinic. She had visited several medical centers since she was a toddler with complaints of language delay, poor weight gain, attention deficit-hyperactivity disorder (ADHD), and delayed walking. Her face showed epicanthal folds, broad nose, wide mouth, micrognathia, and prominent ears. Her parents were healthy and she had no sibling. At 6 years of age, magnetic resonance imaging of her brain and karyotype using peripheral blood were performed and the results showed no abnormal finding. She was investigated for Williams syndrome due to the morphologic features and learning disability: there was no abnormality in 7q11.23 by fluorescence in situ hybridization. The workup for periodic hypokalemic paralysis due to motor delay and symptoms of falling down on her legs; there was no mutation in *CACNA1S* and *SCN4A* genes by DNA sequencing. The screening for lysosomal storage

disease including Pompe disease using dried blood spot considering muscle weakness associated with myopathy; the enzyme levels were in all normal range.

At 7 years and 4 months of age, she undertook a language problem-solving ability test in our Pediatric Neurology Clinic and Department of Rehabilitation. The language problem-solving score was found to be 5 (< 1st percentile). The acceptance vocabulary equivalent age was estimated to be 3 years and 6 months by Peabody Picture Vocabulary Test (< 1st percentile). The accuracy of the consonants and vowels by Urimal test of Articulation and Phonology was 65.11% and 100%, respectively. The nerve conduction velocity and electromyography showed normal electrophysiological findings at 7 years and 4 months of age. The electroencephalography showed normal waking pattern at the age of 8 years and 2 months.

At 10 years and 5 months of age, during the time of visiting our genetics clinic, her height was within the 5-10th percentile and her weight was under the 3rd percentile (Fig. 1A). Her head circumference (HC) was within the 5-10th percentile (based on a 7-year-old girl's standard). The radiological findings suggested scoliosis of the thoracic spine (Fig. 1B). On the social maturity scale, her developmental status was scored as 3 years and 9 months for social age, and the social quotient was 35.98. She had poor performance in learning self-help skills due to poor cognitive and motor development. She had difficulty in performing most of her daily activities, which were all performed under the direction and assistance of her parents. It was difficult for her to write her own name because of the lack of visual perception. There was a delay in providing her training for age-appropriate social skills and adaptation behaviors.

Based on her symptoms and signs, CMA (Affymetrix Cytoscan 750K array, Genome build: Hg 19) was performed and was found to be arr 16p11.2 (29580020_30190029)×3 (Fig. 2). Accordingly, the patient was diagnosed with 16p.11.2 duplication syndrome. Her microarray showed a gain of 610 kb at the 16p11.2 site. We provided genetic counseling to her family. A prenatal genetic test will be necessary for her parents in subsequent pregnancies to rule out the possibility of gonadal mosaicism. Currently, the patient is diagnosed with Grade 2 intellectual disability and she is attending a computerized cognitive rehabilitation class twice weekly. Her visual perception is improving, although memory is further deteriorating and she needs repeated learning.

A nutritional evaluation revealed that she was having moderate malnutrition. The general nutritional requirement at her age is 1,600-1,900 kcal/day, but her oral intake was only 800-1,300 kcal/day. As a result, she consulted nutritionists and her mother was counseled for providing her additional nutritional

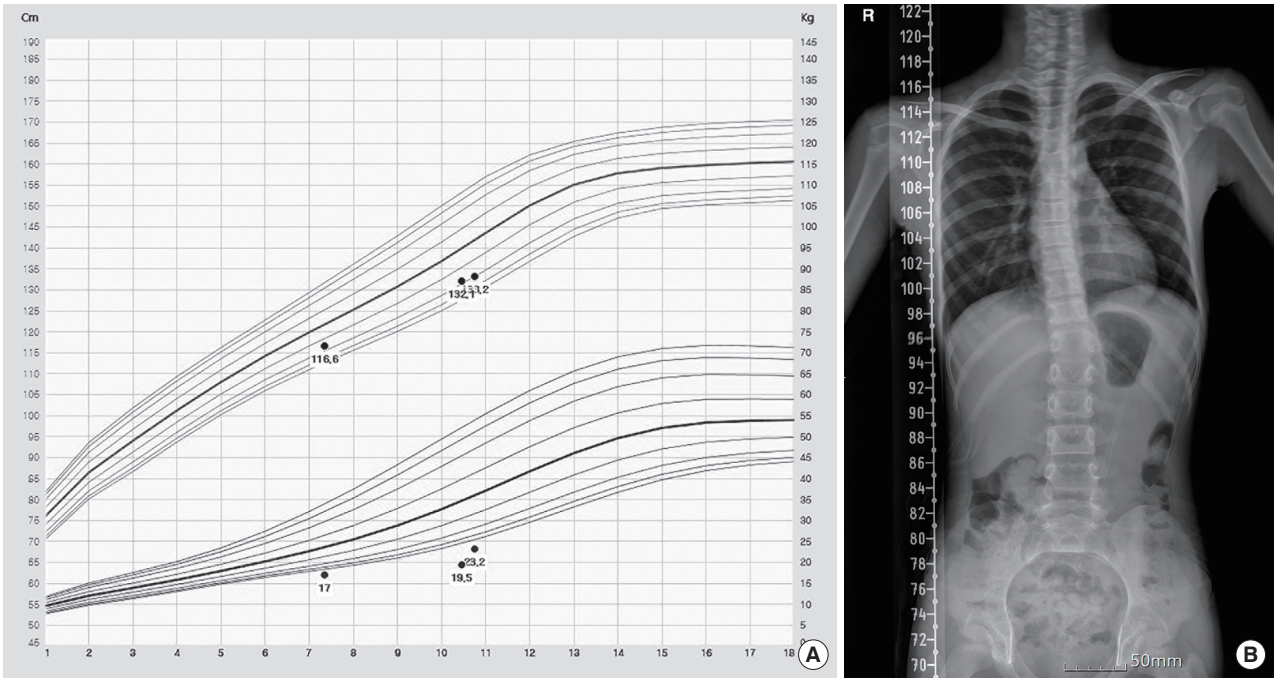


Fig. 1. The growth curve (A) and X-ray findings (B) of a 10-year-old Korean girl with 16p11.2 duplication syndrome. Her height was within the 5-10th percentile (upper curve) and her weight was under the 3rd percentile (lower curve) showing poor weight gain and failure to thrive (A). The radiological findings suggested right thoracolumbar scoliosis with an apex at T12 and a Cobb angle of 6° (B).

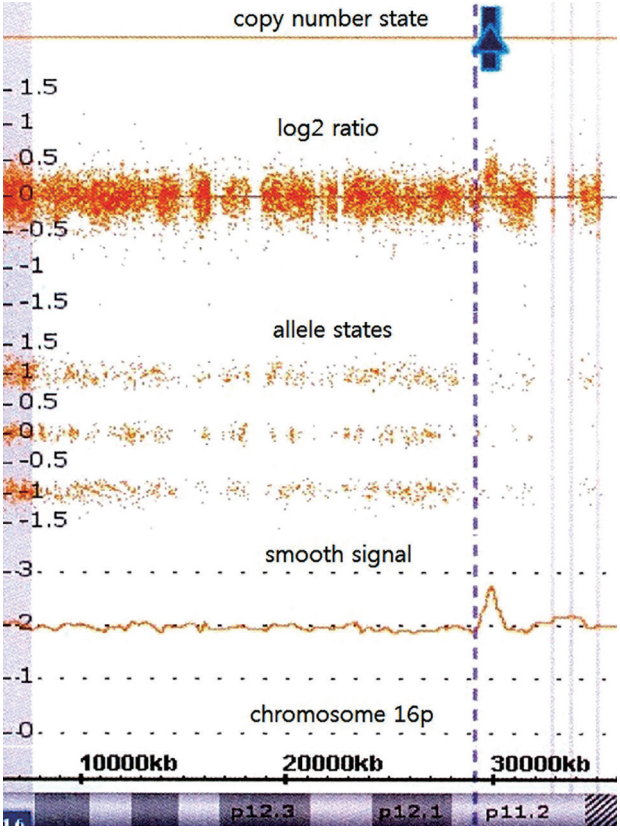


Fig. 2. The chromosomal microarray analysis (Affymetrix Cytoscan 750K array, Genome build: Hg19) of a 10-year-old Korean girl with 16p11.2 duplication syndrome. The patient's microarray showed a gain of 610kb at the 16p11.2 site: arr[GRCh37] 16p11.2 (29580020_30190029)×3.

supplements. During the endocrinological evaluation, there was no abnormal finding in the laboratory test results including insulin-like growth factor-1, insulin-like growth factor binding protein-3, thyroid-stimulating hormone, free thyroxine 4, adrenocorticotropic hormone, cortisol, luteinizing hormone, follicle stimulating hormone, estradiol, 25-hydroxyvitamin D, and parathyroid hormone levels. Her bone age was estimated to be 10.5 years, which was normal. This study was approved by the institutional review board of Keimyung University Dongsan Hospital (Approval No. 2019-04-005).

DISCUSSION

Recurrent 16p11.2 deletions or duplications (approximately 600 kb, chr16; 29.6–30.2 mb-HG19) are risk factors for neurodevelopmental and psychiatric problems [2, 3]. CNVs in 16p11.2 are known to occur in a frequency of 1-3 per 10,000 [4]. About 70% of the 16p11.2 deletions occur *de novo*, while around 70% of the 16p11.2 duplications are familial [5]. The parents of this patient were symptom-free and did not undertake any special investigations. However, a prenatal genetic test to rule out the possibility of gonadal mosaicism will be necessary for her parents for subsequent pregnancies.

Language delay and cognitive impairment are the most common clinical characteristics of 16p11.2 CNVs [6]. In addition, motor delay, seizures, behavioral problems (especially ADHD), congenital anomalies, and autism are frequently observed symptoms in CNVs [6]. The patients with 16p11.2 duplication have a higher frequency of ADHD [1, 7]. The 16p11.2 duplication carriers have a higher frequency of psychiatric disorders and ADHD than the normal controls and the 16p11.2 deletion carriers [1]. The frequency of ASD was similar between the duplication carriers and the deletion carriers [1, 8]. The patient had typical features of 16p11.2 duplication syndrome including language delay, intellectual impairment, and ADHD. However, she does not have any sign of ASD. She is friendly with people, has good eye contact, and maintains a good relationship with her mother.

Interestingly, 16p11.2 deletion and duplication have opposite patterns of HC and body mass index (BMI) [6]. The 16p11.2 deletion is associated with large HC and risk of obesity, while 16p11.2 duplication is associated with small HC and low BMI [6]. Several studies provided evidence supporting the linkage between small HC with schizophrenia and large HC with ASD [9-12]. There are several genes in the site of 16p11.2. Among them, *KCTD13* is suggested as a major driver of head size phenotypes in 16p11.2 CNVs through the regulation of neurogenesis by the animal data [13]; 1) only one gene (*KCTD13*) of the 16p11.2 site showed microcephaly in the overexpression test in vivo; 2) the reciprocal suppression of this locus resulted the mirrored phenotype, macrocephaly; 3) a neurogenic defects were established by the functional analyses [13]. Potassium channel tetramerization domain containing 13 encoded by *KCTD13* interacts with the proliferating cell nuclear antigen, thus might be involved in cell cycle regulation during neurogenesis [14]. This patient also had a small HC without ASD. Although it was difficult to compare her HC directly to normal control due to lack of data on normal HC for Korean children of ≥7 years, she also showed a relatively small HC as compared to her peers. Although she is still young and does not present the symptoms of schizophrenia yet, it is necessary to take appropriate psychiatric measures as there is a possibility that she may develop schizophrenia later.

CONFLICT OF INTEREST

There are no potential conflicts of interest relevant to this article.

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4) For acronyms, provide the full names below the corresponding table. Symbols should be marked with small alphabet letters in the order of its usage, such as, [a, b, c, d, e] with their respective descriptions in the footnote.
5) Tables should be easy to understand while functioning independently.
6) Unnecessary longitudinal lines should not be drawn. Horizontal lines should be refrained from being used as much as possible.

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1) Submit the figures separately from the main text. The resolution power of the pictures and photographs is expected to exceed 300 dpi. Figures should be included with online submissions, either as JPEG, GIF, TIFF, BMP, PICT with RTF manuscripts or embedded in the PDF file.
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Nonessential tables and figures may accompany articles as online-only supplemental files. All online-only supplementary files should be combined in one document file (whenever possible) and uploaded separately during the submission process. This file must be clearly labeled as "Online-Only Supplemental Material." In addition, supplemental onlineonly files must be referenced in the main text of the manuscript at least once (e.g., "Supplemental Table S1").

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