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New Drug Development of Myotonic Muscular Dystrophy

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Myotonic muscular dystrophy is a disease characterized by progressive muscle weakness with myotonia and multiorgan involvement. Two subtypes have been recognized; each subtype is caused by nucleotide repeat expansion. So far, there has been no cure for myotonic muscular dystrophy. In this article, we introduce ongoing clinical trials for new drugs to modify disease course by correcting genetic derangement or its downstream in myotonic dystrophy type 1.

Key words: Myotonic Dystrophy, Genetic Therapy, Drug Development, Muscular Dystrophies, Oligonucleotides

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INTRODUCTION

Myotonic muscular dystrophy is an autosomal dominant disorder characterized by progressive weakness and myotonia, accompanied by multisystemic involvement [1]. It is the most common form of adult-onset muscular dystrophy. Two subtypes have been recognized, each harboring nucleotide expansion of noncoding regions.

Myotonic dystrophy type 1 (DM1) is caused by a large trinucleotide repeat expansion in the untranslated tail of the dystrophia myotonica protein kinase (DMPK) gene. This mutation leads to mis-splicing of mRNA species and results in abnormal cellular processes and diverse symptoms. Muscle weakness in DM1 patients begins from face, ankle dorsiflexor, and finger flexor, which progresses to the other skeletal muscles including respiratory muscle. Frequently accompanied are cardiac arrhythmia, cataract, diabetes mellitus, respiratory dysfunction, and cognitive impairment. Cardiac and respiratory dysfunction reduces median life span to the age of 55 years in DM1 patients. Most severe cases may show neonatal hypotonia with respiratory failure.

Myotonic dystrophy type 2 (DM2) is caused by unstable tetranucleotide repeat expansion (CCTG) in the first intron of the CNBP gene on chromosome 3q21. Like in DM1, the repeat expansion has been shown to cause mRNA splicing errors, which lead to widespread cellular abnormalities and multisystemic symptoms [2]. Unlike DM1, the extent of repeat expansion in DM2 is less related to the disease severity, and anticipation is not observed in DM2. Patients with DM2 show proximal muscle weakness and myalgia.

Current management of myotonic muscular dystrophy is focusing on symptomatic control of myotonia, cardiac care, and physical support of skeletal and respiratory muscle weakness. Multidisciplinary management by cardiologists, ophthalmologists, and endocrinologists for systemic complications is required. There is no cure or disease modifying therapy targeting the pathomechanism of myotonic muscular dystrophy. In this article, we briefly review molecular pathomech-

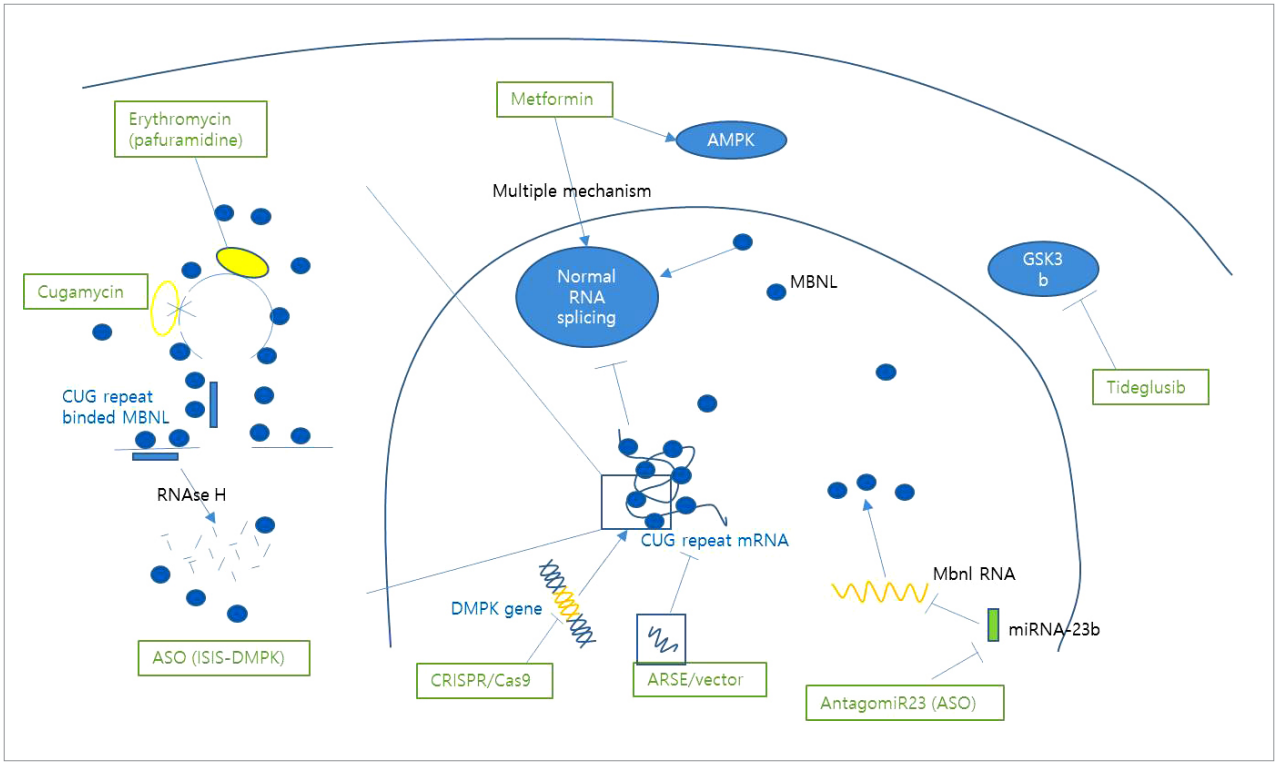


Fig. 1. Drug action site of developing drugs for myotonic dystrophy type 1. Green boxes are developing drugs, blue fonts are substances related to DM1 pathomechanism.

anism of DM1, and introduce emerging novel drugs in clinical development stages to treat DM1 (Fig. 1).

PATHOGENESIS OF MYOTONIC MUSCULAR DYSTROPHY

DM1 is caused by the expansion of the unstable CTG repeat in the 3′-untranslated region (UTR) of DMPK gene on chromosome 19q [1]. Normal individuals have 5 to 37 CTG repeats. Individuals with 38 to 49 CTG repeats are asymptomatic, but have risk of having children with large repeats by the phenomenon known as *anticipation* [3].

There are correlations between longer CTG repeat in DMPK and earlier age onset with more severe clinical manifestations. Patients with repeat expansions between 50 and 150 have late-onset, mild symptoms of DM1. CTG repeat ranges from 50 to 3,000 in patients with DM1. People with less than 70 repeat may not show neurologic symptom until their 6th decade, while those with 70-90 repeats may show mild symptoms after age 40. Congenital DM1 cases usually show more than 1,000 repeats [4]. The CTG repeat length varies widely by organ systems, which can make the correlation of repeat length and clinical severity less predictable. For example, CTG repeat is more stable in leukocytes than in skeletal or cardiac muscles, which can lead to underrepresentation of the result from blood tests compared with the burden of CTG repeat in other organ systems [5].

Expanded CTG repeat of DMPK gene transcribes into expanded CUG repeat on DMPK mRNA, which sequestraters RNA-binding proteins and disrupts splicing machinery. RNA-binding proteins such as muscleblind-like splicing regulator 1 (MBNL1) and CUG binding protein 1 (CUG-BP1) are affected [1]. MBNL1 co-locates in foci with the CUG repeat expansion [6]. Depleted MBNL1 results in abnormal splicing of mRNA transcription, which lead to abnormal expression of other genes [7]. For example, abnormal expression of chloride channel leads to myotonia, and loss of the insulin receptor proteins are associated with insulin resistance [8,9].

CUG repeat upregulates CUGBP1 proteins that also result in various abnormal mRNA splicing [10]. In addition, bi-directional antisense transcription, dysregulation of microRNAs and potentially non-ATG-mediated translation of homopolymeric toxic proteins have been introduced as novel mechanisms of DM1 [11].

DRUG DEVELOPMENT OF MYOTONIC MUSCULAR DYSTROPHY

At this point, there is no therapy that can cure myotonic dystrophy. Multidisciplinary management, involving neurology, cardiology, endocrinology, pulmonology and rehabilitation medicine, is needed to control widespread organ dysfunction. Recent advances in knowledge on the underlying molecular mechanism of myotonic dystrophy guides new approaches for disease specific treatments for DM1. Novel medications such as antisense oligonucleotides and gene therapies try to correct the disease process at the gene level. Small molecules to reduce toxic mRNA products or prevent interaction of CUG repeat and RNA-splicing proteins have been introduced. Some legacy drugs may be used for symptom controls or mitigate the metabolic derangement.

ANTISENSE OLIGONUCLEOTIDES (ASO)

ASOs are short, synthetic, single stranded oligonucleotides or its analogs that bind to a specific sequence of single strand RNA [12]. ASO therapies target the fundamental pathogenetic process, so it has theoretically higher chances of success than therapies targeting downstream pathways. Two distinctive strategies that use ASOs to treat myotonic dystrophy are under investigation: degradation of the mutant RNA transcript via direct targeting of the expanded CUG repeat, and steric hindrance of the toxic RNA to prevent binding and sequestration of critical RNA-binding proteins [12].

ISIS-DMPK2.5 is a 2′-O-methoxyethyl (MOE) gapmer antisense drug that promotes degradation of the mutant DMPK transcript by the RNase H mechanism [13,14]. It targets CUG repeats in the 3′-UTR. The ASOs had 2′-MOE modifications at both ends and a central gap of 10 unmodified DNA nucleotides to allow RNase H activity. In DM1 mice model, administration of this ASO resulted in the reduction of CUG expanded RNA in skeletal muscle, modifying physiologic and histopathologic features [13]. However, the drug failed to achieve desired therapeutic level in skeletal muscle in phase 1/2a clinical trial.

ASO blockers are in the developmental stage. They block the binding of MBNL 1 to the expanded CUG repeats of DMPK without inducing RNase H activity [12]. Peptide conjugated oligonucleotides may overcome inefficient delivery to the target tissue. PGN-EDODM1 hybridizes to the CUG repeat and blocks the interaction of MBNL1 with the toxic hairpin loops [15]. Oligonucleotides conjugated with target organ specific antibodies (AOC-1001) are in the preclinical stage [16].

MicroRNAs are regulators of mRNA expression. In DM1, microRNA-23b-3p (miR-23b) regulates expression of MBNL1 and MBNL2. AntagomiR23, an antisense technology blocking miRNA-23b in preclinical development, increases MBNL1 protein levels [17].

GENE THERAPY

Gene therapy is a medical approach to genetically modify cells to manage the disease by repairing or reconstructing defective genetic material. The genetic informations delivered to the target cells as naked DNA, or by viral vectors or lipid complexes. Furthermore, there have been attempts to apply CRISPR-Cas9 gene splicing technology to manage DM1.

Artificial site-specific RNA endonuclease (ASRE) targeting CUG repeat RNA have been reported. It degrades pathogenic DMPK mRNA with minimal effect on wild type alleles. ARSEs with gene delivery vectors are in preclinical stage [18].

Gene editing machinery including the CRISPR-Cas9 system is also under investigation. Lo Scurudato *et al.* [19] reported a RNA-Cas9 complex consisting of Cas9 from *Staphylococcus aureus* and selected pairs of single guide RNAs derived from DM1 patient muscle cells carrying 2,600 CTG repeats. It resulted in targeted DNA deletion, ribonucleoprotein foci disappearance, and correction of splicing abnormalities in various transcripts [19].

SMALL MOLECULES FOR BLOCKING TOXIC MRNA

Major downstream pathomechanism in myotonic muscular dystrophy is the splicing protein disruption by CUG repeat RNA. Several studies have shown that blocking CUG repeat RNA with oligonucleotides, peptides or small molecules can improve toxic effect of expanded CUG repeat [20].

Nakamori *et al.* [21]. reported that erythromycin, a well-known antibiotic, shows high affinity to expanded CUG repeat and inhibits the binding of MBNL1 to CUG repeat. It is under phase II clinical trial for DM 1 evaluating functional outcome and splicing abnormality. Combination trial of erythromycin and pafuramidine, an experimental drug for pneumocystis pneumonia, has displayed synergistic rescue of mis-splicing in DM1 mice model [22]. Cugamycin, a newly-developed molecule, showed cleavage of expanded CUG repeat while it left short CUG repeat untouched, in mouse model [23].

MEDICATIONS FOR MODIFICATION OF DOWNSTREAM RNA SPLICING PROTEIN

Multisystemic dysfunctions of DM1 are caused by malfunction of RNA splicing proteins induced by toxic RNAs accumulated in the nucleus. There have been trials to modify disease course of DM1 by intervening RNA splicing proteins: inducing MBNL overexpression or CUGBP proteins regulation.

Metformin is a popular drug for diabetes mellitus, and many myotonic dystrophy patients take this medication to manage glucose intolerance. Laustriat *et al.* [24]. reported that metformin change RNA splicing deficit by mechanism of AMP-activated protein kinase (AMPK) activation and downregulation of RNA binding motif protein 3 (RBM3). Metformin has shown beneficial effects on motor improvement of myotonic muscular dystrophy type 1 patients, by several molecular mechanisms on RNA splicing, autophagia, insulin sensitivity or glycogen synthesis. Bassez *et al.* [25] reported phase II clinical trial results that presented metformin at the maximal tolerated dose provided a promising effect on the mobility and gait in myotonic dystrophy patients.

Mutant CUG repeats increases glycogen synthase kinase 3 beta (GSK3b) expression. Upregulated GSK3b causes mis-splicing of effectors responsible for the differentiation of muscle tissue and the formation of synapses in the central nervous system. Tideglusib, a GSK3b inhibitor, influence the regulation of mutant DMPK mRNA and normalize CUGBP1 activity, which lead to favorable outcomes for adult-onset and childhood-onset DM1 patients in recent phase II clinical trial [26].

Chloroquine, which has anti-autophagic activity, up-regulated of MBNL1 and 2 proteins and improved functional outcome in DM1 mice [27].

MEDICATIONS FOR SYMPTOM MANAGEMENT

Mexiletine is a class 1B antiarrhythmic agent with high affinity for muscle sodium channels. Prior studies have demonstrated short-term effects of hand-grip myotonia in DM1 and non-dystrophic myotonia. In a recent randomized controlled study, mexiletine could not improve 6 minute walk distance at 6 months, but still had a positive effect on hand grip myotonia. No effects on cardiac conduction were seen on 6-months follow up period [28].

Possible effectiveness of cannabidiol and tetrahydrocannabinol for myotonia and myalgia have been reported [29]. Pitolisant, a new H3 receptor antagonist, may help manage excessive daytime sleepiness [30].

CONCLUSION

Myotonic muscular dystrophy is a multisystemic disease from noncoding repeat expansion defects. Clinical trials are underway targeting various steps in pathogenesis. Gene therapy and ASOs correct the genetic derangements, while small molecules mitigate downstream mechanisms. Drug repositioning of legacy medications, such as metformin and erythromycin, may provide an alternative approach with safety and accessibility.

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Campomelic dysplasia: A review of a rare lethal genetic disorder

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Campomelic dysplasia (CD) is a rare genetic disorder characterized by multiple skeletal anomalies and the abnormal development of male reproductive organs. To date, the *SOX9* gene is the only known causal gene for CD, and approximately 90 causative mutations in *SOX9* have been identified worldwide. CD is diagnosed based on clinical characteristics of skeletal dysplasia (e.g., short bowed long bones, kyphoscoliosis, bell-shaped thoracic cage with 11 pairs of ribs, and hypoplastic scapulars), typical facial features of Pierre Robin sequence with cleft palate, and gonadal dysgenesis in 46,XY individuals. Most patients with CD exhibit life-threatening respiratory failure owing to laryngotracheomalacia and hypoplastic thorax during the neonatal period. Although fatal complications decrease after infancy, several medical conditions continue to require proper management. A better understanding of this rare but lethal condition may lead to more appropriate treatments for patients.

Key words: Campomelic dysplasia, *SOX9*, Osteochondrodysplasias, Gonadal dysgenesis

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INTRODUCTION

Campomelic dysplasia (CD; OMIM #114290), a rare genetic disorder characterized by skeletal abnormalities and male to female sex reversal, was first described by Maroteaux *et al.* in 1971 [1]. Due to the skeletal anomalies in the airway and thoracic cage, patients with CD often present with life-threatening respiratory failure, particularly during neonatal periods [2]. The incidence rate of CD has been reported at approximately 0.05-0.09 per 10,000 live births [3], and no reliable data exists on the prevalence which is currently estimated as 1:40,000 to 1:80,000 [2]. Data have been reported across case reports and small series, and three Korean cases have been published [4-6]. The *SOX9* (17q 24.3-q25.1) gene is the only known causal gene for CD [7-9]. To date, approximately 90 causative mutations have been identified in *SOX9*, which are listed on the Human Gene Mutation Database (HGMD; http://www.hgmd.org). Most mutations are sporadic and familial CD is rarely found, although CD is inherited in an autosomal dominant manner.

The term "campomelic" comes from the Greek for "bent limb", but this characteristic is neither specific to CD nor a mandatory finding for diagnosis. Bowing of the long bone is present in many other skeletal dysplasias (e.g., osteoporosis imperfections), and is absent in about 10% of cases of CD, a condition called acampomelic CD, which shares the same OMIM number as CD [2].

CLINICAL CHARACTERISTICS

A wide spectrum of pathologic clinical features involving multiple organs is observed in CD, not only skeletal anomalies but also dysmorphic facial features and male sex reversal (Table 1). Skeletal dysplasia, which is the most representative

Table 1. Common clinical and radiographic features of campomelic dysplasia

System	Clinical features	Radiographic features
Head	Relatively large head Dolichocephaly	Ventriculomegaly
Face	High forehead Midface hypoplasia Flattened nasal bridge Long philtrum Cleft palate Micrognathia and microglossia Low-set ears	
Neck	Cervical spine instability Laryngotracheomalacia	Cervical spine dislocation
Thorax	Bell-shaped thorax Scoliosis (kyphoscoliosis)	Scapular hypoplasia Hypoplastic vertebral pedicles Eleven pairs of ribs
Pelvis and genitalia	Ambiguous genitalia or female external genitalia in 46,XY individuals	Narrow vertical iliac bodies Subluxation of hip joints
Extremities	Short, bowed limbs (occasionally upper limb) Pretibial skin dimples Brachydactyly Clubfoot	Bowed femora and tibiae

characteristic of CD, includes bowing and shortening of long bones, hip dislocation, clubfoot, and pretibial skin dimples due to bowing of the lower legs [2]. Skeletal radiographs of patients with CD typically show spine anomalies, scoliosis, hypoplastic scapulae, bell-shaped thorax with 11 pairs of ribs, and vertically oriented narrow iliac wings (Fig. 1).

Patients with CD also exhibit dysmorphic facial features known as the Pierre-Robin sequence (PRS) with cleft palate, including high forehead, midface hypoplasia, hypertelorism, long philtrum, micrognathia, and low-set ears. Another specific characteristic of CD is male to female sex reversal that results from the abnormal development of male reproductive organs. Approximately 75% of 46,XY patients have ambiguous or complete female genitals [2]. Sensorineural and conductive hearing impairment and laryngotracheomalacia are common, and ventriculomegaly, which is usually of the communicating type, is also found in patients with CD. Some patients have reported minor congenital heart defects, such as an atrioseptal defect [4,5].

DIAGNOSIS

There is no consensus on the diagnostic criteria for CD, but clinical diagnosis can be established based on clinical features and radiographic interpretation [2]. In particular, radiographic findings, including short bent limbs, bell-shaped thoracic cages with 11 pairs of ribs, or hypoplastic scapulars, are consistent and reliable diagnostic cues for CD.

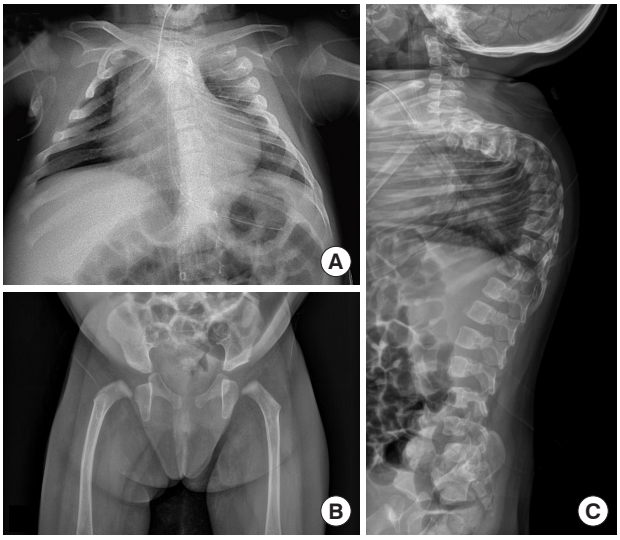


Fig. 1. Radiographs of a 7-month-old girl with campomelic dysplasia showing (A) a bell-shaped thoracic cage, 11 pairs of ribs, scapular hypoplasia, (B) vertical ischia, bowing of the right proximal femur, and (C) thoracic kyphosis.

Similar clinical manifestations between CD and some diseases should be considered during differential diagnosis. For example, patients with spondyloepiphyseal dysplasia congenita, one of the type 2 collagen disorders caused by *COL2A1* mutations, present a dysmorphic faces with cleft palate and short limbs [10]. Also, patients with Stickler syndrome, a mild form of type 2 collagen disorder, have similar facial features to CD. Bowing long bones suggest a rare but fatal skeletal disorders not only in CD but also in other conditions. In a study by Tonni *et al.* [11] molecular and histopathological investigations revealed that four fetuses presented bowing limbs on prenatal ultrasound and were diagnosed with CD with a *de novo* mutation in *SOX9*, osteogenesis imperfecta type II, Cumming syndrome, and femoral-facial syndrome.

SOX9 is the only known causative gene of CD, and the presence of a heterozygous pathogenic variant in *SOX9* can confirm the diagnosis of CD [2]. Sequence analysis of *SOX9* allows the detection of the majority of pathogenic variants. Using chromosomal microarray analysis, large deletions and duplications that cannot be detected by sequence analysis can be identified. Rarely, a reciprocal translocation that involves the *SOX9* locus but does not result in *SOX9* copy number changes can be found through a karyotype [12].

GENES AND MOLECULAR PATHOGENICITY

The *SOX9* gene codes for a high mobility group (HMG) DNA-binding domain-containing transcription factor that plays an important role in multiple organ development, especially in chondrogenesis and sex determination. In 1994, Foster *et al.* established a high resolution map across a 20 Mb region of chromosome 17q24.1-q25.1 that was previously revealed to contain the locus that is responsible for CD [7-9].

SOX9 is a master regulator of chondrogenesis [13]. *SOX9* is expressed in prechondrocytes and chondrocytes during embryogenic development, and is an essential transcription factor that controls chondrocyte differentiation and cartilage formation. *SOX9* is co-expressed with *COL2A1*, which encodes type II collagen, the major cartilage matrix protein, and *SOX9* protein binds to sequences in *COL2A1* and activates chondrocyte-specific enhancers in nonchondrocytic cells [14,15]. As *COL2A1* expression is directly regulated by the *SOX9* protein, mutations in *SOX9* lead to decreased *COL2A1* expression, resulting in skeletal dysplasia.

Moreover, *SOX9* expression is also detected in non-chondrogenic tissues, such as the neural crest, gonad, otic vesicle, lung, notochord, neural tube, pancreas, and cardiac cushions during embryonic development in vertebrates [14,16,17]. These results suggest that *SOX9* mutations are related to deformities that include multiple organs not only skeletal systems in CD. Embryonically, the expression of *SOX9* is closely correlated with the formation of neural crest cells, a group of cells unique to vertebrates that contribute to the development of the craniofacial complex [18]. *SOX9* acts as a craniofacial regulatory element and is associated with large deletions or translocations found in non-syndromic PRS [19]. This can be the basis for explaining the formation of cleft palate and the typical facial features of CD.

In mammalian male development, the *SRY* (sex-determining region on the Y chromosome) gene is fundamental and initiates testis differentiation of the undetermined male gonads [20]. *SOX9* (SRY-related HMG-box gene 9) is another key gene involved in a cascade for testicular formation. Shortly after *SRY* activation, *SOX9* is activated and involved in the male sex development process at various stages, including glycogenesis in pre-Sertoli cells, coelomic epithelium proliferation, mesonephric migration, vasculogenesis, and testicular cord formation [21]. However, *SOX9* gene mutations do not cause sex reversal in all male patients. Approximately 25% of male patients with CD do not exhibit sex reversal [2], as seen in the patient with a normal male phenotype of the two 46,XY karyotypes of Korean patients (Table 2). The cooperative dimerization of *SOX9* with other genes is essential for chondrogenesis but not for gonadal development [22]. In sex development, *SOX9* proteins act as monomers and bind sex-determining genes, such as *SF1*, which might explain why CD is not always accompanied by sex reversal.

GENOTYPE-PHENOTYPE CORRELATIONS

To date, 140 *SOX9* gene mutations have been reported on the HGMD, among which only 87 have been identified as CD-causing mutations. Of these 87 mutations, almost all of them were detected using a sequence analysis of *SOX9*: 39 missense/nonsense mutations, 16 small insertions, nine small deletions, three splicing mutations, one small indel mutation, and one regulatory abnormality. Ten large deletion mutations and eight complex rearrangements have been also reported. In three Korean patients with CD, three different novel heterozygous mutations in the *SOX9* gene have been reported; two were small deletions [4,6] and one was a nonsense mutation [5].

In CD, there is no clear genotypic phenotype correlation re-

Table 2. Summary of *SOX9* gene mutations and the clinical characteristics in three Korean patients with campomelic dysplasia

Patients	Type	Exon	Mutation	Karyotype	Sex phenotype	Medical conditions	Clinical course
1 [4]	Small deletion	3	c.1372del (p.Gln458ArgfsX12)	46,XY	Male	Laryngomalacia Atrial septal defect Recurrent pneumonia	Death at 4 months
2 [5]	Nonsense	3	c.1105C>T (p.Gln369*)	46,XX	Female	Ventriculomegaly Laryngotracheomalacia Atrial septal defect Gastroesophageal reflux	Alive at 40 months Global developmental delay
3 [6]	Small deletion	1	c.235delC (p.Gln79Argfs*31)	46,XY	Female	Tracheal ring Tracheomalacia Dysphagia Hearing impairment	Alive at 2 years Home ventilator Global developmental delay

garding mutation type or location and clinical severity [23]. However, some degree of correlation is observed in some cases. The farther the translocation breakpoint is from *SOX9*, the milder the phenotype, including its impact on male genitalia and skeletal anomalies [24]. This property was observed in a family with acampomelic CD which was transmitted through several generations [25]. Moreover, several cases with isolated disorders of sex development were identified with duplications or deletions involving a region located approximately 600 kb upstream of *SOX9* [26]. Notably, among the 53 mutations not related to CD out of 140 *SOX9* mutations, more than half have been classified as isolated disorders of sex development (n = 29), and other phenotypes of PRS with or without minor skeletal anomalies (n = 17). Most of these mutations are associated with large deletions, large insertions/duplications, and chromosomal rearrangements. These types of *SOX9* mutations appear to be associated with mild phenotypes that do not involve multiple organs.

MANAGEMENT OF MANIFESTATIONS

During the neonatal period, CD is a life-threatening condition primarily because it causes respiratory failure. Fatal respiratory failure is associated with tracheobronchomalacia or cervical instability, rather than with a hypoplastic thoracic cage [2]. Many of the surviving patients require a tracheostomy to relieve laryngotracheomalacia, and home mechanical ventilator support is needed because of the restrictive lung condition caused by hypoplastic thoracic cage, scoliosis, and tracheobronchomalacia. All three Korean patients with CD needed tracheostomy for laryngotracheomalacia. One patient died of respiratory failure at 4 months of age [4]. Of the two surviving patients, one was able to discontinue mechanical ventilation after a closure operation of the atrioseptal defect [5], while the other patient required home ventilator support [6].

Although fatal complications decrease after infancy, mental retardation, global developmental delay, feeding problems, scoliosis, and respiratory complications will continue to need medical intervention. For patients with medical conditions such as cleft palate, cervical instability, hip subluxation, and clubfoot, proper management is required. Hearing aids are needed in patients with hearing impairments. Regular radiographic examinations of the spinal curvature are necessary to determine the progression of scoliosis. For cervical instability and progressive kyphoscoliosis that compromises lung function, surgical treatment is required, whereas bracing is usually not helpful [27]. Patients that are 46,XY phenotypic females mostly have internal male genital organs. Gonadectomy is recommended because of the increased risk of malignant changes, as reported in a phenotypic female who developed gonadoblastoma at age 3 [28].

FAMILY COUNSELING

Most reported *SOX9* mutations are *de novo* mutations found in sporadic cases. However, due to the autosomal dominant inheritance manner of CD, a genetic approach to the proband's parents and siblings is also required. As some families with mosaicism have been reported [29], prenatal evaluation and diagnosis are recommended for future pregnancies as there is a risk of recurrence in these families. For fetuses at risk of CD, fetal ultrasound allows the detection of clues, such as increased nuchal transparency, micrognathia, short bowed limbs, and hypoplastic scapulae [30]. Molecular genetic testing through DNA analysis or chromosomal analysis from amniocentesis is confirmative.

CONCLUSION

CD is a very rare genetic disorder, but it is lethal in affected patients, especially during the neonatal period. This review focused on the clinical and genetic characteristics of patients with CD. A better understanding of patients with CD will allow clinicians to provide more appropriate care to patients. Early diagnosis and management of the associated complications in affected patients can prevent deterioration of their clinical conditions and improve their quality of life.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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Genetics of Prader-Willi Syndrome

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Prader-Willi syndrome (PWS) is a rare genetic disorder which lead to severe neurodevelopmental, endocrine, and metabolic impairment. PWS is genetic disorder related to genomic errors which lead to inactivation of paternally-inherited genes on chromosome 15q11-q13. Epigenetic mechanisms are also involved in PWS, and epigenetic therapies are under investigation. Here we provide review about genetics of PWS, focused on genes involved in pathophysiology of PWS. We will also summarize epigenetics and genetic counseling of PWS.

Key words: Prader-Willi Syndrome, Genetics, Phenotypes

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OVERVIEW

Prader-Willi syndrome (PWS; OMIM 176270) has an estimated prevalence of 1/10,000-1/30,000, and males and females are affected equally in all ethnic groups [1]. The PWS critical region is located on chromosome 15q11-q13. Normally only paternally inherited genes on this region are expressed, and PWS is caused by the abnormal expression of one or more of these genes [2,3]. Genetic mechanisms of PWS are paternal deletion of the 15q11-q13 region; maternal uniparental disomy 15; and imprinting defects [4]. Deletions in PWS are further divided into type-1 and type 2 deletion (Fig. 1).

PWS affects multiple body systems. Prenatal growth is usually normal. Fetal movement is decreased, and there is increased need of assisted delivery or cesarean section [5,6]. At birth weight and body mass index (BMI) are 15% lower than average. Severe hypotonia during infancy can lead to poor suck and failure to thrive. Hypotonic status improve after infancy, but mild-to-moderate hypotonia persists throughout life [7,8].

As they grow up, they show hyperphagia due to lack of satiety with obsessive food seeking. They become severely obese unless food intake is strictly controlled. In uncontrolled cases, obesity, and its complications are the major causes of morbidity and mortality. Most PWS patients show developmental delay and intellectual disability [9].

Endocrine dysfunction is also common in PWS. Growth hormone (GH) deficiency is present in about 74% of cases [10,11]. GH replacement therapy has positive effects not only on growth and body composition but also on development, behavior, and nocturnal respiratory abnormalities, although a careful respiratory follow up is mandatory during long-term GH administration [12,13]. Hypogonadism is thought to have a hypothalamic origin, manifested as hypogenitalism, incomplete pubertal development and infertility [14]. Other endocrine abnormalities such as hypothyroidism, central adrenal insufficiency and type 2 diabetes can also occur [9].

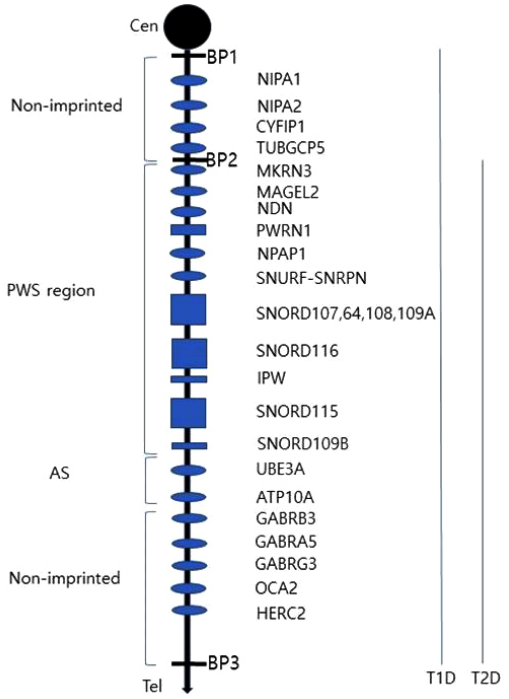


Fig. 1. Chromosome map of 15q11.2-13.1 region. BP1, breakpoint 1; BP2, breakpoint 2; BP3, breakpoint 3; Cen, Centromere; Tel, Telomere. Type 1 deletion (T1D) extend from BP1 to BP3, and type 2 deletion (T2D) extend from BP2 to BP3.

MOLECULAR GENETICS AND DIAGNOSIS

The PWS region is located spans on the long arm of chromosome 15, with length of about 6Mb (Fig. 1). Within this region, at least 2.5 Mb comprises genes which are expressed differentially depending on parental origin. This region also includes several non-coding RNAs, which are believed to be involved in the regulation of alternative splicing [4,15].

Most common genetic cause of PWS patients is deletion of 15q11-q13 from the paternal origin (65-75%) [15,16]. Maternal uniparental disomy (mUPD) accounts for 20-30% of cases, which occurs when both chromosomes 15 are inherited from the mother [6,7]. The least common mechanism (1-3%) is imprinting defects (ID), caused by epimutations or microdeletions in the PWS-imprinting center (PWS-IC).

DNA methylation analysis is usually the first step for diagnosis of PWS DNA methylation analysis can detect all three types. but it cannot distinguish the molecular class (i.e., deletion, UPD, or ID). So further investigations like FISH or CMA to determine molecular class is required. When chromosomal analysis with FISH or CMA rather than DNA methylation was first performed and reveals a deletion in the PWS/AS region (i.e., 15q11.2-q13), it is still necessary to perform DNA methylation analysis to distinguish PWS with Angelman Syndrome.

GENOTYPE-PHENOTYPE RELATIONSHIPS

There is no symptom of PWS which occurs only in specific genotype. But there are some clinical characteristics which show differences in frequency or severity according to genotype. Feeding problems, sleep disturbances, hypopigmentation and speech and language deficits are more common in deletion [17]. Type 1 deletion patients are known to have better academic performance and intellectual abilities, and more compulsiveness than individuals with type 2 deletion [18,19]. mUPD patients show higher incidence of post-term delivery, psychosis and autism spectrum disorder. On the other hand, typical PWS facial appearance or hypopigmentation are less common in mUPD patients [15].

GENES INVOLVED IN PWS

The 15q11.2-q13 region has four distinct regions divided by three common deletion breakpoints [20] (Fig. 1): 1) a proximal nonimprinted region contain four biparentally expressed genes (*NIPA1*, *NIPA2*, *CYF1P1*, and *GCP5*. 2) The "PWS region" contain five polypeptide coding genes (*MKRN3*, *MAGEL2*, *NECDIN*, and the bicistronic *SNURF-SNRPN*), *C15orf2*, a cluster of C/D box small nucleolar RNA genes (snoRNAs), and several antisense transcripts. 3) The "Angelman syndrome (AS) region" contain the preferentially maternally expressed genes *UBE3A* and *ATP10A*. 4) A distal nonimprinted region contain a cluster of three GABA receptor genes, the gene for oculocutaneous albinism type 2 (*OCA2*), *HERC2* [15].

MKRN3

The Makorin Ring Finger Protein 3 (*MKRN3*, *ZNF127*) gene encodes a zinc finger protein of the Makorin family. This gene is paternally expressed. This gene is known to inhibit initiation of puberty, and loss of function mutations in *MKRN3* are recognized as the main genetic cause of central precocious puberty [21]. *MKRN3* is thought to be related with hypogonadism and infertility in PWS.

MAGEL2

Hyperphagia is commonly observed in individuals with PWS, associated with a defect in the hypothalamic arcuate nucleus. Interaction of neuropeptide Y (NPY) and agouti-related pep-

tide (AgRP) stimulates food intake, whereas proopiomelanocortin (POMC) reduces it. Loss of *MAGEL2* expression inhibits POMC neurons, thus food intake is less repressed [22,23]. But patients with point mutations on the paternal allele of *MAGEL2* does not usually show the high appetite and severe obesity though other clinical characteristics of PWS are present, which can disassociate *MAGEL2* and the hyperphagia.

Loss of expression of *MAGEL2* also impairs reproductive function in mice. *MAGEL2*-null females showed extended and irregular estrous cycles, and males displayed decreased testosterone levels, and reduced pheromone detection. These results suggest that lack of expression of *MAGEL2* contributes to the reproductive deficiencies observed in PWS.

NDN

The Necdin (*NDN*) gene encodes a DNA binding protein necdin, which is postulated as a key regulator of GnRH levels both in vitro and in vivo, modulating essential intracellular processes for neurite and axonal outgrowth [24-26]. Also, *NDN* paternal-deficient mice showed irregular breathing and sleep apneas. *NDN* might be a genetic factor contributing to apneas and respiratory dysfunctions of PWS.

lncRNAs

Within the Prader-Willi region there are a series of long noncoding RNAs (lnc RNAs) which are characteristically more than 200 nucleotides long. They are thought to be involved in epigenetic modifications of DNA, and regulation of gene expression at transcriptional and post-transcriptional levels [27-29]. One of the lnc RNAs, Prader-Willi Region Non-Protein Coding RNA 1 (*PWRN1*), is biallelically expressed in the testis and kidneys, and monoallelic expressed in the brain [30]. *PWRN1* is thought to have an indirect role on the imprinting mechanism through keeping the paternal allele in an open chromatin configuration, allowing assess to transcription factors [31].

NPAP1

The Nuclear Pore Associated Protein 1 (*NPAP1*) is biallelically expressed in adult testis and monoallelically expressed in fetal brain, including the hypothalamus which is related to several endocrine features of PWS [30,32]. This gene is also associated with the Nuclear Pore Complex (NPC), in which the main function is to regulate macromolecular transport between the nucleus and the cytoplasm. NPCs also participates in several nuclear processes, such as gene regulation, mRNA biogenesis, and cell cycle control.

SNURF-SNRPN

SNURF-SNRPN is a complex bicistronic gene encoding two different proteins. SNURF is encoded by exons 1-3 and produces a small nuclear protein of unknown function [33], exons 4-10 correspond to the *SNRPN* portion and encode the protein SmN, involved in mRNA splicing [34].

Small Nucleolar RNAs (snoRNAs) are within the *SNURF-SNRPN* transcript. snoRNAs participate in DNA methylation, alternative splicing and post-transcriptional regulation [4,35]. The PWS region encompasses five single copy snoRNA genes (*SNORD64*, *SNORD107*, *SNORD108*, *SNORD109A*, and *SNORD109B*) and two snoRNA gene clusters (*SNORD115* and *SNORD116*). Although most of the SNORDs are ubiquitously expressed in human tissues, *SNORD115* and *SNORD109B* are expressed exclusively in the brain.

SNORD116 has emerged as a critical, and possibly, determinant candidate in PWS. Patients with small deletions (150-200 kb) or translocations showed PWS phenotype [36-38]. Snord116-KO mice also displayed PWS features such as post-natal growth retardation and hyperphagia [39,40]. Mice model *SNORD116* was knocked-out specifically in NPY neurins in the hypothalamic arcuate nucleus showed the same overall phenotype observed in mice lacking *SNORD116* globally; low birth weight, increased body weight gain in early adulthood, increased energy expenditure and hyperphagia [41]. This suggests that *SNORD116* control NPY neuronal functions, and thus food intake and energy homeostasis. Also, *Snord116*-deficient mice showed decreased activity of the hypothalamic prohormone convertase PC1 impairing the prohormone processing of proinsulin, pro-GH-releasing hormone, and proghrelin [42]. In addition, Snord116-deficient mice displayed loss or shift in methylation dynamics of CpG islands in the cerebral cortex dependent on the circadian cycle [43].

SNORD115 gene encodes the serotonin receptor 5-HT2C [35]. *5-HT2C* gene encodes G protein-coupled receptor specific to the brain, whose activation is associated with a variety of physiological processes, such as dopamine modulation, anxiety, sleep regulation, satiety response, energy balance, and locomotor activity [44]. Lack of *SNORD115* is not sufficient to cause PWS, but a phenotypic effect when absent along with other genes in the PWS critical region cannot be excluded. 5-HT2C receptor knockout mice that developed hyperphagia and late-onset obesity [45,46]. The absence of *SNORD115* expression in PWS accompanied by the impairment of the 5-HT2C recep-

tor activity may be partly responsible for some of the behavioral and metabolic features of PWS.

EPIGENETICS

Maternal microdeletions of the Angelman syndrome imprinting control region (AS-ICR) I causes Angelman syndrome [47, 48]. The AS-ICR contains alternate 5′ noncoding exon for *SNRPN* that are uniquely expressed in oocytes [48,49]. Oocyte-specific transcription leads to methylation and transcriptional silencing of the maternal allele of the PWS-ICR. In addition, some individuals with AS have imprinting mutations that deletes a binding site for the transcription factor *SOX2* [50]. These studies of AS imply that AS-ICR has an important role for establishing silencing the maternal allele of the imprinted genes within the PWS locus.

There are several additional epigenetic marks differentiated by parental origin at the PWS-ICR. Histone H3 lysine 9 (H3K9) methyltransferase *SETDB1* associates with the transcription factor *ZNF274* bound to sites within the 5′ cluster of *SNORD116* repeats [51]. Knockdown or inhibition of either *SETDB1* or *ZNF274* induced *SNORD116* transcript expression from the normally silent maternal allele [51-53].

Epigenetic Therapies

The general strategy for epigenetic strategies for PWS involves de-repressing the maternal silent PWS-ICR to activate *SNRPN* and *SNORD116* transcription [54]. Several inhibitors of *EHMT2/G9a*, a histone 3 lysine 9 methyltransferase were able to reactivate the expression of paternally expressed *SNRPN* and *SNORD116* from the maternal chromosome, both in cultured PWS cell lines and in a PWS mouse model [55,56]. Inhibition of *SETDB1* using shRNA knockdown resulted in partial reactivation of *SNORD116* and *116HG* in PWS-derived iPSC cell lines and neurons [51]. In addition, when inactivated *ZNF274* using CRISPR/Cas9 in PWS-derived iPSC lines, reactivation of both *SNRPN* and *SNORD116* resulted, as well as a reduction of H3K9me3 at the PWS-ICR [53]. These studies suggest that epigenetic strategies for unsilencing maternal SNORD116 are potential candidates for treatments of PWS.

GENETIC COUNSELING

Recurrence risk of PWS differs according to genotype. Sporadic deletion cases have < 1% risk of recurrence, while recurrent risk in structural abnormalities of chromosome 15 (such as translocations, ring formation, isochromosome or inversions) can be as high as 25-50%.

Occurrence of mUPD 15 is typically de novo and recurrence risk is low (< 1%). However, there is possibility that the father has a Robertsonian translocation involving chromosome 15. This can result in a sperm nullisomic for 15, which would result in an embryo with mUPD 15. So, the father of the child with mUPD should be recommend to perform chromosomal analysis.

Rarely, a small marker chromosome is present in a patient with mUPD 15 [57]. In these patients, both parents' karyotype should be examined, as these small marker chromosomes may increase the risk for nondisjunction and UPD [58].

The majority (approximately 85%) of PWS patients with an ID have a de novo epigenetic mutation (IIIb) with the recurrence risk below 1%. However, in approximately half of these with microdeletion in the IC (IIIa), the IC deletion is familial and the recurrence risk is 50%. Therefore, for patients with imprinting defects, IC analysis by MS-MLPA (Methylation-Specific Multiplex Ligation-dependent Probe Amplification). MS-MLPA or DNA sequencing should be performed to investigate the exact origin [1,59]. And fathers of patients with an IC deletion should be recommended with DNA methylation and dosing analysis (or sequence analysis) [15].

NOTES

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Review of Genetic Diagnostic Approaches for Glanzmann Thrombasthenia in Korea

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Inherited platelet function disorders (IPFDs) are a disease group of heterogeneous bleeding disorders associated with congenital defects of platelet functions. Normal platelets essential role for primary hemostasis by adhesion, activation, secretion of granules, aggregation, and procoagulant activity of platelets. The accurate diagnosis of IPFDs is challenging due to unavailability of important testing methods, including light transmission aggregometry and flow cytometry, in several medical centers in Korea. Among several IPFDs, Glanzmann thrombasthenia (GT) is a most representative IPFD and is relatively frequently found compare to the other types of rarer IPFDs. GT is an autosomal recessive disorder caused by mutations of *ITGA2B* or *ITGB3*. There are quantitative or qualitative defects of the GPIIb/IIIa complex in platelet, which is the binding receptor for fibrinogen, von Willbrand factor, and fibronectin in GT patients. Therefore, patients with GT have normal platelet count and normal platelet morphology, but they have severely decreased platelet aggregation. Thus, GT patients have a very severe hemorrhagic phenotypes that begins at a very early age and persists throughout life. In this article, the general contents about platelet functions and respective IPFDs, the overall contents of GT, and the current status of genetic diagnosis of GT in Korea will be reviewed.

Key words: Blood platelet disorders, Thrombasthenia, Platelet function tests, High-throughput nucleotide sequencing, Whole exome sequencing, Whole genome sequencing

REVIEW ARTICLE

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INTRODUCTION

Platelets are small fragmented blood cells without nucleus, which are originated from megakaryocytes of bone marrow [1]. Normal platelets play most important roles in primary hemostasis in case of vascular damage, and they are also involved in immune responses, inflammatory reactions, and wound healing [1]. The process of primary hemostasis occurs though several steps with many essential molecules [2]. After vascular damage and exposure of subendothelial collagen, circulating platelets of plasma adhere to the collagen of exposed subendothelium [2]. This step 'adhesion' is mediated by the interaction of platelet surface receptors and adhesive proteins; 1) binding of Glycoprotein (GP) Ia/IIa (integrin α2β1) and collagen at low shear rates and 2) binding of GPIb/V/IX complex and von Willebrand factor (vWF) at high shear rates [3]. After adhesion, platelet 'activation' occurs by adenosine diphosphate (ADP) and thromboxane A2 (TXA2) through signal transduction of G-protein coupled receptors, tyrosine kinase, or GPIIb/IIIa (integrin αIIbβ3) [3]. Activated platelets make their shape irregular with pseudopodia and centralization of granules [1]. The α-granules contain vWF, fibrinogen, coagulation factors, mitogenic factors, and angiogenic factors [4]. The dense granules (δ-granules) contain ionized calcium, ADP, ATP, serotonin and epinephrine [4]. The 'secretion' of these platelet granules promotes the recruitment of circulating platelets into the initial plug [5]. And the cross-linking between the receptor GPIIb/IIIa of platelet and ligands (vWF and fibrinogen) results platelet

'aggregation' which make platelets firmly connected [3]. Further, coagulation factors bind to the phosphatidylserine of platelet phospholipid bilayer membrane, and then generate thrombin in secondary hemostasis; 'procoagulant activity' of platelets [1]. Thrombin formation on the platelet surface make more stable hemostatic plug [1].

If there is a congenital problem with platelets causing defects during these various processes, life-long hemostatic disorders occur, and they are called as 'inherited platelet function disorders (IPDSs)'. According to the function of platelets, IPFDs are classified as follows [6,7]; 1) Defect of platelet adhesion (Bernard–Soulier syndrome and pseudo-von Willebrand disease), 2) Defect of platelet activation (ADP receptor $P2Y_{12}$ defect and TXA2 receptor defect), 3) Defect of secretion of granules (Gray platelet syndrome, Paris–Trousseau/Jacobsen syndrome, Chediak–Higashi syndrome, and Hermansky–Pudlak syndrome), 4) Defect of platelet aggregation (Glanzmann thrombasthenia [GT]), 5) Defect of procoagulant activity (Scott syndrome). The function of platelets, involved genes, inheritance pattern, and defects of platelets of respective IPFDs are summarized in Table 1.

Among several IPFDs, GT is a most representative IPFD with phenotypes of severe repetitive bleeding episodes throughout the patient's life. The accurate diagnosis and differential diagnosis of IPFDs is challenging owing to the unavailability of essential testing methods, including light transmission aggregometry and flow cytometry, in several medical centers in Korea. Although each IPFD is very rare, but among them, GT is a relatively frequently found disease. Thus, in this review, I review the current status of genetic diagnosis of GT in Korea along with the overall contents of GT.

GT

GT (OMIM #273800) was firstly presented as 'hereditary hemorrhagic thrombasthenia without reduction of platelet numbers' by Eduard Glanzmann in 1918 [8]. It is an autosomal recessive disorder caused by mutations of *ITGA2B* or *ITGB3* [9]. It is a very rare autosomal recessive IPFD which occurs with a prevalence of 1 in 1,000,000 individuals [10]. However, Glanzmann thrombasthenia often show higher prevalence up to 1 in 200,000 individuals in some populations due to consanguineous marriages [8]. In patients with Glanzmann thrombasthenia, there are quantitative or qualitative defects in the GPIIb/IIIa complex of platelet, which is the binding receptor for von Willbrand factor, fibrinogen, and fibronectin [11,12]. Thus, patients with Glanzmann thrombasthenia have normal platelet count and normal platelet morphology. But they have severely decreased platelet aggregation in response to ADP, epinephrine, serotonin, thrombin, or collagen. Thus, GT patients have life-long, severe hemorrhagic phenotypes [9].

By the large scale study about the clinical manifestations of patients with Glanzmann thrombasthenia, most patients had typical bleeding symptoms during the first year of life [13]. The median age at diagnosis of Glanzmann thrombasthenia was about 7 years old [13]. Severe persistent epistaxis and easy bruising were the most common symptom in patients with Glanzmann thrombasthenia [11,13]. And menorrhagia was severe during the menstruation in the female patients with Glanzmann thrombasthenia [11]. Although intracranial hemorrhage was rare, approximately 1% of the patients with Glanzmann thrombasthenia had hemorrhage of central nervus system [11,13]. In total, over 80% of patients with Glanzmann

Table 1. Classification of IPFDs according to the platelet functions

Disease	Function of platelet	Defect	Genes	Inheritance
Bernard–Soulier syndrome	Adhesion	GPIb/V/IX	*GPIBA, GPIBB, GP9*	AR (rarely AD)
Pseudo-von Willebrand disease	Adhesion	GPIbα	*GPIBA*	AD
ADP receptor $P2Y_{12}$ defect	Activation	ADP receptor	*P2RY12*	AR
TXA2 receptor defect	Activation	TXA2 receptor	*TBXA2R*	AD
Gray platelet syndrome	Secretion	α-granule	*NBEAL2*	AR (rarely AD)
Paris–Trousseau/Jacobsen syndrome	Secretion	α-granule	*FLI1*	AD
Chediak–Higashi syndrome	Secretion	Dense granule	*LYST*	AR
Hermansky–Pudlak syndrome	Secretion	Dense granule	*HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3, BLOC1S6*	AR
Glanzmann thrombasthenia	Aggregation	GPIIb/IIIa	*ITGA2B, ITGB3*	AR
Scott syndrome	Procoagulant activity	PS expression	*ANO6*	AR

AR, autosomal recessive; AD, autosomal dominant; IPFDs, inherited platelet function disorders; GP, glycoprotein; ADP, adenosine diphosphate; TXA2, thromboxane A2; PS, phosphatidylserine.

thrombasthenia needed red blood cell transfusion [11].

Because of the platelet GPIIb/IIIa defect, the platelets of Glanzmann thrombasthenia exhibit normal count and normal morphology, but platelet function analyser-100 measurements is significantly abnormal with prolonged closure times in both ADP/collagen and adrenaline/collagen cartridges [14]. In platelet aggregation test by light transmission aggregometry, only platelet agglutination in ristocetin (binding of GPIb/IX and von Willebrand factor) is intact, but the platelet aggregation is severely diminished in response to ADP, epinephrine, and collagen [13,14]. Flow cytometry using antibodies to GPIIb (CD41) and GPIIIa (CD61) is useful for diagnosis of Glanzmann thrombasthenia [14]. The genetic test for 2 genes for Glanzmann thrombasthenia, *ITGB2A* and *ITGB3*, are diagnostic. Glanzmann thrombasthenia typically develops as loss-of-function mutations in *ITGB2A* or *ITGB3* genes, encoding GPIIb or GPIIIa of platelets, respectively [9]. However, very rarely, gain-of-function mutations in *ITGB3* had also been reported, which results enhanced fibrinogen binding, thus severe hemorrhagic phenotype [15].

The mainstay of treatment for the patients with Glanzmann thrombasthenia is platelet transfusion [8]. In patients with IPFDs, platelet transfusion is standard modality to control bleeding episodes and is also helpful for perioperative care for prophylaxis [6]. However, adverse events such as transfusion-transmitted infections, allergic reactions, or development of antibodies to platelet surface proteins or HLA antigens should be considered. Thus, transfusion of leukocyte-depleted platelet from the HLA-matched single donor is the most appropriate method to decrease alloimmunization [16]. And recombinant activated factor VII (rFVIIa, Novoseven™) had been approved for use in patients with GT in case of hemorrhagic episodes or prophylaxis before invasive procedures in 2004 [8]. The rFVIIa is recommended by the European Medicines Agency recommend for the patients with GT who have platelet antibodies or refractory hemorrhages despite of platelet transfusions [8]. The rFVIIa, alone or combination with platelet transfusion and/or anti-fibrinolytic agents, is efficient and safe treatment for all patients with GT [17]. In Korea, the use of rFVIIa at 90 μg/kg/dose every 2 hours was approved for the treatment of bleeding or prophylactic manage before invasive procedure in patients with GT with platelet antibodies or platelet refractoriness. In addition, adjuvant anti-fibrinolytic agents also can be used. Anti-fibrinolytic agents (tranexamic acid or aminocaproic acid) are effective for mucosal bleeding and minor surgical procedures [18]. They also can be used in adjunctive therapy for major bleeding episodes with other modalities [18]. And there have been successful hematopoietic stem cell transplantations in patients with GT [16,18]. In patients with IPFDs associated with severe repetitive hemorrhagic problems or high potential for marrow aplasia or malignant transformation, hematopoietic stem cell transplantation can be considered as a curative treatment [14].

There are some rarer types of IPFDs with similar phenotypes compare to Glanzmann thrombasthenia; Bernard–Soulier syndrome, leukocyte adhesion deficiency type III, or *RASGRP2*-related platelet dysfunction [19]. Light transmission aggregometry and flow cytometry are the first-line screening tests recommended by the International Society of Thrombosis and Haemostasis for differential diagnosis of IPFDs [20]. Light transmission aggregometry is known as the gold standard method for diagnosing IPFDs with high sensitivity and specificity [20]. It requires a large sample volume for diagnosis [20]. Further, it is expensive and time-consuming with poor reproducibility [20]. On the contrary, flow cytometry can be conducted with a small sample volume with high sensitivity and specificity [20]. However, it is also expensive and laborious with high inter-laboratory variability [20].

There is a big problem that these two essential tests are only available for research purposes in a few Korean medical centers due to the nature of the Korean national health insurance system. Thus, it is very difficult to accurately diagnose and identify each type of IPFD cases in Korea. The prevalence Korean IPFDs and the distributions of their genetic abnormalities remain also unknown. Therefore, the Benign Hematology Committee of the Korean Pediatric Hematology Oncology Group (KPHOG) conducted a multicenter study for diagnosing IPFDs by next-generation sequencing (NGS) [21], and the results will be discussed in the next chapter.

KOREAN STUDY FOR GT

Although light transmission aggregometry and flow cytometry are recommended first-line tests by ISTH for differential diagnosis of IPFDs [20], only very few Korean centers can fully conduct these essential tests. In other words, it is very difficult to accurately diagnose the patients with IPFD in Korea. Only anecdotal cases of GT had been genetically confirmed and reported in Korea in the past [7,22]. Therefore, the Benign Hematology Committee of the Korean Pediatric Hematology Oncology Group (KPHOG) conducted the multicenter study for Korean IPFDs using NGS from March 2017 to December 2020

Table 2. Baseline clinical information and genetic variants of Korean patients with Glanzmann thrombasthenia by KPHOG study

Clinical information	N
Male : Female	6 : 4
Age of symptom onset (months, range)	1 (0–48)
Bleeding symptoms	
Easy bruising	7
Gum bleeding	6
Whole body petechiae after birth	5
Persistent epistaxis	3
Delayed wound healing	2
Hematoma after vaccination	1
Bleeding after procedure	1
Melena	1
Anal bleeding	1
Hematemesis	1
Muscle hematoma	1
Genetic variants	
ITGB3	
c.1913+5G>T	9 (45%)
c.1451G>T (p.Gly484Val)	1 (5%)
c.917A>C (p.His306Pro)	1 (5%)
c.1595G>T (p.Cys532Phe)	1 (5%)
ITGA2B	
c.2333A>C (p.Gln778Pro)	3 (15%)
c.2975del (p.Glu992Glyfs*)	1 (5%)
c.257T>C (p.Leu86Pro)	1 (5%)
c.1750C>T (p.Arg584*)	1 (5%)
c.1184G>T (p.Gly395Val)	1 (5%)
c.2390del (p.Gly797Valfs*29)	1 (5%)

KPHOG, Korean Pediatric Hematology Oncology Group.

[21]; this study aimed for differential diagnosis and finding causative genetic variants of Korean IPFDs. Targeted exome sequencing, followed by whole genome sequencing was performed. Clinical manifestations were also collected. As a result, unrelated 10 families of GT were found and the causative genetic variants were identified. The median age of symptom onset was very early (1 month after birth), and the most common hemorrhagic symptoms are easy bruising, gum bleeding and whole body petechiae after birth. These clinical manifestations are described in Table 2.

Identified variants of Korean GT patients by KPHOG study are also shown in Table 2. Among the identified variants, c.1913+5G>T of *ITGB3* was the most common (9/20, 45%) [21]. This variant was found as homozygotes in three unrelated patients, and heterozygotes in other three unrelated patients [21]. Park *et al.* [22] also previously had reported that homozygote of c.1913+5G>T in *ITGB3* was found repeatedly among four unrelated Korean GT patients. The variant c.1913+5G>T in *ITGB3* had also been described as g.29107G>T by Tanaka *et al.* [23]; which causes aberrant splicing, thus resulting in a premature stop codon [23]. Because the variant c.1913+5G>T in *ITGB3* is most commonly found and is also frequently found as a homozygote in KPHOG study [21], it is thought to be the founder mutation of Korean GT [22]. Next, c.2333A>C (p.Gln778Pro) of *ITGB2B* was second common (3/20, 15%) and was identified as heterozygote in three unrelated GT patients. This variant had been reported in both Korean patients [22] and Japanese patients with GT [24]. Considering its relatively high minor allele frequency (0.012% in East Asia by ExAC database), it is suggested as Asian founder mutation of GT [21].

Other known variants of GT from Japanese patients, c.917A>C (p.His306Pro) of *ITGB3* [25,26] and c.257T>C (p.Leu86Pro) of *ITGA2B* [27] were found in this Korean study [21]. And c.1750C>T (p.Arg584*) of *ITGA2B* is found in both Korea [22] and Japan [28]. The variant c.2975del (p.Glu992Glyfs*) of *ITGA2B* had been previously reported in Korean subject [22]. In addition, four novel variants - c.1451G>T (p.Gly484Val) and c.1595G>T (p.Cys532Phe) of *ITGB3* and c.1184G>T (p.Gly395Val) and c.2390del (p.Gly797Valfs*29) of *ITGA2B* - were identified in four unrelate subjects with GT [21]. Based on the results of this study, KPHOG is planning to establish a Korean registry of IPFDs. In addition, KPHOG is seeking ways to expand the application of NGS for accurate diagnosis of IPFDs.

CONCLUSION

Platelets have important functions in primary hemostasis with wound healing and formation of immune barrier [1]. Various genetic variants are associated with platelet function and respective IPFDs are rare and hemorrhagic symptoms are similar [6,7]. Further, the modalities for accurate diagnosis and differential diagnosis of each IPFDs are difficult for access in Korea [21]. Over the past few decades, many researchers worldwide have elucidated the etiology of different types of IPFDs [29,30]. Various IPFD-associated genes have been identified and molecular characterization of these disorders is ongoing using NGS [29,30]. With this information, we can better understand the IPFDs.

The prevalence of genetically confirmed IPFDs in Korea has not yet been reported. This may be because the prevalence of IPFDs in Koreans is very low, and the accessibility to the diagnostic tests is also low. These problems indicate the need to establish a network among the Korean physicians for the accurate diagnosis and comprehensive management of the patients

with IPFDs. A domestic IPFD study by KPHOG showed that the genetic confirmation of IPFDs was possible using NGS. Therefore, the application of NGS for IPFDs is thought to be useful for accurate and differential diagnosis of each IPFDs. Although this study is only the beginning, it is expected to be useful for future large-scale research and establishment of the Korean IPFD registry.

CONFLICTS OF INTEREST

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

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