

Diagnosis of Schaaf-Yang syndrome in Korean children with developmental delay and hypotonia

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Abstract

Schaaf-Yang syndrome (SYS) is a recently identified disorder caused by a loss-of-function mutation in a maternally imprinted gene, *MAGEL2*, at 15q11.2q13. Due to its extreme rarity and wide range of clinical severity, clinical suspicion is difficult for a physician. In the current study, its frequency among the Korean pediatric patients with developmental delay (DD) or intellectual disability (ID) was assessed. As the first report of Korean patients with SYS, our study aims to increase the awareness of this condition among the physicians taking care of the pediatric patients with DD/ID and hypotonia.

The patients diagnosed with SYS by whole-exome sequencing (WES) among the 460 Korean pediatric patients with DD/ID were included, and their clinical and molecular features were reviewed.

Four patients (0.9%) were diagnosed with SYS. Profound DD (4 patients), multiple anomalies including joint contractures and facial dysmorphism (4 patients), generalized hypotonia (3 patients), and severe respiratory difficulty requiring mechanical ventilation (3 patients) were noted in most cases, similar to those in previous reports. Sleep apnea (2 patients), autistic features (2 patients), a high grade of gastroesophageal reflux (1 patient), and seizures (1 patient) were found as well. A total of 3 different truncating *MAGEL2* mutations were identified. A previously-reported mutation, to be the most common one, c.1996dupC, was found in 2 patients. The other 2 mutations, c.2217delC and c.3449_3450delTT were novel mutations. As *MAGEL2* is maternally imprinted, 2 patients had inherited the *MAGEL2* mutation from their respective healthy fathers.

SYS is an extremely rare cause of DD/ID. However, hypotonia, joint contractures, profound DD/ID and facial dysmorphism are the suggestive clinical features for SYS. As a maternally imprinted disorder, it should be reminded that SYS may be inherited in form of a mutation from a healthy father.

Abbreviations: ACMG = American College of Medical Genetics, ASD = autism spectrum disorder, DD = developmental delay, ID = intellectual disability, IRB = Institutional Review Board, *MAGEL2* = melanoma-antigen-subfamily-like-2, MRI = magnetic resonance imaging, PWS = Prader-Willi Syndrome, SYS = Schaaf-Yang syndrome, WES = whole-exome sequencing.

Keywords: genomic imprinting, *MAGEL2*, Schaaf-Yang syndrome

1. Introduction

The gene *MAGEL2* is one of the protein-coding genes located on the Prader-Willi syndrome ([PWS]; OMIM #176270) domain at chromosome 15q11-q13. This chromosomal region is an imprinting region, and *MAGEL2* is a maternally imprinted gene encoding the melanoma-antigen-subfamily-like-2 protein.^[1]

MAGEL2 is part of a large ubiquitin complex that controls endocytosis, receptor recycling, and cell-surface localization.^[1] The paternal genomic deletion or maternal uniparental disomy at 15q11-13 are responsible for PWS, whereas pathogenic intragenic *MAGEL2* mutations result in phenotypes of the Schaaf-Yang syndrome ([SYS]; OMIM #615447).^[2] Therefore,

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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maternal imprinting pathway of *MAGEL2* must be considered when interpreting its variants regarding parental inheritance.^[2]

In 2013, Schaaf et al reported the first 4 patients with truncating mutations in the paternal copy of *MAGEL2*.^[3] The phenotypic characteristics of these patients partially resembled those of patients with PWS; these characteristics include neonatal hypotonia, feeding problems, delayed development (DD), and intellectual disability (ID) and were referred to as “PW-like syndrome”.^[3] With increasing numbers of individuals with pathogenic truncating variants of *MAGEL2*, their distinct phenotypic profile can be identified more reliably. Compared to PWS patients, SYS patients have higher incidences of autism spectrum disorder (ASD) and arthrogryposis.^[4,5] Moreover, SYS patients are characterized by a wide phenotypic spectrum despite numerous common clinical symptoms including varying degrees of ID and of language and motor development.^[3–5]

The prevalence of SYS is unknown. By SEP 2020, only more than 120 individuals with pathogenic variants of *MAGEL2* were reported worldwide.^[2–18] Due to the small number of reported cases, the underlying pathological mechanisms and genotype-phenotype correlation in *MAGEL2*-related disorders remain to be elucidated. Moreover, clinical suspicion for SYS is not easy in pediatric patients with hypotonia and DD/ID due to physicians’ unfamiliarity.

Here we report the first 4 Korean SYS patients with *MAGEL2*-intra-genic mutations, which was found in 0.9% out of the pediatric patients with DD/ID. The clinical features of these patients with SYS were described in detail. The Mutations were identified in all 4 cases by whole-exome sequencing (WES), which was confirmed by family member testing. The 2 mutations were novel. Our report aims to increase the awareness of this condition among the physicians taking care of the pediatric patients with DD/ID and hypotonia.

2. Methods

2.1. Patients

Patients (age <19 years) who had DD/ID and undiagnosed with chromosomal analysis and routine metabolic work-up such as plasma amino acid analysis, plasma acylcarnitine analysis, and urine organic analysis at the Asan Medical Center Children’s Hospital, Seoul, Korea underwent WES as in Figure 1. Among them, the patients diagnosed with SYS were included in this study. The cognitive, neurological, developmental, and physical spectrum of phenotypes using data were reviewed with their medical records.

2.2. Genetic analyses

Informed consent for genetic testing was obtained from patients or their legal guardians. WES was performed using genomic DNA isolated from either whole blood or saliva. All exons of all human genes (approximately 22,000) were captured using a SureSelect kit (Version C2; Agilent Technologies, Inc., Santa Clara, CA, USA). The captured genomic regions were sequenced using a NovaSeq platform (Illumina, San Diego, CA, USA). Raw genome sequencing data analyses included alignment to the reference sequence (NCBI genome assembly GRCh37; accessed in February 2009). Mean depth of coverage was 100-fold with 99.2% coverage higher than 10-fold. Variant calling, annotation, and prioritization were performed as previously described.^[19] In brief, the similarity

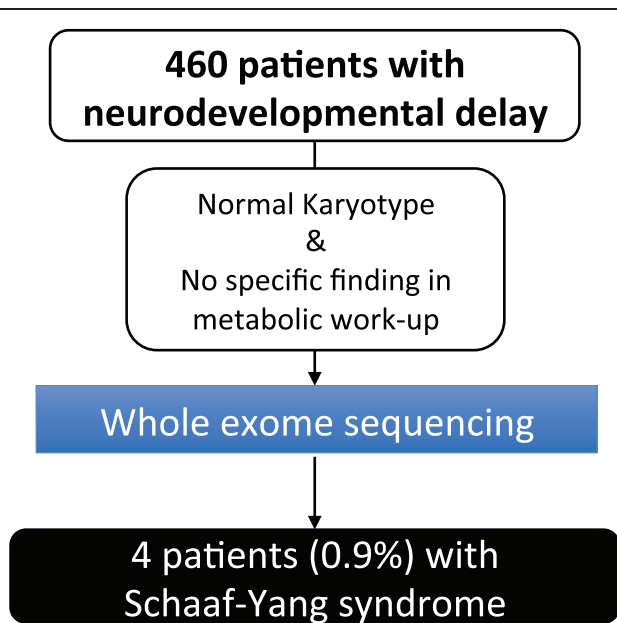


Figure 1. A flowchart of the whole exome sequencing in 460 Korean patients with delayed development and intellectual disability.

between patient’s phenotype and symptoms associated with disease caused by prioritized variants according to the American College of Medical Genetics (ACMG) guidelines^[20,21] was integrated and automated by all computational process.^[19]

For *MAGEL2* gene sequencing, genomic DNA was isolated from peripheral blood using PUREGENE DNA isolation kit (Qiagen, Hilden, Germany). The *MAGEL2* gene was amplified by PCR using primers designed with primer3 cgi v.3.0, Whitehead Institute (<http://bioinfo.ut.ee/primer3-0.4.0/>) and a reference sequence (NCBI GenBank accession number NT_026446.14). The *PHOX2B* gene was amplified exon-by-exon including promoter region by PCR using primers designed with primer3 and NT_006238.11 as reference sequence. To analyze whole mitochondrial sequence, 24 parts of the mitochondrial DNA were amplified by PCR using primers by Rieder et al.^[22] DNA sequencing was performed using a BigDye Terminator V3.1 Cycle Sequencing Ready reaction kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions.

To analyze (CTG)_n expansion of *DMPK* gene, PCR was performed by use of the primers 5’-CAGTTCACAACCGCTCC-GAGC-3’ and 5’-CGTGGAGGATGGAACACGGAC-3’. Subsequently PCR-Southern blot was performed using biotin-labeled (CTG)₁₀ probe (COSMO genetech, Japan) and the DNA-Detector Southern blotting kit (KPL, Maryland, USA). To analyze (CGG)_n expansion of *FMR1* gene, AmpliEx PCR/CE FMR1 (Asurgen, Austin, TX, USA) was used according to the manufacturer’s instructions.

This study was approved by the Institutional Review Board (IRB) of Asan Medical Center (IRB 2018-0180, 2018-0574, and 2017-0988).

3. Results

Among the 460 Korean individuals subjected to WES due to DD/ID, 4 individuals (0.9%) were diagnosed with SYS due to a mutation in the *MAGEL2* gene.

The clinical and genetic features were described in Table 1.

3.1. Clinical characteristics of 4 patients with SYS

3.1.1. Patient #1. The patient was the second child of healthy, non-consanguineous Korean parents. At the time of his birth, the mother was 41 years old. After an uneventful pregnancy, the boy was born at term by vaginal delivery and weighed 3270 g (percentile 13.0) with a length of 50 cm (percentile 15.1) and head circumference of 36 cm (percentile 27.2). No family history of neurological diseases existed apart from cloacal anomaly in the patient's sister. After birth, dysmorphic features were observed including frontal bossing, prominent ears, sparse hair and eyebrows, inverted nipples, distal arthrogryposis, club feet, and small hands and feet. Neurological examination revealed severe global hypotonia with retained reflexes. During the first week of life, the patient needed oxygen and mechanical ventilation therapy owing to recurrent apnea. Strength of sucking was weak, and nasogastric-tube feeding was required during the first 1.5 months of life. Brain magnetic resonance imaging (MRI) revealed asymmetrical lateral ventricles with a mildly dysmorphic shape (Fig. 2); however, no other parenchymal lesions were observed. Abdominal ultrasound and metabolic screening were normal. His thyroid function was normal. The patient was able to stand with support at 11 months of age but was not able to stand without support until aged 36 months (Table 2). At the most recent follow-up at age 38 months, he did not utter any meaningful words and presented autistic features. Body height and head circumference were persistently below the 3rd percentile.

3.1.2. Patient #2. The patient was the first-born of twins born after in-vitro fertilization and had healthy, non-consanguineous parents. At the time of giving birth, the mother was 43 years old. The boy was born at 37 weeks of gestation by cesarean section

and weighed 2530 g (below the 3rd percentile). A highly arched palate, severe camptodactyly of the 1st, 3rd, 4th, and 5th fingers of both hands, and bilateral equinovarus were observed. After birth, the patient experienced numerous apneic episodes and needed respiratory assistance. Brain MRI showed a minimally dysmorphic lateral ventricle without dilatation (Fig. 2). Laryngoscopy produced a laryngeal cleft type 1 and vocal cord palsy, and polysomnography revealed severe obstructive sleep apnea. In addition, a nasogastric-tube was installed due to poor sucking, recurrent vomiting, decreased gastrointestinal motility, and severe gastro-esophageal reflux. Laparoscopic pyloromyotomy was performed at 2 months of age. Until aged 6 months, the patient suffered from intermittent apneic episodes and required tube feeding. His thyroid function was normal. Body height, weight, and head circumference were below the 3rd percentile, and the boy was unable to support his head (Table 2).

3.1.3. Patient #3. The patient was the fourth child of healthy, non-consanguineous 34-year-old parents. The pregnancy was spontaneous. The prenatal period was complicated by polyhydramnios, and his weight at birth was 3600 g (70th percentile). The patient had 3 healthy sisters. After birth, he experienced respiratory failure requiring resuscitation. Generalized hypotonia was noted, with dysmorphic features including a coarse face, flat nasal root, large ears, camptodactyly of the 3rd, 4th, and 5th fingers, clinodactyly, and cryptorchidism. He was able to support his head at 6 months of age, sat upright with support at age 10 months, stood with support at age 14 months, and walked without support at 35 months of age. The patient uttered his first meaningful word at age 36 months (Table 2). At the most recent evaluation at age 47 months, his height was in the 25th percentile,

Table 1

Molecular and clinical phenotypes of 4 individuals with truncating *MAGEL2* mutations.

Sex	Patient #1 male	Patient #2 male	Patient #3 male	Patient #4 female	Previous publications ^{2,4,5}
Disease onset age	0 months	0 months	0 months	19 months	
Molecular diagnosis					
Mutation Nucleotide	c.1996dupC	c.1996dupC	c.2217delC	c.3449_3450delTT	
Protein	p.Gln666ProfsTer47	p.Gln666ProfsTer47	p.Ser739Ter	p.Phe1150TrpfsTer4	
Mutation reported previously	Reported	Reported	Not reported	Not reported	
Inheritance	Paternal	De novo	De novo	Paternal	
Prenatally problem					
History of polyhydramnios			•		N/A
Postnatal difficulties					
Neonatal hypotonia	•	•	•		97%
Respiratory distress requiring mechanical ventilator	•	•	•		55%
Feeding problems		•			84%
Clinical phenotypes					
Facial dysmorphism	•	•	•	•	81%
Joint contractures	•	•	•		88%
Macrocephaly				•	N/A
Microcephaly	•	•			N/A
Brain MR abnormality	•	•			N/A
Developmental problems					
Central or sleep apnea	•	•			76%
Gastroesophageal reflux		•			57%
Chronic constipation			•		71%
Failure to thrive	•	•	•		N/A
Delayed development/Intellectual disability	•	•	•	•	100%
Autistic features	•		•		78%
Seizures			•		33%

N/A = information not provided or otherwise unavailable.

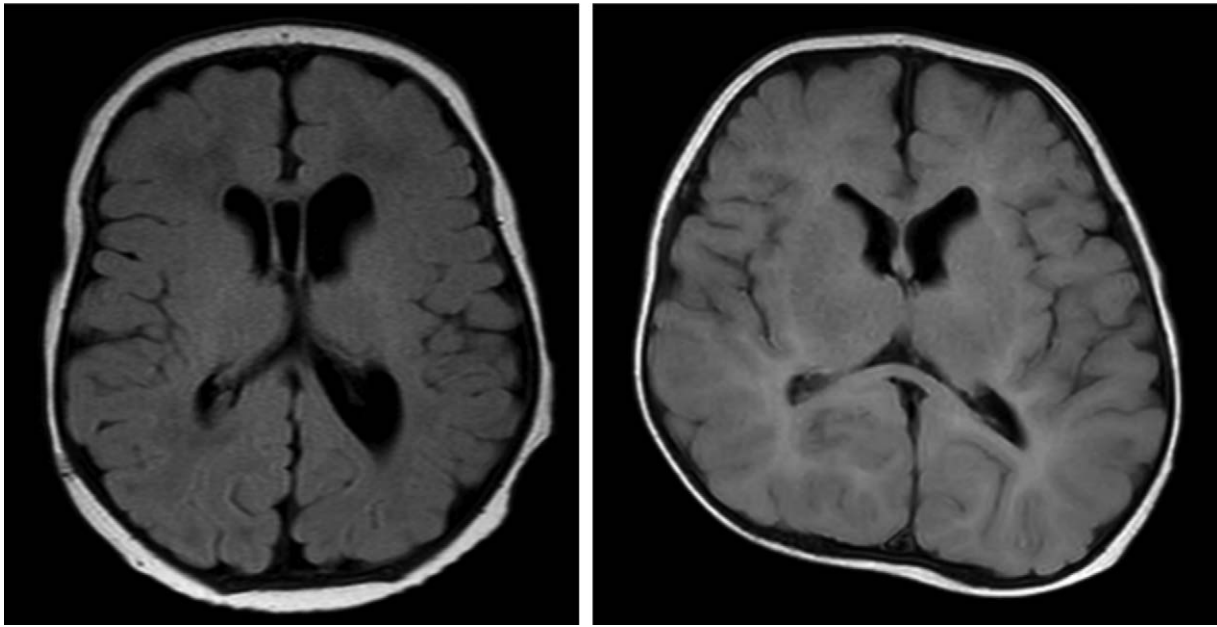


Figure 2. T2 fluid attenuated inversion recovery (FLAIR) axial image of patient #1 (left; at one month of age) and patient #2 (right; at 4 months of age). Brain magnetic resonance imaging (MRI) showed asymmetrical lateral ventricles with mild dysmorphic shape in both patients.

weight was in the 75th percentile, and head circumference was in the 25th percentile. His thyroid function and blood sugar levels were normal. He could not utter a two-word sentence and exhibited autism spectrum disorder and experienced generalized nonmotor absence seizures.

3.1.4. Patient #4. The female patient had healthy, non-consanguineous 31-year-old parents and a healthy younger brother. The girl was born at term with a weight of 3070 g (28th percentile). The pregnancy was spontaneous. She stood without support at age 17 months and walked without support at age 20 months. She uttered the first meaningful word at age 24 months but was not able to pronounce a two-word sentence until aged 34 months (Table 2). At 34 months, her height was in the 75th percentile, and weight and head circumference were in the 97th percentile. Hypertelorism and thick eyebrows were observed. Brain MRI did not reveal any significant abnormal findings. Her thyroid function and blood sugar levels were normal.

3.2. Molecular genetic analyses

All 4 patients showed normal karyotypes. To identify potential genetic causes for DD with hypotonia and respiratory difficulties, the mitochondrial genome of patient #1, the gene *PHOX2B* of patient #2, the *DMPK* gene of patient #2, and the *FMR1* gene of patient #4 were sequenced; however, no mutation was observed.

WES was performed at ages 12 months (patient #1), 1 month (patient #2), 30 months (patient #3), and 19 months (patient #4). WES yielded 111,390 (range, 105,155–114,769) variants on average in each patient. After filtering-out variants with frequency of 5% or higher of minor allele frequency, approximately 10,996 (10,313–11,429) variants on average were remained in each patient. After excluding variant with low impact including likely benign, benign, and non-coding variant with low evidence according to the ACMG guidelines and filtering by known inheritance pattern and gene matched with known disease up to date, 51 (41–64) disease-variant pairs on average remained. Finally, candidate genetic variants were

Table 2

Developmental outcomes of the 4 patients with Schaaf-Yang syndrome.

	Patient #1	Patient #2	Patient #3	Patient #4
Age at most recent examination (Dec 2019)	38 months	6 months	47 months	36 months
Motor development				
Head control	5 months	x	6 months	NA
Roll-over	9 months		7 months	NA
Sit alone with tripod	7 months		10 months	NA
Stand with support	11 months		14 months	NA
Standing independently	x		17 months	17 months
Walking independently	x		35 months	20 months
Language development				
First word	x		36 months	24 months
First two-word sentence	x		x	x

NA = information not provided or otherwise unavailable.

selected based on the relationship between the gene and patient phenotypes, and only the variants in the *MAGEL2* gene (NM_019066.4) remained.

All patients had a heterozygous truncating variant in the *MAGEL2* gene, which was c.1996dupC (p.Gln666ProfsTer47) in patients #1 and #2, c.2217delC (p.Ser739Ter) in patient #3, and c.3449_3450delTT (p.Phe1150TrpfsTer4) in patient #4. The variant c.1996dupC has been previously described,^[2,5–8,11,14,15] whereas c.2217delC and c.3449_3450delTT have never been reported. The allele frequency of p.Gln666ProfsTer47 was 0.002% in gnomAD (<https://gnomad.broadinstitute.org/>); however, those of p.Phe1150TrpfsTer4 and p.Ser739Ter have not been reported previously. p.Gln666ProfsTer47 is classified as a “pathogenic” variant, and p.Ser739Ter and p.Phe1150TrpfsTer4 are categorized as “likely pathogenic” variants, according to the ACMG Guidelines.^[23] All variants observed in the present study were confirmed by Sanger sequencing. The variants were not detected in DNA isolated from peripheral leukocytes of the parents of patients #2 and #3, whereas the fathers of patients #1 and #4 were heterozygous for the respective variant (Fig. 3).

There was no other mutation in 59 medically actionable genes for secondary reporting recommended by ACMG guidelines.^[24]

4. Discussion

The current study describes the first Korean SYS patients. SYS is an ultra-rare genetic disorder with unknown prevalence.^[2–18] In our patient cohort, 0.9% of patients with DD/ID can be expected to suffer from SYS.

As the first report of SYS in Korean population, although only 4 patients are described, our report helps to understand the common clinical and genetic characteristics among the affected patients.

Clinical manifestations in the 4 patients were similar to those described in previous reports (table 1).^[2,4,5] The 3 male infants showed generalized hypotonia, severe respiratory difficulty requiring mechanical ventilation, profound DD, and multiple anomalies including joint contractures and facial dysmorphism from birth. The girl presented slightly delayed motoric and language development and mild dysmorphic characteristics. Also, feeding problems requiring nasogastric tube feeding, gastro-esophageal reflux, chronic constipation, and failure to thrive were noted. Furthermore, neuropsychiatric symptoms such as autism and seizures were observed. The patients showed either micro- or macrocephaly. Brain imaging revealed asymmetrical lateral ventricles with mild dysmorphic shape.

SYS has been referred to as “PW-like syndrome” as SYS and PWS share major clinical symptoms such as neonatal hypotonia, feeding difficulties, failure to thrive, respiratory distress, and DD/ID.^[25,26] However, joint contractures and higher prevalence of life-threatening respiratory distress are more commonly observed in SYS than in PWS.^[5] During childhood, ASD is more common in SYS,^[4] and SYS patients generally do not exhibit over-eating habits with severe obesity as do PWS patients.^[25–27] Most SYS patients exhibit DD/ID^[5]; however, the level of ID varies substantially from mild to profound.^[5] On average, children with SYS were able to sit independently at age 18 months, to crawl at 31 months, and to walk independently at 50 months. Previously, SYS patients were found to utter their first word at 36

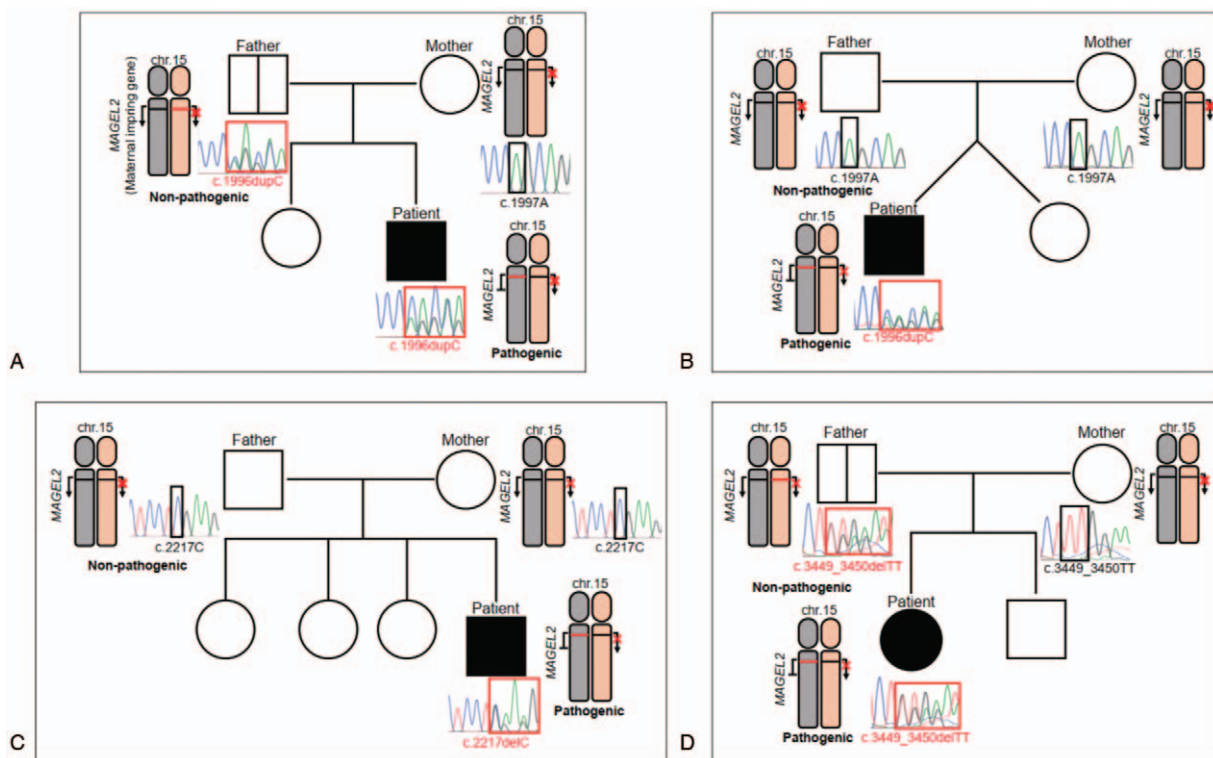


Figure 3. Family tree of 4 patients with Schaaf-Yang syndrome (SYS). In patients #1 (A) and #2 (B), a heterozygous frameshift mutation, c.1996dupC, was detected. Two novel mutations were detected in patient #3 (C; c.2217delC) and patient #4 (D; c.3449_3450delTT). The respective fathers of patients #1 and #4 were heterozygous for the respective mutations.

months and use first two-word sentences at 40 months of age.^[4] However, not all clinical characteristics necessarily occur in every patient. In our case series, patient #4 only showed motoric DD and signs of autism but no other symptoms.

A recent study showed that phenotypic severity may depend on the respective location of the truncating mutation, suggestive a genotype-phenotype association.^[5] In the largest number of cases, as reviewed by McCarthy et al, approximately half the patients with a *MAGEL2* mutation showed the variant c.1996dupC,^[5] which occurred in 4 out of 9 East Asian families as used in our study.^[10,14,15,18] Individuals with a c.1996dupC variant of *MAGEL2* show higher prevalence of joint contractures, feeding difficulties, respiratory dysfunction, and more profound DD/ID.^[5] Furthermore, deletion of the same nucleotide causes intrauterine fetal or perinatal demise.^[5]

The gene *MAGEL2* is maternally silenced through epigenetic regulation and is a maternally imprinted gene. Clinicians or medical geneticists might erroneously consider SYS to be a disorder in an autosomal dominant trait and that a de novo mutation event is the cause; however, a healthy father can carry a *MAGEL2* mutation and pass on the mutation to his child, as observed in 2 of our patients and in previous SYS cases.^[3] Fathers can thus carry a mutation in their maternal allele, which is silenced (Fig. 3). No difference in terms of phenotypic severity has been observed between paternally inherited and de novo mutation carriers.^[2,3,6–15]

Based on experimental studies, Wevrick et al suggested that *Magel2*-null mice show similar symptoms to those in human PWS patients, including neonatal growth retardation, excessive weight gain after weaning, impaired hypothalamic regulation, and reduced fertility.^[28,29] However, the precise pathomechanisms of SYS have yet to be elucidated. Further identification and investigation of cases with *MAGEL2* mutations will help understand the pathogenic mechanisms and genotype-phenotype correlations of SYS.

There are some limitations to our study. Due to the small number of patients, our report does not represent the general clinical and genetic characteristics of SYS patients in Korean population. With more cases identified, the full spectrum of clinical and genetic features of SYS needs to be understood in the perspectives of ethnic background.

5. Conclusions

SYS is an extremely rare genetic disorder with a variety of musculoskeletal and neurodevelopmental phenotypes, accounting for 0.9% of DD/ID. However, hypotonia, joint contractures, DD/ID and facial dysmorphism are the suggestive clinical features for SYS. As a maternally imprinted disorder, it should be reminded that SYS may be inherited in form of a mutation from a healthy father. After this first report of SYS in the Korean population, identification of more cases will help understand the clinical and molecular characteristics of this extremely rare genetic condition among the different ethnic groups.

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