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A Recurrent Variant in *MAGEL2* in Five Siblings with Severe Respiratory Disturbance after Birth

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Established Facts

- Schaaf-Yang syndrome (SHFYNG) is caused by heterogeneous truncating mutations in the paternal allele of the *MAGEL2* gene located at 15q11q13, the Prader-Willi syndrome region.
- Respiratory distress is a common manifestation of SHFYNG, and patients with c.1996dupC variants show a higher prevalence of respiratory dysfunction compared to those without c.1996dupC variants.

Novel Insights

- We report 5 family members affected with SHFYNG who displayed severe respiratory distress at birth and passed away within 4 months of life (7, 15, 110, 7, and 8 days) due to apnea or respiratory failure.
- The underlying mechanisms for respiratory disease may include peripheral (muscular) and central (CNS respiratory center) causes, which require further study.

Keywords
MAGEL2 · Respiratory failure · Whole-exome sequencing

Abstract
Schaaf-Yang syndrome (SHFYNG) is caused by truncating mutations in the paternal allele of the *MAGEL2* gene located in the Prader-Willi syndrome region. We report 5 newborns affected with SHFYNG in one family. Trio exome analysis revealed a heterozygous c.1996dupC frameshift mutation in *MAGEL2* inherited from the unaffected father. The phenotypes showed strong resemblance, especially for severe respiratory disturbance requiring mechanical ventilation at birth. After discharge from the hospital, 4 of the patients died of respiratory insufficiency within 1 or 2 weeks after birth, and 1 child died after 110 days of aggravated apnea. Apnea or respiratory failure was the main cause of early death in this family. Respiratory distress is a common manifestation of SHFYNG, especially in patients with c.1996dupC mutations. Hypotonia is a main cause of respiratory disturbance, and we propose another possible cause affecting the respiratory center of the brain.

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MAGEL2 is an imprinted paternally expressed gene located in the 15q11q13 Prader-Willi syndrome (PWS) critical region. Heterozygous truncating mutations in the paternal allele of *MAGEL2* lead to Schaaf-Yang syndrome (SHFYNG; MIM 615547), which is characterized by intellectual disability/development delay, feeding difficulty, hypotonia, contractures, and behavioral abnormalities – an entity with some resemblance to PWS. A de novo nonsense mutation in *MAGEL2* was found in 2 patients with a severe arthrogryposis phenotype [Mejlachowicz et al., 2015] and in a patient initially diagnosed with Opitz-C syndrome [Urreizti et al., 2017]. The SHFYNG phenotype can be highly variable. In a severe case, a patient affected with SHFYNG died of respiratory distress at postnatal day 2, and 3 fetuses died in utero diagnosed with arthrogryposis multiplex congenital [Mejlachowicz et al., 2015]. Thus, further investigation of the natural course of SHFYNG and its associated mortality is necessary.

We report 5 newborns affected with SHFYNG in one family with severe respiratory disturbance from at requiring respiratory support; after discharge from the hospital, 4 of them died of respiratory insufficiency after 1 or 2 weeks. One child died at 110 days after birth of aggravated apnea.

Clinical Report and Methods

The proband was the fifth child of a nonconsanguineous healthy couple. The family history was striking (Fig. 1A). Four siblings died after birth of respiratory insufficiency (clinical presentation is listed in Table 1). The pregnancy was uneventful, and delivery occurred at 39 weeks' gestation by C-section due to breech presentation. Birth weight was 3,350 g. Neonatal anoxia occurred at birth, and after emergency treatment, she presented with irregular breathing, cyanosis, a poor response, and weak crying. She was admitted to the neonatal intensive care unit for respiratory and feeding support. The physical examination noted frontal bossing, low-set ears, microretrognathia, a high-arched palate, short neck, a wide nipple distance, decreased elbow extension of the upper arms, hypotonia, small hands, camptodactyly of the third and fourth fingers, and bilateral polydactyly. She died at postnatal day 8 after discharge from the hospital of respiratory distress (Fig. 1C).

Whole-exome capture was performed on 3 μg of genomic DNA per individual using the xGen Exome Research Panel (Integrated DNA Technologies, Skokie, IL, USA). The resulting libraries were sequenced on a HiSeq 4000 system (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations for paired-end 150-bp reads. The minimal amount of data was 8 Gb per sample. A standard bioinformatics pipeline applies GATK to call SNVs and small indels. This pipeline filtered out the high-frequency variants based on several population databases (the 1000 Genomes Project, ExAC, GnomAD, EVS, and an in-house database containing 500 exomes). Filtered variants were analyzed following different inheritance patterns to generate 3 candidate gene lists. The imprinted gene list was downloaded from Geneimprint (http://geneimprint.com/site/genes-by-species). Variants in the imprinted gene list were studied specifically based on the imprinted pattern. Selected variants in the family were confirmed by Sanger sequencing. Amplification of the corresponding genomic regions was conducted by a general PCR method. The primer sequences are available upon request.

Candidate variants were confirmed using Sanger sequencing. PCR primer sequences and protocols are available upon request. Amplified fragments were sequenced using a 96-capillary 3730xl system (Applied Biosystems, Foster City, CA, USA).

Results and Discussion

We report 5 siblings affected with SHFYNG with severe respiratory disturbance at birth. Subsequent trio exome analysis revealed a heterozygous frameshift variant in *MAGEL2* – NM_019066.4:c.1996dupC (p.Gln666Profs*47) – previously described in other patients with SHFYNG inherited from the unaffected father. Sanger sequencing of PCR products with primers flanking the mutation confirmed the mutation in the 2 affected patients (II-3 and II-5) and showed that it was inherited from the unaffected father (Fig. 1D). c.1996dupC is a recurrent variant that has been found in 35 of 78 reported patients with SHFYNG [Schaaf et al., 2013; Buiting et al., 2014; Soden et al., 2014; Mejlachowicz et al., 2015; Fountain et al., 2017; Urreizti et al., 2017; Bayata et al., 2018; Jobling et al., 2018; Matuszewska et al., 2018; McCarthy et al., 2018a, b; Takuji et al., 2018; Tong et al., 2018].

All family members showed a high phenotypic similarity, and 4 children died within 2 weeks due to respiratory failure; 1 child died day 110 due to apnea. Apnea or respiratory failure was the main cause of early death in this family. To date, 3 individuals with SHFYNG have passed away due to the abovementioned symptoms, including a male patient with the c.1996dupC variant, who died at 9 months of age of an unknown cause of death, although apnea was suspected [Fountain et al., 2017]; another patient who carried a de novo variant in c.2118delT and died 2 days after birth of respiratory distress [Mejlachowicz et al., 2015], and another patient with the c.1996insC variant who died after 2 months due to apnea [Tong et al., 2018]. In a genotype-phenotype association study [McCarthy et al., 2018b], respiratory distress was found to be a common manifestation of SHFYNG; 58% of the patients required intubation during their lifetime, 55% required the use of mechanical ventilation, and 18% required a tracheostomy. Furthermore, patients with c.1996dupC mutations show a higher prevalence of respi-

Table 1. The clinical presentation of the affected siblings in this family

	II-1	II-2	II-3	II-4	II-5
Sex	M	M	F	M	F
Gestation age	36 week, 6 days	36 weeks	40 weeks, 4 days	31 weeks	39 weeks, 1 day
Birth weight, g	2,600	3,290	3,450	1,790	3,350
Respiratory disturbance	Neonatal anoxia, admitted to NICU, after rescue, respiratory and feeding support required	Tachypnea, cyanosis and foaming at the mouth soon after birth, admitted to NICU after rescue, respiratory and feeding support required	Weak crying, poor response, groan and foaming at the mouth soon after birth, admitted to NICU, feeding assistance required during hospital, after discharge, incidental apnea to frequent and aggravated apnea	Tachypnea, cyanosis occurred 50 min after birth, admitted to NICU after rescue, respiratory assistance required	Neonatal anoxia, after rescue, admitted to NICU, respiratory assistance required.
Neonatal pneumonia	+	+	+	–	+
Feeding difficulty	+	+	+	+	+
Hypotonia	+	+	–	+	+
Camptodactyly	Bilateral: 2nd–4th finger joint	Left: 3rd–4th finger joint	Left: 3rd–4th finger joint, decreased elbow extension	Left: 3rd–4th finger joint	Left: 3rd–4th finger joint, decreased elbow and knee extension
Polydactyly	–	–	–	–	Bilateral polydactyly
Hypogonadism	+	+	/	+	/
Facial dysmorphism	Robin sequence, low-set ears	–	Toothed gum, high-arched palate, microretrognathia, frontal bossing, small hands and feet	Micrognathia, high-arched palate, low-set ears	Frontal bossing, low-set ears, high-arched palate, short neck, small hands
Age at death, days	7	15	110	7	8

NICU, neonatal intensive care unit.

ratory dysfunction; 75% of the patients in the c.1996dupC cohort required intubation compared to 41% of the individuals with truncating variants other than c.1996dupC, and 71% of patients with a c.1996dupC variant required mechanical ventilation compared to 37% without c.1996dupC variants. These data demonstrate a high prevalence of respiratory distress in SHFYNG, especially in patients with c.1996dupC variants.

A major cause of respiratory disease is hypotonia, which is a common symptom in SHFYNG and PWS. In PWS, 88% of the patients present with hypotonia [Gunay-Aygun et al., 2001], and neonatal hypotonia is a nearly universal finding amongst patients with SHFYNG (97%). The higher prevalence of hypotonia in SHFYNG and PWS is most likely responsible for some complications in these 2 disorders, such as feeding difficulties, respiratory dysfunction, and skeletal abnormalities. In a *Magel2*-null mouse model, it was found that the loss of MAGEL2 contributed to hypotonia and musculoskeletal abnormalities in PWS and SHFYNG by reducing skeletal muscle strength and endurance in adult mice [Kamaludin et al., 2016]. *Magel2* is highly expressed in the murine adult nervous system, strongly expressed in the tongue, and moderately expressed in the abdominal wall muscle, the diaphragm, and the vertebral axis muscle system. Therefore, the loss of *Magel2* causes hypotonia in respiratory muscle, which results in decreased respiratory effort and hypoventilation [Kamaludin et al., 2016; McCarthy et al., 2018a].

In a previous report on PWS patients, 12% of the infants with PWS required intubation, and 33% required mechanical ventilation [Bar et al., 2017]. Children and adults with PWS have breathing defects with an irregular

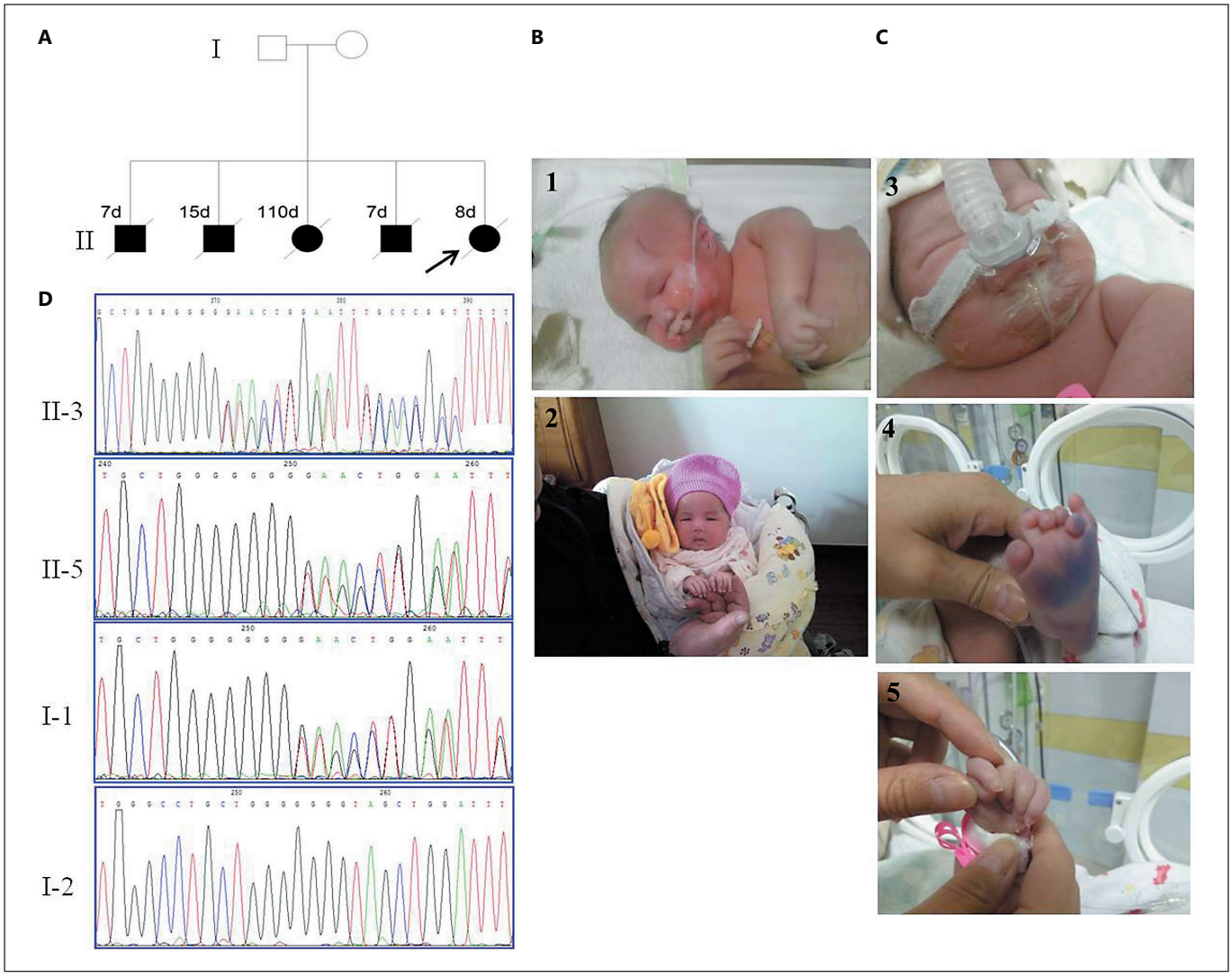

Fig. 1. A Pedigree of the family. **B** Facial features of patient II-3 at 2 days (B1) and 57 days (B2) old. **C** Clinical features of patient II-5 at 2 days (C3–C5) showing camptodactyly of the third and fourth fingers and polydactyly. **D** Heterozygous *MAGEL2* mutations: Sanger sequencing of patients II-3 and II-5 and her parents, NM_019066.4 c.1996dupC (p.Gln666Profs*47).

rhythm, frequent sleep apnea, and blunted respiratory responses to hypoxia and hypercapnia [Nixon and Brouillette, 2002]. Among several mouse models of PWS, only those with a Necdin deletion or Necdin (*Ndn*) knock out could reproduce the respiratory phenotype of PWS (central apnea and a blunted response to respiratory challenges). Serotonin contributes to CNS development and affects the maturation and function of the brainstem respiratory network. It has been suggested that Necdin may trigger breathing difficulties in PWS by affecting the serotonin system. Necdin deficiency in neonatal mice alters the serotonergic modulation of the respiratory rhythm generator [Zanella et al., 2008]. We further checked the variant list and found no pathogenic mutations in *Ndn*. The *Magel2* gene is structurally similar to *Ndn*, and its mRNA is enriched in the mouse brain, especially within the hypothalamus and brainstem. Both Necdin *Ndn* and *Magel2* are type II MAGE members and are adjacent to each other in the PWS region. These data raise the possibility that respiratory disease in patients with *MAGEL2* truncating variants originates from dysregulated serotonin metabolism in the CNS respiratory system, which needs further study. However, severe respiratory deficiency in infants lacking MAGEL2 is not recapitulated in

the *Magel2*-null mouse model [Kamaludin et al., 2016], which does not support the abovementioned speculation. Of course, the clinical features in the *Magel2*-null mouse model are less representative of the low prevalence of their respective phenotypes in human patients with truncating *MAGEL2* mutations. It is hypothesized that the deletion of the entire *MAGEL2* gene could have a milder effect than a truncating mutation, and the deletion of the complete paternal copy of the gene and promoter could lead to leaky expression of the maternal copy of the *MAGEL2* gene, as suggested based on studies of mice lacking the paternal *Magel2* copy [Matarazzo et al., 2017]. In addition, a patient with a *MAGEL2* deletion was reported to have a milder phenotype than in those with PWS or SHFYNG with point mutations in *MAGEL2* and without respiratory disturbance [Buiting et al., 2014]. It is possible that a mouse model with a truncating variant, which is not available so far, could provide more information.

In conclusion, we report 5 siblings with SHFYNG presenting with severe respiratory distress at birth and death within 4 months of life (7, 15, 110, 7, and 8 days) due to apnea or respiratory failure. The underlying mechanisms for respiratory disease may have central (CNS respiratory center) and peripheral (muscular) causes and require further study.

Acknowledgment

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Statement of Ethics

This study was conducted according to institutional policies; written informed consent was obtained from the family for all clinical studies and for publication.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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