Early onset critically ill infants with Schaaf-Yang syndrome: a retrospective study from the China neonatal genomes project and literature review

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Background: Schaaf-Yang syndrome (SYS) is a recently identified rare neurodevelopmental disorder characterized by neonatal hypotonia, feeding difficulty, joint contractures, autism spectrum disorder and development delay/intellectual disability. It is mainly caused by truncating variants in maternally imprinted gene *MAGEL2* within the Prader-Willi syndrome critical region 15q11-q13. Clinical diagnosis of SYS is difficult for clinicians due to its rarity and highly variable phenotypes, while unique inheritance patterns also complicate genetic diagnosis. To date, no published papers have analyzed the clinical consequences and molecular changes in Chinese patients.

Methods: In this study, we retrospectively investigated the mutation spectrums and phenotypic features of 12 SYS infants. The data were from a cohort of critically ill infants from the China neonatal genomes project (CNGP), sponsored by Children's Hospital of Fudan University. We also reviewed relevant literature.

Results: Six previously reported mutations and six novel pathogenic variations of *MAGEL2* were identified in 12 unrelated infants. Neonatal respiratory problems were the major complaint for hospitalization, which occurred in 91.7% (11/12) cases. All babies displayed feeding difficulties and a poor suck postnatally, and neonatal dystonia was present in 11 of the cases; joint contractures and multiple congenital defects were also observed. Interestingly, we found that 42.5% (57/134) of the reported SYS patients, including ours carried variants in the c.1996 site, particularly the c.1996dupC variant. The mortality rate was 17.2% (23/134), with the median age of death between 24 gestational weeks in fetuses and 1-month-old in infants. Respiratory failure was the leading cause of death in live-born patients (58.8%, 10/17), especially during the neonatal period.

Conclusions: Our findings expanded the genotype and phenotype spectrum of neonatal SYS patients. The results demonstrated that respiratory dysfunction was a typical characteristic among Chinese SYS neonates

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that should attract physicians' attention. The early identification of such disorders allows early intervention and can further provide genetic counseling as well as reproductive options for the affected families.

Introduction

Genetic diseases frequently occur in neonatal intensive care units and often progress extremely rapidly, not allowing for timely diagnosis and treatment, leading to morbidity and even mortality (1,2). Most genetic diseases may already manifest at neonatal age, but the diagnosis can be complicated by variable and nonspecific phenotypes, especially in critical conditions (3-5). Moreover, some genetic conditions may be silent or only partially expressed and not easily recognizable during the neonatal age. One example is imprinting disorders, a group of rare diseases caused by common alterations affecting imprinted genes or chromosomal regions (6). The underlying molecular changes include four types: copy number variations of the imprinted region, uniparental disomy, aberrant methylation marks (epimutations), and point mutations in imprinted genes (6-8). Twelve imprinting disorders have been recognized based on different clinical findings and/or the association of specific imprinting sites with molecular interference (9).

Schaaf-Yang syndrome (SYS) (OMIM 615547) is a recently identified imprinting disorder characterized by a broad range of symptoms, including prenatal polyhydramnios, fetal akinesia, neonatal hypotonia, respiratory distress, arthrogryposis, moderate-to-severe development delay/intellectual disability (DD/ID) and autism spectrum disorder (10,11). It is caused by heterozygous truncating mutations of *MAGEL2*, a paternally-expressed gene located within the Prader-Willi Syndrome critical region 15q11-q13 (12,13). Approximately 50% of patients inherit a *MAGEL2* pathogenic variant from a clinically unaffected father, and others are *de novo*. Notably, some of the specific variants are life-threatening, and the patient may die in the neonatal period.

In 2013, when SYS was first described in four individuals (14), it was originally named Prader-Willi-like syndrome, considering that *MAGEL2* was also a candidate gene of Prader-Willi syndrome (OMIM176270) (15-17). Later, as emerging cases were reported, it was suggested as an entirely new disease, renamed as SYS (18). Unlike classic Mendelian genetic disorders, DNA methylation of the maternal allele of *MAGEL2* results in monoallelic expression from the paternally inherited copy (19). In other words, pathogenic phenotypes occur only if the active paternal allele is affected while that on the maternal is not.

Up to now, more than 100 SYS patients have been reported by papers worldwide. As the disease was poorly understood, a proportion of patients were misdiagnosed or missed leading to an underestimate of the actual number of patients with SYS. A deeper understanding of SYS's clinical consequences and molecular basis significantly impacts diagnostics, focused medical care, and targeted counseling for the affected family.

The objectives of this study were to analyze clinical features and genetic findings of Chinese SYS patients. Furthermore, we investigated the correlation between clinical features and genotype in the SYS population worldwide.

Highlight box

Key findings
- Respiratory dysfunction was typical characteristics among critically ill Chinese patients with Schaaf-Yang syndrome (SYS). In addition, respiratory failure was the leading cause of death in live-born patients, especially during the neonatal period.

What is known and what is new?
- SYS is a rare imprinting disorder caused by truncated mutation on paternal allele of *MAGEL2*. In recent years, increasing patients were identified by next generation sequencing.
- This study described the clinical features and genetic findings of 12 critically ill Chinese SYS infants. As well, the genotype-phenotype correlation in the SYS population worldwide was explored.

What is the implication, and what should change now?
- A deeper understanding of SYS's clinical consequences and molecular basis is significant for pediatricians, which may help provide early diagnostics, focused medical care, and targeted counseling for the affected family.

We present this article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4396/rc).

Methods

Patients and setting

Individuals with *MAGEL2* pathogenic variants were enrolled based on the genotype from the China neonatal genomes project (CNGP) (NCT03931707) cohort. Neonates from the NICU or the neonatology ward of the maternity hospital with congenital anomalies and suspected genetic disease were referred to the Clinical Genetics Laboratory of Children's Hospital of Fudan University for a genetic test and were enrolled in CNGP (20). The test period was from August 2016 to August 2021. Patients' clinical manifestations were obtained from electronic medical records and telephone follow-ups. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the ethics committee of the Children's Hospital, Fudan University (No. 2021-45). Individual consent for this retrospective analysis was waived.

Variant analysis using clinical exome sequencing (CES)/ exome sequencing (ES)

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol. DNA fragments were enriched for CES using the Agilent ClearSeq Inherited Disease panel kit (covering 2,742 genes, test in #2, #3, #5, #6, #7, #8, #9, #11, #12) or proband ES using the Agilent SureSelect XT Human All Exon 50 Mb kit (tested in #1, #4, #10). For details on the sequencing and analysis, please see the published paper by Wang *et al.* and Yang *et al.* (21,22). The test method was decided by the physicians' and parents' choices based on the test price and year.

Raw data were mapped to the human reference genome (GRCh37/hg19). Variants were annotated by ANNOVAR and VEP software, with a minor allele frequency of less than 3% according to either the 1,000 Genomes Project or the Exome Aggregation Consortium (ExAC) and in-house database (23). The pathogenicity of the candidate variant was analyzed according to the standards and guidelines recommended by the American College of Medical Genetics and Genomics (ACMG) (24) and also described in our previous paper (25).

Methylations-sensitive digestion of MAGEL2 followed by Sanger sequencing

The DNA sample was treated with methylation-sensitive restriction endonucleases digestion prior to Sanger sequencing (14). There are four cleavage sites CCGG around the mutation locus (c.1912C>T and c.1996dupC) for endonucleases HpaII (New England Biosystems). HpaII could digest only the unmethylated paternal allele of *MAGEL2*, while the methylated maternal allele could not. This way, the mutated allele can be determined.

Then, DNA fragments were amplified by long-range PCR with primers LR_magel2_F (GAGAATTCCACCATCGCCACTAACC) and LR_magel2_R (CAGTCCCTGCAACTTCCCACTTTCT), which were provided in the previous study (14). Nested PCR amplified specific mutation loci. The following pairs of DNA primers, which could amplify the 1260bp region near the mutation site (c.1912C>T and c.1996dupC), were used for nested PCR: MAGEL2-1260-F: GCACTGCCCTTCCATCATCT; MAGEL2-1260-R: GAGACACTTGCGACCTCAGACA—further analyzed by Sanger sequencing using an automated sequencer (3500XL Genetic Analyzer, Applied Biosystems, Waltham, MA, USA). Sequence analysis was performed using Mutation Surveyor (Soft Genetics®, State College, PA, USA).

Statistical analysis

Descriptive statistics were used to illustrate the number of patients enrolled, the age at test (the median). The other variables described in the methodology were presented as frequencies and percentages.

Results

General characteristics of patients

Six females and six males, a total of twelve patients, received genetic diagnoses of SYS in the 42257 CNGP cohort. The test age ranged from 1 day to 4 months (median: 10 days). Nine individuals presented symptoms during the neonatal period; the other three patients were 39-day-old, 2-month-old and 4-month-old, respectively. Three babies (#8, #10, #11) were born prematurely and the other nine were in full term. Four families of #1, #4, #9, and #10 had histories of abnormal pregnancy. Four patients (#1, #4, #5, #7) died.
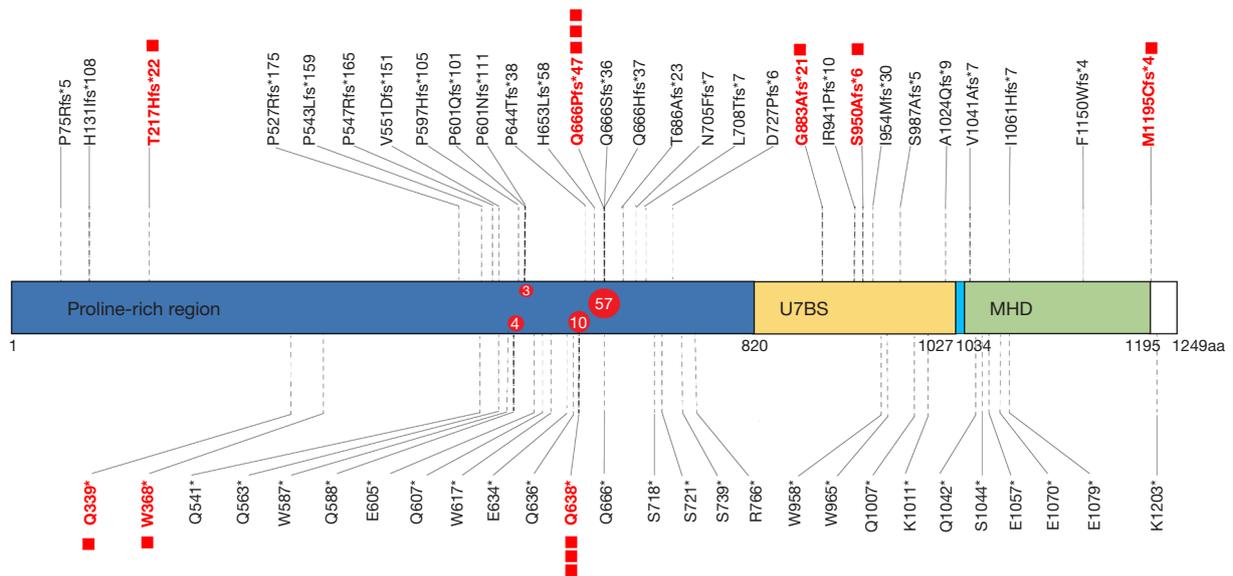
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Figure 1 The structure of MAGEL2 protein and the distribution of the pathogenic variants. MAGEL2 protein contains a proline-rich region (from aa 1 to 819), USP7 binding site (U7BS, from aa 820 to 1034), and MAGE homolog domain (MHD, from residues 1027 to 1195). Region aa 1027 to 1034 represents overlap of domain U7BS and MHD. The pathogenic mutations in *MAGEL2* are depicted by their positions. Above the box are frameshift mutations, while below the box are termination mutations. The red dots indicate that more than one person has the mutation at the site. The larger, the more cases, and the digit in the dots means case number. The red squares represent the variants in our patients.

Genetic findings

A genetic diagnosis of SYS was identified in 12 unrelated infants in our CNGP cohort. Of these, 6 were previously reported mutations, and 6 were novel pathogenic variations in the *MAGEL2* gene. The novel variants of *MAGEL2* (NM_019066.5) include c.3583delA (p. Met1195CysfsTer4), c.1015C>T (p. Gln339Ter), c.648delG (p. Thr217HisfsTer22), c.2847_2883del37 (p. Ser950AlafsTer6), c.2646delC (p. Gly883AlafsTer21), and c.1104G>A (p. Trp368Ter), were identified in patient #7, #8, #9, #10, #11, and #12 respectively (*Figures 1,2*). These variants have not been reported in the ExAC, 1000 genome databases and our in-house databases. All variants were classified as pathogenic according to the criteria of ACMG. Two previously described variants: c.1912C>T (p. Gln638Ter) and c.1996dupC (p. Gln666ProfsTer47) were detected in #1, #2, #3 and #4, #5, and #6, respectively. All these variants were truncated, including 7 frameshifts and 5 stop-gains. Four variants were confirmed paternally inherited (#1, #2, #4, and #10) and three were *de novo* (#3, #5, and #6). According to recent genome annotation (UniProt Q9UJ55), nine variants were located in the

proline-rich region, two in USP7 binding site domain (U7BS), and one (Met1195CysfsTer4) was in MAGE homolog domain (MHD) (*Figure 1*).

Clinical manifestation

Major complaint

Respiratory problems were the main reasons for hospitalization in 91.7% (11/12) of our cases. Expressly, five patients were admitted to the hospital due to neonatal anoxia, two were respiratory distress and three were neonatal pneumonia. The other two patients, #7 was for unexplained newborn moaning and #12 was for recurrent feeding problems. All babies displayed feeding difficulties and a poor suck postnatally. Neonatal dystonia was present in 11 of the cases, and two patients (#2 and #3) showed hypertonia. Joint contractures, a relatively specific manifestation of this disorder, appeared in 58.3% (7/12) cases. In addition, brain MRI in 9/12 of patients was abnormal. The anterior horn of the left lateral ventricle is slightly wider than the contralateral and the T1W1 stripe hyper signal was present in the occipital region in #1. Bilateral ventricular hypodensity was observed in #4. A few
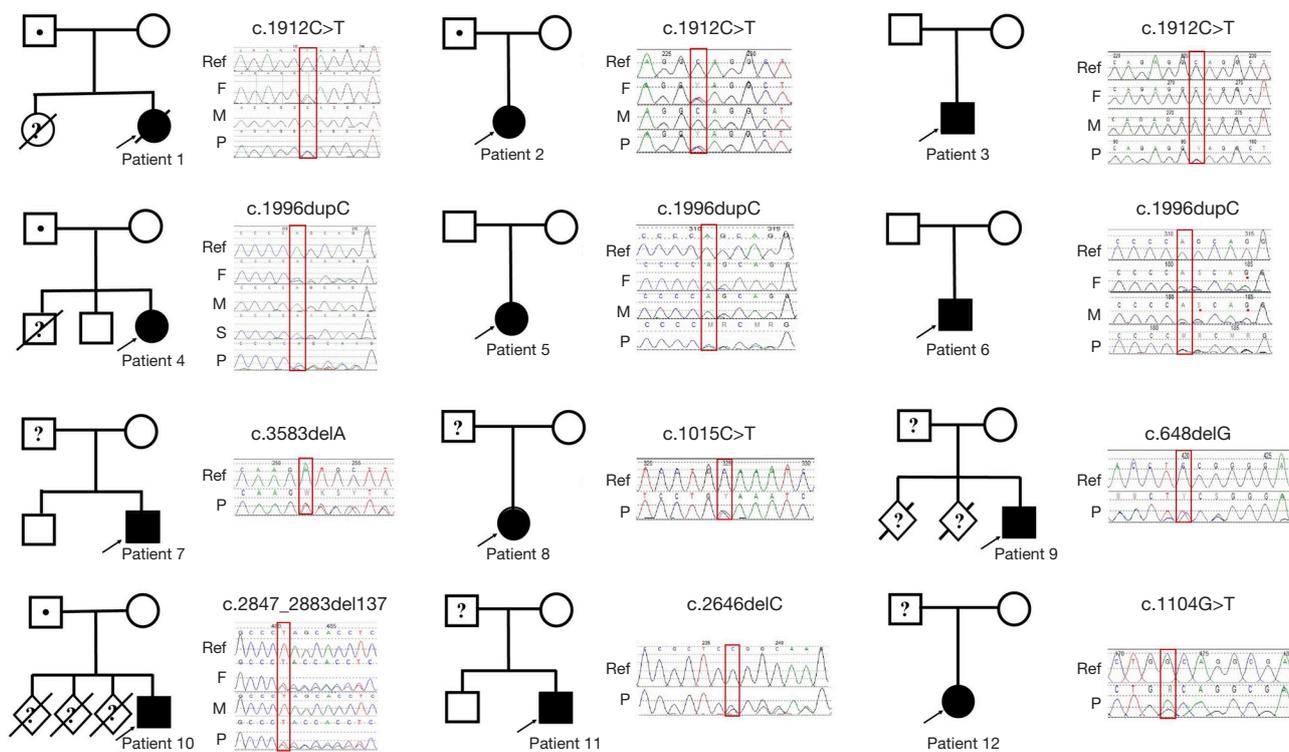
Figure 2 Pedigree and genotypes of *MAGEL2* variations analyzed by Sanger sequencing of patients. Sanger results of the variant are shown in the right of each pedigree. Circles are females, squares are males, triangles are fetuses. Empty symbols for nonaffected individuals, filled in black for affected individuals. Crossed symbols for deceased individuals, arrow points to the proband. Ref, reference; F, female; M, male; P, patient.

lesions near the anterior and posterior horns of bilateral ventricles as well as full lateral ventricles (especially the left one), were observed in #5. B-ultrasound of #6 suggested lateral ventricle widening at 36 gestational weeks. And #9 showed a symmetrically reduced density of white matter in bilateral frontal and parietal lobes. In addition, three patients displayed unusual electroencephalograms and seizures. Anemia and hypoglycemia occurred in two patients (#5, #8), and hyperbilirubinemia appeared in four patients (#1, #6, #9, #11). The clinical features and genotypes of the patients with *MAGEL2* mutations in our study are summarized in *Table 1*.

Physical characteristics

Limb deformities, including clubfoot, small hand/feet and brachydactylia, were present in 4 patients (#1, #2, #4, #10). Craniofacial deformities were observed in 6 patients (#1, #2, #3, #4, #10, #12), including micrognathia, frontal bossing and almond eyes. Genitourinary abnormalities were found in 6 patients: cryptorchidism in #3 and #10, bilateral hydrocele in #7, vulva pigmentation in #1, #4

and #5. Patient 10 had congenital abnormalities of the urinary system; the left renal pelvis was slightly dissected and the left renal collecting system was dissected by 3.7 mm. In addition, congenital laryngeal cartilage dysplasia was observed in #1 and #6. Patient 8, who was indicated fetal intestinal dilatation with the widest 29 mm by prenatal B-ultrasound, got a diagnosis of congenital duodenal atresia and intestinal malrotation, which required early surgical intervention. A congenital palpebral fissure appeared in both eyes in #6 and the diameter of congenital microcornea was 8 mm. When it comes to cardiovascular system, small atrial septal defect was observed in 10/12 patients. The minimal lesions in 6 patients were closed spontaneously during follow-up (except for the fatal cases).

Hotspot and fatal variants

Unrelated cases #1, #2 and #3 bore the identical recurrent heterozygous stop gain variant c.1912C>T (p. Gln638Ter). These patients shared the majority of manifestations, such as anoxia, persistent respiratory distress and poor response soon after birth. Patients #4, #5 and #6 harbored identical

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Table 1 The genotype and phenotype of twelve patients in this study

	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	Summary
Variant	c.1912 C>T p.Gln638Ter	c.1912 C>T p.Gln638Ter	c.1912 C>T p.Gln638Ter	c.1996dupC p.Gln666Profs*47	c.1996dupC p.Gln666Profs*47	c.1996dupC p.Gln666Profs*47	c.3583delA p.Met1195Cysfs*4	c.1015C>T p.Gln339Ter	c.648delG p.Thr217Hisfs*22	c.2847_2883del37 p.Ser950Alafs*6	c.2646delC p.Gly883Alafs*21	c.1104G>A p.Trp368Ter	
Mutation type	Stop gain	Stop gain	Stop gain	Frameshift	Frameshift	Frameshift	Frameshift	Stop gain	Frameshift	Frameshift	Frameshift	Stop gain	5 stopgains/ 7 frameshifts
PMID/novel	27195816 29359444	27195816 29359444	27195816 29359444	30302899 31397880	30302899 31397880	30302899 31397880	Novel	Novel	Novel	Novel	Novel	novel	6 published/ 6 novel
Zygosity	Het	Het	Het	Het	Het	Het	Het	Het	Het	Het	Het	Het	12 Het
Inheritance	Paternal	Paternal	De novo	Paternal	De novo	De novo	NA	NA	NA	Paternal	NA	NA	4 paternal/ 3 de novo/5 NA
Gender	F	F	M	F	F	M	M	F	M	M	M	F	6 F/6 M
Test age	4-d-old	30-d-old	5-d-old	16-d-old	21-d-old	39-d-old	2-d-old	1-d-old	1-d-old	1-d-old	2-m-old	4-m-old	9 newborns/ 3 infants
Perinatal characteristics													
Family history	Yes	NA	NA	Yes	NA	No	No	No	Yes	Yes	No	No	4 family history
Gravidity and parity	G2P2	NA	NA	G3P3	NA	G1P1	G2P2	G1P1	G3P1	G6P4	G2P2	G1P1	
Antenatal examination/ amniotic fluid situation	Hydrops fetal	Normal	Normal	Normal	Amniotic fluid II contamination	At 36 weeks, B-ultrasound showed enlargement of lateral ventricles; amniotic fluid III contamination	Normal	B ultrasound revealed fetal intestinal dilatation with a maximum width of 29 mm. The amniotic fluid is excessive and bloody	Amniotic fluid III contamination	Polyhydramnios	Oligohydramnios	Normal	
Gestation age	39 w	38 w+6	39 w	39 w+3	37 w+5	37 w+1	39 w	33 w+2	40 w	36 w+2	29 w	37 w	
Birth weight (g)	3,800	3,480	3,430	3,650	3,080	3,300	2,950	1,880	2,680	3,660	1,350	2,900	
HC (cm)/age	36/4 d (2 SD)	34/30 d (−3 SD)	34/5 d	NA	32/21 d (>−2 SD)	37/30d	33/2 d (−1 SD)	29.5/1 d (−3 SD)	34/1 d (−1 SD)	NA	NA	NA	
Major complaint	Neonatal anoxia	Respiratory distress	Neonatal pneumonia	Neonatal pneumonia	Neonatal anoxia	Neonatal anoxia	Neonatal groan	Neonatal anoxia	Neonatal pneumonia	Neonatal anoxia	Respiratory distress	Feeding problems	
Neonatal anoxia	+	+	+	+	+	+	−	−	−	+	+	−	8/12
Respiratory distress	+	+	+	+	+	+	−	+	−	+	+	−	9/12
Intubation/mechanical ventilator	+	+	+	+	+	+	−	+	−	+	+	−	9/12
Cyanosis	+	+	+	+	+	+	−	+	+	+	+	−	10/12
Neonatal pneumonia	+	+	+	+	+	+	+	−	+	−	+	−	9/12
Infection	+	+	+	+	+	+	+	−	+	−	+	−	9/12
Feeding problems	+	+	+	+	+	+	+	+	+	+	+	+	12/12

Table 1 (continued)

Table 1 (*continued*)

	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	Summary
Poor suck in infancy	+	+	+	+	+	+	+	+	+	+	+	+	12/12
Hypotonia	+	–	–	+	+	+	+	+	+	+	+	–	9/12
Hypertonia	–	+	+	–	–	–	–	–	–	–	–	–	2/12
Joint contractures	+	+	–	+	+	+	–	–	–	+	+	–	7/12
Craniofacial deformity	–	Microg	Microg, high arche palate	–	Microg	–	–	–	–	Microg	–	Microg	5/12
Brachydactylia	–	+	–	+	+	–	–	–	–	–	–	–	3/12
Small hand/feet	–	+	–	+	–	–	–	–	–	–	–	–	2/12
Neonatal encephalopathy	+	+	–	+	+	–	+	–	–	+	+	–	7/12
Brain imaging anomalies	+	+	–	+	+	+	+	+	–	+	+	–	9/12
Seizure	–	–	+	+	–	–	–	–	–	+	+	–	4/12
Genital anomalies	+	–	+	+	+	–	+	–	+	+	–	–	7/12
Cardiac anomalies	ASD, PDA	ASD	PDA, PFO, PH, spontaneous closure	ASD, VSD, PH	ASD, PFO, PDA	ASD, PDA, spontaneous closure	ASD, PFO, PDA, PH	ASD, PFO, PDA, PH; spontaneous closure	ASD, PFO; spontaneous closure	ASD, PDA, spontaneous closure	ASD, PFO, PDA, spontaneous closure	–	11/12
Outcome	Died	Loss of follow-up	Alive	Died	Died	Alive	Died	Alive	Alive	Alive	Alive	Alive	4 died/7 alive/ 1 loss of follow-up
Others	Hyperbilirubinemia, hydrops fetal, congenital laryngeal chondromalacia	–	High blood ammonia	Blood tandem mass spectrometry showed increased concentrations of various amino acids and acylcarnitine	Hypoglycemia, anemia	Hyperbilirubinemia, cholestasis, microcornea, congenital laryngeal chondromalacia	Hypoglycemia, hepatomegaly, Hemophagocytic syndrome	Anemia, intestinal dilatation	Hyperbilirubinemia, the left renal pelvis is widened to 6.9 mm in width	The left renal gathering system was separated by 3.7 mm	Hyperbilirubinemia	Myopia	–

NA, not available; F, female; M, male; HC, head circumference; SD, standard deviation; Microg, micrognathia; ASD, atrial septal defect; PDA, patent ductus arteriosus; PFO, patent foramen ovale; PH, pulmonary hypertension; VSD, ventricular septal defect.

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pathogenic mutation of c.1996dupC (p. Gln666ProfsTer47), as well; they presented with very similar clinical phenotypes, including congenital malformations and complicated conditions, specifically neonatal hypotonia, poor spontaneous respiration, contractures and pneumonia. These six patients received respiratory and nutritional support soon after birth. Patients # 1, #4 and #5 died early in life due to respiratory failure. In the follow-up, other surviving patients were observed to have varying degrees of DD/ID.

Literature review and genotype-phenotype correlation analysis

Up to now, more than 100 individuals have been reported globally. We summarized the clinical and genetic features of 134 patients with reported SYS, including ours. The variants types and detailed information were unavailable in thirty-five patients in McCarthy's study (12) and not included in our study.

The minimum age of onset was just one day, while the maximum was 36 years. There were no significant racial/ethnic or sex differences. Chinese patients accounted for 17.9% (24/134). In total 93.3% of patients (112/120) presented with neonatal hypotonia, 88.8% (119/134) with joint contractures, 82.7% (67/81) with facial dysmorphism, 73.9% (70/95) with respiratory dysfunction and 76.0% (38/50) with autism spectrum disorder (Table S1). Besides, 26 of 49 (53.1%) patients showed abnormal brain MRI including dysplastic or thin corpus callosum, pituitary gland appeared hypoplastic, ventriculomegaly, atrophy of cerebral white matter, generalized brain atrophy, decreased myelination, hypothalamic hypoplasia and hypoplastic vermis. Seizures occurred in 35.6% (31/87) of all individuals.

Among the 134 patients, truncating mutations were detected in 132 individuals. The remaining two individuals carried partial deletion/inversion and missense variants, respectively. In addition, 42.5% (57/134) cases harbor variants in the c.1996 site, including 50 cases with c.1996dupC, 6 cases with c.1996delC, and 1 case with c.1996_1997dupCA. The most common variant, c.1996dupC, counts for 37.3% (50/134). Furthermore, ten patients (7.5%) harbored c.1912C>T (p. Gln638Ter), four patients (3.0%) carried c.1762C>T (p. Gln588Ter), and two patients (1.5%) bored c.2179_2180delGA (p. Asp727ProTer6). Three frameshift variants (c.1800delT, c.1801_1802delCC, c.1802delC) were identified in three patients (2.3%) that lead to a truncated amino acid in Pro601GlnfsTer101, Pro601AsnfsTer111 and Pro601GlnfsTer101, respectively. Other isolated variants are scattered on the gene (*Figure 1*).

So far, 23 cases diagnosed with SYS have been reported deceased (26-32). The genotype and clinical features of these cases are summarized in *Table 2*. The median age of death was 24 gestational weeks in fetuses and one-month-old in infants. Notably, 78% (18/23) of them carried the variant in the c.1996 site, including twelve c.1996dupC and six c.1996delC. The other five variants were c.1628delC, c.1850G>A, c.1912C>T, c.2118delT and c.3583delA. Six aborted fetuses shared identical variants and similar phenotypic expressions with fetal akinesia, severe arthrogryposis and multiple joint contractures, overlapping fingers, club feet, and dysmorphic facial features, including low-set ears and micrognathia. Respiratory failure was the first cause of death in live-born patients (58.8%, 10/17), especially during the neonatal period. The girl who carried c.1628delC died five days after admission due to cardiovascular failure and also manifested dyspnea. The patient with c.1850G>A died of sleep apnea caused by her extreme obesity. A 2-month-old boy carrying c.1996dupC died due to dyspnea. What calls for special attention is that those truncating mutations (c.3583delA excepted) are located in the middle point of the whole gene.

Discussion

In this study, 12 infants with heterozygous truncating mutations of *MAGEL2* were identified using next-generation sequencing in 42,257 individuals CNGP cohort. Our patients were characterized by early onset and were seriously ill. They attained early diagnosis with a median test age of just 10 days. The 12 critically ill babies presented respiratory distress, dystonia, joint contractures, feeding difficulty, and multiple congenital defects in the neonatal period, which is the most vulnerable time in a child's life. However, most individuals reported previously showed symptoms in infancy not receiving a definite diagnosis until childhood and adolescence (33).

MAGEL2 is highly expressed in the brain especially in the hypothalamus, and is essential in neuropeptides, neuronal signaling transduction and neuronal axonal growth (34-38). SYS was therefore defined as a neurodevelopmental disease (18). In fact, SYS is a multi-system involved disorder with variable clinical manifestations. We found that the typical symptoms of SYS include neonatal hypotonia, joint contractures, facial dysmorphism, and respiratory

Table 2 The summary of 23 dead SYS cases

Ref	Gender	Age of death	Cause of death	Variant	Inheritance	Gestational age	Decreased fetal movement	Respiratory distress	Intubation/ ventilation	Hypotonia	Feeding problems	Cardiovascular anomalies	Joint contractures	Facial deformity	Genital anomalies
This study	F	2 m	Respiratory failure	c.1912C>T	Paternal	39 w	+	+	+	+	+	ASD, PDA, PH	+	–	+
	F	1 m	Respiratory failure	c.1996dupC	Paternal	NA	NA	+	+	+	+	ASD, PDA, PH	+	+	+
	F	1 m	Respiratory failure	c.1996dupC	De novo	NA	NA	+	+	+	+	ASD, VSD, PDA	+	+	+
	M	7 d	Hemophagocytic syndrome	c.3583delA	NA	39 w	NA	–	–	–	+	ASD, PFO, PDA, PH	–	–	+
Chen X	F	20 d	Respiratory failure	c.1996dupC	De novo	40 w	+	+	+	+	+	VSD, ASD, PH	+	+	–
	F	98 d	Respiratory failure	c.1996dupC	De novo	40 w	–	+	+	+	+	VSD, PFO	+	+	–
Xiao B	M	7 d	Respiratory failure	c.1996dupC	Paternal	36 w	NA	+	+	+	+	NA	+	+	+
	M	15 d	Respiratory failure	c.1996dupC	Paternal	36 w	NA	+	+	+	+	NA	+	–	+
	F	110 d	Apnea	c.1996dupC	Paternal	40 w	NA	+	+	–	+	NA	+	+	NA
	M	7 d	Respiratory failure	c.1996dupC	Paternal	31 w	NA	+	+	+	+	NA	+	+	+
	F	8 d	Respiratory failure	c.1996dupC	Paternal	39 w	NA	+	+	+	+	NA	+	+	NA
McCarthy*	F	8 y	NA	c.1996dupC	Paternal	NA	–	NA	NA	+	+	NA	+	+	–
Guo	M	35 g.w.	NA	c.1996delC	Paternal	35 w	+	NA	NA	+	+	–	+	+	+
Kleinendorst	F	23 m	Obesity/sleep apnea	c.1850G>A	De novo	40 w	+	+	NA	+	+	NA	+	+	NA
Tong	F	11 m	Cardiovascular failure	c.1628delC	Paternal	40 w	+	+	+	+	+	NA	+	+	–
	F	2 m	NA	c.1996dupC	De novo	37 w + 4 d	NA	+	+	+	+	NA	+	+	+
Fountain	F	21 g.w.	Fetal akinesia	c.1996delC	Paternal	21 w + 4 d	+	NA	NA	NA	NA.	–	+	+	–
	M	14 g.w.	Fetal akinesia	c.1996delC	Paternal	14 w + 6 d	+	NA	NA	NA	NA	–	+	+	–
	M	9 m	Suspected apnea	c.1996dupC	Paternal	NA	NA	NA	NA	NA	+	NA.	+	+	+

Table 2 (*continued*)

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Table 2 (*continued*)

Ref	Gender	Age of death	Cause of death	Variant	Inheritance	Gestational age	Decreased fetal movement	Respiratory distress	Intubation/ ventilation	Hypotonia	Feeding problems	Cardiovascular anomalies	Joint contractures	Facial deformity	Genital anomalies
Mejlachowicz	F	24 g.w.	Fetal akinesia	c.1996delC	Paternal	24 w	+	✓	✓	✓	✓	✓	✓	✓	✓
	F	24 g.w.	Fetal akinesia	c.1996delC	Paternal	24 w	+	✓	✓	✓	✓	✓	✓	✓	✓
	M	27 g.w.	Fetal akinesia	c.1996delC	Paternal	27 w	+	✓	✓	✓	✓	✓	✓	✓	✓
	F	2 d	Respiratory distress	c.2118delT	De novo	NA	+	+	+	+	+	NA	+	+	NA

*, this patient of McCarthy's study had been reported previously as patient 2 by Fountain *et al.* in 2016. F, female; M, male; g.w., gestational age; NA, not available; ASD, atrial septal defect; PDA, patent ductus arteriosus; PH, pulmonary hypertension; PFO, patent foramen ovale; VSD, ventricular septal defect.

dysfunction, consistent with McCarthy's cohort (12), In the natural progression of the disease, all patients showed varying degrees of developmental delay and over 75% displayed with an autism spectrum disorder.

Newborn hypotonia is a hallmark trait in over 90% of SYS patients, which is related to multi-system phenotypes such as joint contracture, respiratory impairment, feeding abnormalities and reduced movement. The underlying causes involve the central nervous systems and peripheral nervous systems (39). *MAGEL2* is expressed in cells of mesodermal origin that contribute to the formation of fat, muscle, and bone (40). It is found that Magel2-null mice had increased fat mass, less lean mass, and reduced bone mass which could principally be caused by differentiation defects (40,41). In addition, the expression of atrophy gene *Murf1*, which is important for regulation of ubiquitin-mediated protein degradation in skeletal muscle, was elevated in Magel2-null mice and may result in imbalance of protein synthesis and degradation (40,42).

Respiratory dysfunction is another concern that is highly disruptive to 78% of SYS patients' daily life and represents the most common fatal cause in SYS infants. Recurrent respiratory distress remains a problem during infancy, with frequent exacerbations during common viral infections. Over 90% of babies in our cohort required oxygen supplementation and non-invasive or invasive respiratory supports, which is consistent with McCarthy's findings. In addition, patients with c.1996dupC had a higher rate of requiring intubation or mechanical ventilation, almost twice as high as the other variants (12). Until now, the underlying pathology of dyspnea remains elusive, but it can be determined that it is multifactorial, involving both central and peripheral mechanisms. For one thing, *MAGEL2* is highly expressed in the hypothalamus; truncating mutations lead to hypothalamus dysfunction and an inability to adjust the respiratory rate to compensate for the increase in carbon dioxide and a decrease in oxygen levels (43); for another, hypotonia leads to weak respiratory musculature and depressed respiratory response.

Exploration of individuals' phenotypic features and mutation positions revealed further genotype-phenotype association. Hotspot mutation and the most serious variant are associated with the c.1996 site. We found that the c.1996 mutation occurred in nearly 50% of patients and in 78% of deaths. Specifically, c.1996dupC is the most common and severe mutation in SYS patients with higher prevalence of respiratory distress, joint contractures, and more severe ID/DD than other variants (exclude c.1996delC) (12).

While c.1996delC was proven to be embryonically lethal, all six fetuses were aborted in the middle of pregnancy due to hypokinesia and severe arthrogryposis (28). *MAGEL2* gene is GC-rich, especially the c.1990_c.1996 consequence containing seven consecutive cytosines. Why *MAGEL2* mutations occur most often in the c.1996 site and why c.1996dupC and c.1996delC mutations lead to such markedly different phenotypes need to be studied.

Another finding was that critical illness and death tended to occur in individuals with mid-truncated mutations because hotspot variants and fatal mutations are concentrated in the midpoint of protein. McCarthy *et al.* indicated that the severity of SYS depended on the specific location of the truncating mutation (12). Since *MAGEL2* is a single-exon gene, mutations that cause premature stop codons are not predicted to lead to nonsense-mediated mRNA decay (11,44). Frameshift mutations of the c.1996 site encode a mid-truncated MAGEL2 protein that is no longer associated with the C-terminal and may therefore impair the dynamics of the ribosome for liquid-liquid phase separation, leading to pathological aggregation of the MAGEL2 protein in RNA-rich particles. However, c.3583 cite is an exception which is located near the end of C-term, and even weirder is that our patient with c.3583delA did not appear in respiratory distress; instead, he presented hemophagocytic syndrome. These findings suggest that truncated mutation at specific loci appears to be incompatible with life.

Our study has several limitations. First, it was retrospective and lacked a control group. Clinical data were collected principally through chart review, which may have led to under- or overestimates of acute changes in management. Second, our patients were too young to assess their language development, behavioral performance and neurocognition.

Conclusions

Our study identified twelve critically ill Chinese SYS infants with six novel deleterious truncating variants in *MAGEL2*, expanding SYS's genotype and phenotype spectrum. The results demonstrated that respiratory dysfunction and dystonia were typical characteristics among Chinese SYS patients which should attract physicians' attention. The early identification of such a disorder is critical as it allows early intervention with specific care and treatment. More importantly, improving genetic diagnosis can provide genetic counseling and prenatal diagnosis for families.

Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4396/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4396/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4396/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of the Children's Hospital, Fudan University (No. 2021-45) and individual consent for this retrospective analysis was waived.

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