Received: 17 July 2022 | Revised: 22 February 2023 | Accepted: 23 February 2023

DOI: 10.1111/cge.14320

SHORT REPORT

CLINICAL GENETICS WILEY

Two new cases with novel pathogenic variants reflecting the clinical diversity of Schaaf-Yang syndrome

Ceren Alavanda[1] | Esra Arslan Ateş[2] | Zehra Yavaş Abalı[3] | Bilgen Bilge Geçkinli[1] | Serap Turan[3] | Ahmet Arman[1]

[1]Department of Medical Genetics, School of Medicine, Marmara University, Istanbul, Turkey

[2]Department of Medical Genetics, Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Istanbul, Turkey

[3]Department of Pediatric Endocrinology and Diabetes, School of Medicine, Marmara University, Istanbul, Turkey

Correspondence
Ceren Alavanda, Department of Medical Genetics, School of Medicine, Marmara University, Pendik, Istanbul 34890, Turkey.
Email: cerenalavanda@gmail.com

Abstract

Schaaf-Yang syndrome (SHFYNG) is a rare pleiotropic disorder, characterized by hypotonia, joint contractures, autism spectrum disorders (ASD), and developmental delay/intellectual disability. Although it shares some common features with Prader-Willi Syndrome, joint contractures, and ASD were more commonly detected in in this syndrome. Recently, it was shown that truncating variants in the paternal allele of the *MAGEL2* gene cause SHFYNG. Here, we present two patients diagnosed with SHFYNG syndrome having two different novel truncating variants in the *MAGEL2* gene, one paternally inherited and one de novo. One patient had obesity, brachydactyly and dysmorphic features, and the other patient presented with contractures, severe hypotonia and early death. This is the first report of Turkish SHFYNG syndrome cases presented to emphasize the phenotypic diversity of the syndrome.

KEYWORDS
MAGEL2, novel, Prader-Willi-like syndrome, Schaaf-Yang syndrome, SHFYNG

1 | INTRODUCTION

Prader-Willi syndrome (PWS-OMIM #176270) is one of the well-known syndromes characterized by typical dysmorphic features, neonatal hypotonia, feeding difficulties in early life, hyperphagia leading to excess weight gain, and hypogonadism.[1] Chromosome 15 contains a critical region for PWS which is 6 MB long. This region encompasses paternally expressed, maternally imprinted genes like *MKRN3*, *MAGEL2*, *NDN*, *NPAP1*, *SNURF*, *SNRPN*, and small nucleolar RNA (*snoRNA*) genes.[2] Recently, it was shown that truncating variants in the GC-rich single-exon melanoma antigen L2 (*MAGEL2*) gene cause a PWS-like phenotype and autism.[3] This syndrome was called Schaaf-Yang syndrome (SHFYNG-OMIM #615547).[4,5] There are some common clinical features in SHFYNG and PWS, such as neonatal hypotonia, feeding problems, and developmental delay. The major difference of these syndromes are autism and joint contractures. Although more than 100 patients have been reported in the literature, the identification of new cases will lead to a better understanding of this syndrome. In this study, we report two new cases diagnosed with SHYNG by detecting two novel truncated variants and their clinical findings.

2 | METHODS

2.1 | Anthropometric measurements

Weight and height were measured in all subjects, using a wall-mounted calibrated Harpenden Stadiometer (Holtain Limited, Crosswell, UK) and electronic scale (sensitivity to 0.1 kg). All measurements were expressed as SDS according to national standards.[6,7]

2.2 | Genetic studies

Informed consent was obtained from the parents of the patients. G-banded karyotype analysis was performed at the 550 band level.

Genomic DNA was isolated from the peripheral blood of both patients' and patients' parents'. Isolation was performed using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) in according to the manufacturer's instructions. Methylation-specific multiplex ligation-dependent probe amplification kit from MRC Holland (Amsterdam, the Netherlands) was used for PWS methylation analysis. ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) was used for sequence the *MC4R* and *LEPR* genes. Clinical Exome Sequencing (CES) was performed using SOPHIA Clinical Exome Solution V2 (Boston, USA) via Illumina Nextseq 550 platform (San Diego, CA, USA). Data were analyzed using the Sophia DDM-V4 (Boston, USA) data analysis platform. The following filtering steps were applied: (1) All missense, nonsense, frameshift, indels, in-frame, no-start or no-stop variants, (2) Synonymous or intronic variants that predicted to affect the splice sites, (3) Rare variants with minor allele frequency <1.0% in population studies [1000 Genome (1000G), ESP5400, and Genome Aggregation Database (GnomAD)]. In silico tools (Mutation Taster-MT, PolyPhen-2, PROVEAN, Sorting Intolerant from Tolerant-SIFT, Human Splicing Finder) were used to predict the pathogenicity of the candidate variants. The Human Gene Mutation Database and ClinVar databases were used to determine whether the detected variants were novel or previously reported. Segregation analysis was performed via Illumina Miseq Platform (San Diego, CA, USA).

The method previously reported by Schaaf et al.[3] was used to show whether the variant detected in the Patient 2's father was on the paternal or maternal allele. The methylation sensitive restriction enzyme *SmaI* used in this method to digest the unmethylated paternal *MAGEL2* allele. Therefore, only the maternal allele can be obtained in PCR after restriction. For *SmaI* enzyme digestion, 1 ng DNA of the Patient 2's father was digested with 10 units of *SmaI* restriction enzyme at 30°C for overnight. PCR was performed using the digested template DNA, forward GAGAATTCCACCATCGCCACTAACC and reverse CAGTCCCTGCAACTTCCCACTTTCT primers. PCR was carried by total volume of 25 µL reaction containing digested 75 ng of genomic DNA, 2.5 µL 10× buffer, 1.5 mM MgCl2, 0.5 µM forward primer, 0.5 µM reverse primer, 0.2 mM dNTP, and 2 units Taq polymerase. The PCR sample was denatured at 94°C for 1 min (1×) for initial denaturation and main PCR cycle was denaturation at 98°C for 10 sec, annealing-extension at 68°C for 15 min (all cycle 30×) and final extension was done at 72°C for 10 min (1×). The PCR product was visualized by gel electrophoresis and sequenced by Illumina Miseq Platform (San Diego, CA, USA).

3 | PATIENTS AND RESULTS

3.1 | Patient 1

A 15-year-old girl was referred to the medical genetics department because of obesity, intellectual disability, and dysmorphism. Her parents were second cousins and she was the first child of healthy parents. Prenatal history was uneventful. She was born at term with a birth weight of 3300 g (0.5 SD). She had no history of failure to thrive in infancy or respiratory insufficiency. She began to sit independently at 6 months and started walking at 18 months. She spoke her first words at the age of 30 months. In dysmorphological examination, coarse face, high forehead, bitemporal narrowing, deep-set eyes, prominent nasal bridge, bulbous nasal tip, short philtrum, thin lips, prominent jaw, posteriorly rotated ears, short neck, obesity, tapering fingers, brachydactyly, bilateral sandal gaps between the first and second toes were detected (Figure 1A–D). Physical examination revealed acanthosis nigricans on the neck and striae on the abdominal region. Her family stated an increase in weight gain starting from the age of 3 months. At the age of $6^{2/12}$ years her weight, length, and body mass index (BMI) were 38.7 kg (3.4 SDS), 116.4 cm (0.1 SDS), 24.4 kg/m^2 (3.3 SDS), respectively. At the age of $16^{5/12}$ her weight, height, and BMI were 129.4 kg (6.38 SDS), 156.5 cm (1.03 SDS), 52.83 kg/m^2 (4.79 SDS), respectively (Figure 1G). The bone age was determined as 8.8 years at the age of $6^{2/12}$. During follow-up, a GnRH stimulation performed due to advanced bone age and early breast development revealed prepubertal results. Her age was $12^{8/12}$ at menarche and menstrual cycles were irregular. Her fasting blood glucose, insulin, HbA1c, liver transaminases, thyroid function tests and a metabolic workup were normal. Due to progressive weight gain, and clinical findings of insulin resistance such as acanthosis nigricans, oral glucose tolerance test was performed and revealed insulin resistance. Metformin treatment was commenced at the age of 13 years 9 months; however, she was noncompliant to treatment and weight gain was progressive. Ambulatory blood pressure monitorization was planned due to high blood pressure in outpatient examinations (BP:155–75 mmHg, systolic >99p, >2.3 SDS). After the evaluation by the pediatric psychiatrist, it was determined that the patient did not have autism spectrum disorder but she had attention deficit hyperactivity disorder and low school performance. Her intelligence quotient was 80. There were no abnormalities in other systemic evaluations. Clinical findings of the Patient 1 were summarized in Table 1.

Her karyotype was 46, XX, and methylation analysis for PWS was normal. No pathogenic variant was identified in the *LEPR* and *MC4R* genes. A novel heterozygous c.1732C>T (p.Gln578*) pathogenic variant in *MAGEL2* (NM_019066) gene was detected in CES (Figure 2B). This variant was not reported in GnomAD, ESP5400, G1000. According to ACMG criteria, this variant was "Likely Pathogenic" (PVS1, PM2). Combined annotation dependent depletion (CADD) score of this variant was 12.08.[8] Segregation analysis revealed that this variant had arisen de novo (Figure 2C,D). In the CES analysis, no homozygous or heterozygous variant was found in any other gene that could explain the patient's clinic.

3.2 | Patient 2

A 16 month old male patient with overriding fingers, developmental delay and hypotonia was referred to our clinic. He was the first

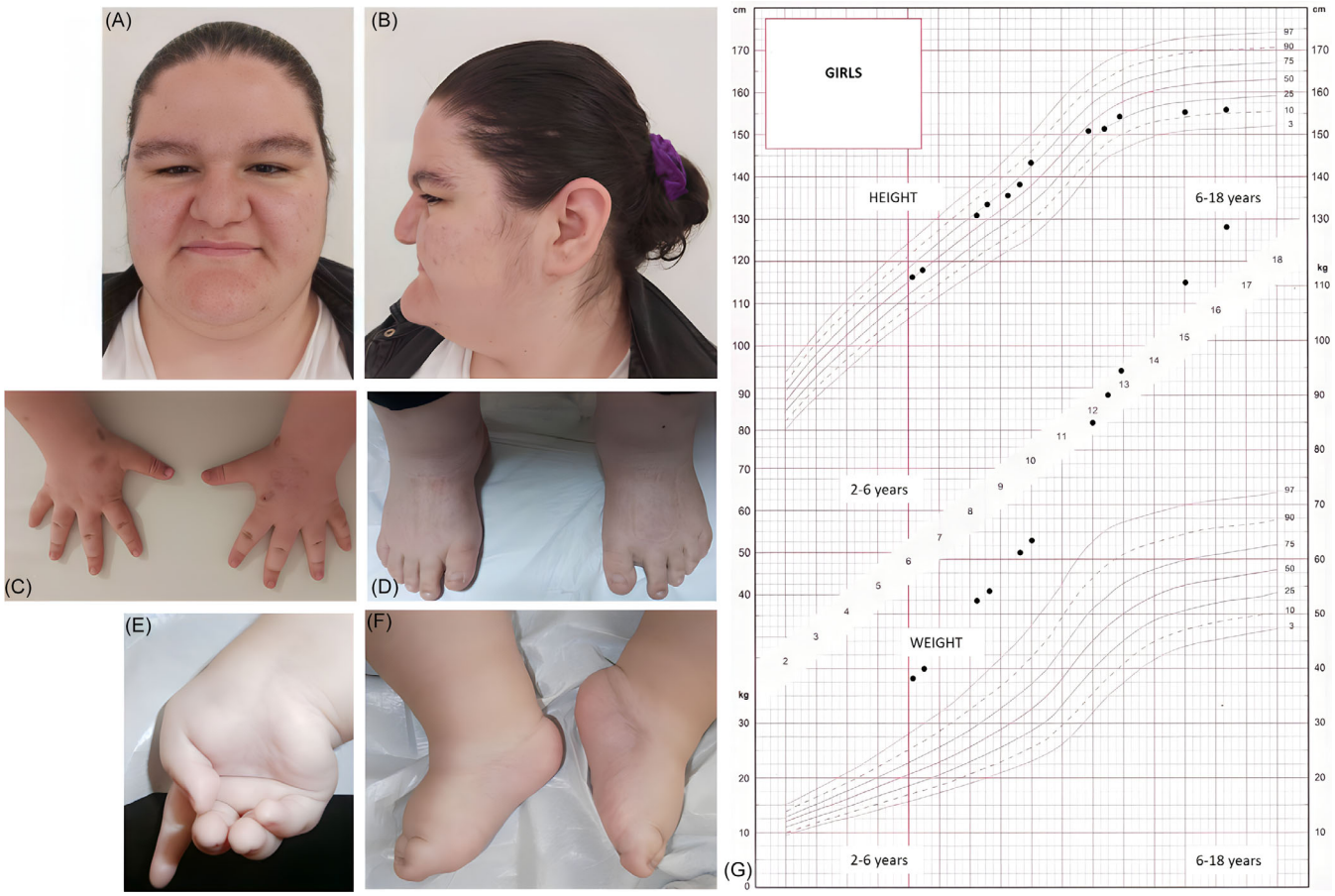

FIGURE 1 Dysmorphic features of the two patients. Patient 1: (A) Coarse face, high forehead, bitemporal narrowing, deep set eyes, prominent nasal bridge, bulbous nasal tip, short philtrum, thin lips; (B) Prognathism, posteriorly rotated ears, short neck; (C) Tapering fingers, brachydactyly; (D) Bilateral sandal gaps, brachydactyly. Patient 2: (E) Overriding fingers due to contractures, brachydactyly; (F) Small feet, rocker-bottom feet. (G) Height and weight percentiles measured during the follow-up of the Patient 1. [Colour figure can be viewed at wileyonlinelibrary.com]

child of healthy and nonconsanguineous parents. Prenatal history was uneventful. He was born by vaginal delivery at 7.5 months gestational age due to fetal stress. According to mother's statement, his birth weight, height and OFC were 2500 gr (3.27 SD), 49 cm (3.32 SD), 34 cm (4.21 SD), respectively.[9] Meconium aspiration and fetal asphyxia were detected. He was hospitalized in the neonatal intensive care unit for 4.5 months. Percutaneous endoscopic gastrostomy was performed. At the time of examination, he had severe hypotonia and there was no unassisted sitting, walking, or speaking. In his dysmorphological examination nystagmus, prominent forehead, prominent ridge over the metopic suture, bitemporal narrowing, low posterior hairline, micrognathia, posteriorly rotated ears, overriding fingers (Figure 2E), tapering fingers, simian crease in both hands, brachydactyly, small feet, rocker-bottom feet (Figure 2F), micropenis and bilateral undescended testis were detected. His anthropometric measurements were; height: 74 cm (−2.09 SD), weight:10 kg (−0.84 SD), OFC: 44 cm (−2.69 SD), and penile length: 1.7 cm (<3p).[9] In his neonatal echocardiography, patent foramen ovale, mild tricuspid insufficiency and mild pulmonary hypertension were detected. At the age of one, his echocardiography and abdominal ultrasonography (USG) were normal. Cranial USG revealed a 3 mm choroid plexus cyst at the right choroid plexus. Nystagmus was detected on ophtalmological examination. First electroencephalogram (EEG) was performed at the age of 16 months and showed irregular background activity without epileptic discharges. After 2 months without treatment the second EEG was reported as normal. Clinical features of the Patient 2 were summarized also in the Table 1. While the genetic tests were going on, the patient died at the age of 20 months due to an unknown reason.

His karyotype analysis and methylation testing for PWS were normal. CES revealed heterozygous c.1882C>T (p.Gln628*) variant in *MAGEL2* (NM_019066) gene (Figure 2F). This variant was not reported in GnomAD, ESP5400, G1000. According to ACMG, this variant was "Likely Pathogenic" (PVS1, PM2). CADD score of this variant was 36.[8] Segregation analysis revealed that the variant was inherited from his healthy father (Figure 2G,H). After *SmaI* enzyme digestion, a PCR product of 5746 bp was visualized by gel

TABLE 1 Clinical summary and genetic analysis results of the two SHFYNG patients.

Clinical findings	Patient 1	Patient 2
Prenatal manifestations		
Decreased fetal movement	–	–
Fetal akinesia	–	–
Growth		
Short stature	+	+
Obesity	+	–
Excessive weight gain after neonatal period	+	N/A
Infancy		
Failure to thrive in infancy	–	+
Poor feeding in infancy	–	+
Respiratory problems in infancy	–	+
Head		
Prominent ridge over the metopic suture	–	+
Face		
Coarse facies	+	–
Frontal bossing	+	+
High forehead	+	+
Bitemporal narrowing	+	+
Retrognathia	–	+
Micrognathia	–	+
Prominent jaw	+	–
Abnormal philtrum	+	+
Myopathic facies	–	+
Horizontal groove over the chin	–	+
Ears		
Low-set ears	–	–
Eyes		
Hypertelorism	–	–
Downslanting palpebral fissures	+	+
Epicanthal folds	–	–
Myopia	–	–
Strabismus	–	+
Almond-shaped eyes	+	+
Short palpebral fissures	+	+
Bushy eyebrows	+	+
Deep-set eyes	+	+
Ptosis	–	–
Nystagmus	–	+
Microcornea	–	–
Hyperopia	–	–
Exotropia	–	–
Nose		
Prominent nasal bridge	+	–
Depressed nasal bridge	–	+
Broad nasal root	+	+
Mouth		
Open mouth	–	+
Thick lips	–	–
Thin upper lip	+	+
Cleft palate	–	–
Gastrointestinal		
Gastroesophageal reflux	–	–
Constipation	+	+
Genitourinary		
Micropenis	N/A	+
Cryptorchidism	N/A	+
Skeletal		
Joint contractures	–	+
Arthrogryposis	–	–
Decreased bone mineral density	–	N/A
Scoliosis	–	–
Kyphosis	+	–
Brachydactyly	+	+
Tapering fingers	+	+
Camptodactyly	–	+
Overlapping digits	–	+
Clinodactyly	+	–
Rocker-bottom feet	–	+
Cognitive/Neurologic		
Delayed psychomotor development	+	+
Intellectual disability, mild to severe	+	+
Hypotonia	+	+
Speech articulation defects	+	N/A
Sleep apnea	–	–
Seizures	–	+
Kraniyal MRI abnormalities	–	+
Autistic features	–	N/A
Temperature instability	–	–
Endocrine features		
Growth hormone deficiency	–	–
Hypothyroidism	–	–
Glucose intolerance	+	–
Hypoglycemia	–	–
Hypogonadism	+	–
Genetic analysis of *MAGEL2* gene	Heterozygous c.1732C>T (p.Gln578*) novel pathogenic variant	Heterozygous c.1882C>T (p.Gln628*) novel pathogenic variant

Abbreviation: N/A, not applicable.

electrophoresis (Figure 2I). After sequencing it was shown that the patient's father carried this variant on his maternal allele (Figure 2J).

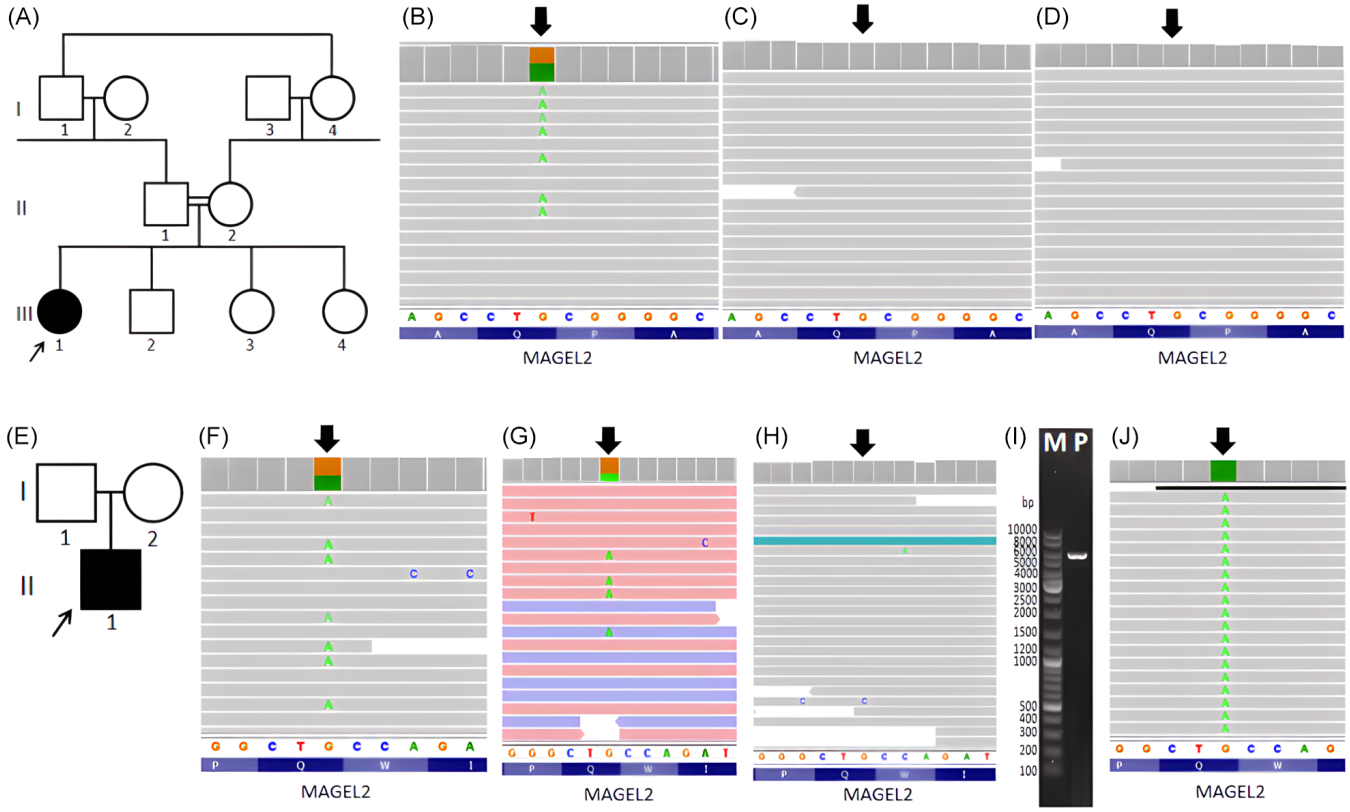

FIGURE 2 Pedigrees of the patients and Integrative Genomics Viewer (IGV) visualizations of the detected novel variants. (A) Pedigree of Patient 1. (B) Novel heterozygous c.1732C>T (p.Gln578*) pathogenic variant in *MAGEL2* (NM_019066) gene. (C), (D) Segregation analysis showed that this variant was de novo. (E) Pedigree of Patient 2. (F) Heterozygous c.1882C>T (p.Gln628*) novel pathogenic variant in *MAGEL2* (NM_019066) gene. (G), (H) Segregation analysis showed that the variant was inherited from the healthy father and mother had wild type allele. (I) Gel electrophoresis of 5746 bp PCR product after *SmaI* digestion (M: Marker, P: PCR product). (J) DNA sequencing result showed that patient's father carried this variant on his maternal allele. [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

There is a significant but incomplete phenotypic overlap between PWS and SHFYNG. Joint contractures which were seen in 88% of SHFYNG patients can be an early finding hinting at a possible SHFYNG.[10] Hyperphagia (35%) and obesity (30%–50%) are seen less common in SHFYNG compared with PWS.[11] In this study, Patient 1 had obesity without hyperphagia. The underlying mechanism of obesity is not clear yet. However, dysfunction of hypothalamic pathways related to energy metabolisms and apetite may explain obesity without hyperphagia. Intellectual disability and developmental delay are more severe in SHFYNG.[10] autism spectrum disorders frequency is 25% in PWS,[12] however 89% in SHFYNG.[13] In a recent study,[14] a patient with abnormal background activity in EEG which returned to normal in 3 months was reported. EEG findings of our second patient were consistent with this literature. Consequently, temporary abnormal background activity of EEG may be related to SHFYNG.

Early deaths are more common in SHFYNG. Patient 2 had no obesity or arthrogryposis multiplex congenita, so respiratory failure or sudden infant death syndrome may be the reason of death.

Regular follow-up of Patient 1 for 10 years shows that height and BMI increase significantly with age (Figure 1G), providing supporting information for the literature.[15] In addition, as seen in Patient 1, it is important to follow up patients in terms of hypertension and menstrual irregularity.

To the best of our knowledge, patients with SHFYNG were reported for the first time from Turkey in this study. Long-term follow-up of our patient constitutes an important example for the growth curves of these patients. Furthermore, this study introduced two novel pathogenic variants to the literature and reflected the phenotypic diversity of SHFYNG.

AUTHOR CONTRIBUTIONS

Ceren Alavanda: Formal analysis; investigation; methodology; writing—original draft. **Esra Arslan Ateş:** Writing; Editing; Data curation; investigation; methodology; visualization. **Zehra Yavaş Abalı:** Endocrine evaluation; writing—original draft. **Bilgen Bilge Geçkinli:** Data curation; methodology; writing—review and editing. **Serap Turan:** Endocrine evaluation; writing—review and editing. **Ahmet Arman:** supervision; writing—review and editing.

ACKNOWLEDGMENTS

We would like to thank the patients' families for participating in this work.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14320.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Ceren Alavanda https://orcid.org/0000-0002-7327-3849

REFERENCES

1. Holm VA, Cassidy SB, Butler MG, et al. Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics*. 1993;91(2):398-402.
2. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genet Med*. 2012;14(1):10-26. doi:10.1038/gim.0b013e31822bead0
3. Schaaf CP, Gonzalez-Garay ML, Xia F, et al. Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. *Nat Genet*. 2013;45(11):1405-1408. doi:10.1038/ng.2776
4. Fountain MD, Schaaf CP. Prader-Willi syndrome and Schaaf-Yang syndrome: neurodevelopmental diseases intersecting at the MAGEL2 gene. *Diseases*. 2016;4(1):2. doi:10.3390/diseases4010002
5. Cheon CK. Genetics of Prader-Willi syndrome and Prader-will-like syndrome. *Ann Pediatr Endocrinol Metab*. 2016;21(3):126-135. doi:10.6065/apem.2016.21.3.126
6. Demir K, Konakçı E, Özkaya G, et al. New features for child metrics: further growth references and blood pressure calculations. *J Clin Res Pediatr Endocrinol*. 2020;12(2):125-129.
7. Bundak R, Furman A, Gunoz H, Darendeliler F, Bas F, Neyzi O. Body mass index references for Turkish children. *Acta Paediatr*. 2006;95:194-198.
8. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014;46(3):310-315. doi:10.1038/ng.2892
9. Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu MM, et al. Body weight, length and head circumference at birth in a cohort of Turkish newborns. *J Clin Res Pediatr Endocrinol*. 2012;4:132-139. doi:10.4274/jcrpe.693
10. McCarthy J, Lupo PJ, Kovar E, et al. Schaaf-Yang syndrome overview: report of 78 individuals. *Am J Med Genet A*. 2018;176(12):2564-2574. doi:10.1002/ajmg.a.40650
11. Fountain MD, Aten E, Cho MT, et al. The phenotypic spectrum of Schaaf-Yang syndrome: 18 new affected individuals from 14 families. *Genet Med*. 2017;19(1):45-52. doi:10.1038/gim.2016.53
12. Bennett JA, Germani T, Haqq AM, Zwaigenbaum L. Autism spectrum disorder in Prader-Willi syndrome: a systematic review. *Am J Med Genet A*. 2015;167A(12):2936-2944. doi:10.1002/ajmg.a.37286
13. Thomason MM, McCarthy J, Goin-Kochel RP, Dowell LR, Schaaf CP, Berry LN. Neurocognitive and neurobehavioral phenotype of youth with Schaaf-Yang syndrome. *J Autism Dev Disord*. 2020;50(7):2491-2500. doi:10.1007/s10803-018-3775-7
14. Mizuno S, Yokoyama K, Yokoyama A, Nukata T, Ikeda Y, Hara S. Longitudinal analysis of electroencephalography pattern changes in an infant with Schaaf-Yang syndrome and a novel mutation in melanoma antigen L2 (MAGEL2). *Mol Genet Genomic Med*. 2022;10(6):e1932. doi:10.1002/mgg3.1932
15. Marbach F, Elgizouli M, Rech M, et al. The adult phenotype of Schaaf-Yang syndrome. *Orphanet J Rare Dis*. 2020;15(1):294. doi:10.1186/s13023-020-01557-8

How to cite this article: Alavanda C, Arslan Ateş E, Yavaş Abalı Z, Geçkinli BB, Turan S, Arman A. Two new cases with novel pathogenic variants reflecting the clinical diversity of Schaaf-Yang syndrome. *Clinical Genetics*. 2023;1-6. doi:10.1111/cge.14320