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MOWAT-WILSON SYNDROME: DEEP PHENOTYPING AND MOLECULAR CHARACTERISATION OF TWELVE NEW INDIVIDUALS

MOWAT-WİLSON SENDROMU: ONİKİ YENİ OLGUNUN FENOTİPİK VE MOLEKÜLER KARAKTERİZASYONU

Umut ALTUNOĞLU[1], Nilay GÜNEŞ[2], Gözde Tutku TURGUT[3], Tuğba KALAYCI[3], Ayça Dilruba ASLANGER[3], Murat DERBENT[4,†], Serpil ERASLAN[5], Birsen KARAMAN[3,6], Zehra Oya UYGUNER[3], Beyhan TÜYSÜZ[2], Yasemin ALANAY[7], Hülya KAYSERİLİ[1]

[1]Koç University School of Medicine (KUSOM), Department of Medical Genetics, İstanbul, Türkiye
[2]İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Pediatric Genetics, İstanbul, Türkiye
[3]İstanbul University, İstanbul Faculty of Medicine, Department of Medical Genetics, İstanbul, Türkiye
[4]Başkent University, Faculty of Medicine, Pediatric Genetics Unit, Department of Pediatrics, Ankara, Türkiye
[5]Koç University Hospital, Genetic Diseases Evaluation Center, İstanbul, Türkiye
[6]İstanbul University, Institute of Child Health, Department of Pediatric Basic Sciences, İstanbul, Türkiye
[7]Acıbadem University, School of Medicine, Department of Pediatrics, Pediatric Genetics, İstanbul, Türkiye
[†]deceased

ORCID IDs of the authors: U.A. 0000-0002-3172-5368; N.G. 0000-0002-7787-3630; G.T.T. 0000-0002-2573-6431; T.K. 0000-0002-9963-5916; A.D.A. 0000-0003-1770-1762; S.E. 0000-0002-7674-7384; B.K. 0000-0001-8640-0176; Z.O.U. 0000-0002-2035-4338; B.T. 0000-0002-9620-5021; Y.A. 0000-0003-0683-9731; H.K. 0000-0003-0376-499X

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ABSTRACT

Objective: Mowat-Wilson syndrome (MOWS) is a rare multisystem malformation syndrome characterised by distinctive facial features, moderate to severe intellectual disability, and variable findings including callosal anomalies, ocular features, genital anomalies, congenital heart defects, and Hirschsprung's disease. Pathogenic variants in the *ZEB2* gene are implicated in the aetiology, with nearly all cases arising sporadically due to *de novo* variants. In addition to its low prevalence, the broad clinical spectrum observed among patients can make the diagnostic process challenging. This study aims to expand the clinical and molecular spectrum of MOWS by elucidating the characteristics of a new cohort.

Material and Methods: Twelve patients with a clinical diagnosis of MOWS were included in the study. Following obtaining normal karyotype results, molecular analysis of *ZEB2* was performed using Sanger sequencing.

ÖZET

Amaç: Mowat-Wilson sendromu (MOWS); tanınabilir yüz özellikleri, orta-ağır zihinsel yetersizlik ve korpus kallozum anomalileri, oküler tutulum, genital anomaliler, konjenital kalp defektleri ve Hirschsprung hastalığı gibi multisistemik bulgularla karakterize nadir bir malformasyon sendromudur. Etiyolojide *ZEB2* genindeki patojenik varyantlar rol oynamakta olup, neredeyse bütün vakalar sporadik olarak *de novo* varyantlardan kaynaklanmaktadır. Hastalığın düşük prevalansının yanı sıra etkilenmiş olgularda gözlenen geniş klinik spektrum tanı sürecini zorlaştırabilmektedir. Bu çalışmada yeni bir MOWS kohortu tanımlayarak bu hastalığın klinik ve moleküler spektrumunu genişletmeyi amaçladık.

Gereç ve Yöntem: Çalışmaya MOWS klinik tanısı almış 12 olgu dahil edildi. Normal karyotip sonuçlarının elde edilmesinin ardından *ZEB2* geni Sanger dizi analizi yöntemiyle incelendi.

Corresponding author/İletişim kurulacak yazar: Umut ALTUNOĞLU – ualtunoglu@ku.edu.tr

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Results: Anthropometric measurements at birth and subsequent visits largely aligned with the national and MOWS growth charts, respectively. All patients exhibited moderate to severe intellectual disability and shared a characteristic facial gestalt. In addition to the well-described features, very rare or previously undescribed abnormalities comprising persistent left superior vena cava, choanal stenosis, shawl scrotum, and ocular anomalies were observed. Skin pigmentation defects were noted at significantly higher frequencies than those previously reported. Two patients displayed atypical features overlapping with CHARGE and Aicardi syndromes. We identified 12 heterozygous variants in *ZEB2*, five of which were novel.

Conclusion: Deep phenotyping data of 12 patients enabled the identification of previously uncertain clinical associations and underrepresented features. The novel pathogenic variants identified here expand the molecular spectrum of *ZEB2*.

Keywords: Mowat-Wilson syndrome, *ZEB2*, intellectual disability, CHARGE syndrome, Aicardi syndrome

Bulgular: Doğum ve tekrarlayan klinik değerlendirmelerde alınan antropometrik ölçümlerin ulusal ve MOWS'a özgü büyüme eğrileriyle büyük ölçüde uyumlu olduğu gözlendi. Tüm hastalarda orta ila ağır zihinsel yetersizlik ve karakteristik yüz görünümü mevcuttu. Hastalığın iyi bilinen bulgularına ek olarak; persistan sol süperior vena kava, koanal stenoz, şal skrotum ve atipik oküler anomaliler gibi çok nadir veya daha önce MOWS spektrumunda tanımlanmamış bulgular izlendi. Pigmentasyon bozukluklarının literatür verisine kıyasla belirgin şekilde daha yüksek sıklıkta olduğu gözlendi. İki hastada, CHARGE ve Aicardi sendromları ile örtüşen atipik klinik bulguların varlığı dikkat çekiciydi. Hastalarda, *ZEB2* geninde beşi daha önce tanımlanmamış olmak üzere 12 heterozigot varyant tespit edildi.

Sonuç: On iki hastanın derin fenotipleme verileri, daha önce MOWS ile klinik ilişkisi net şekilde ortaya konulmamış veya hastalık spektrumunda çok nadir olduğu düşünülen klinik bulguların tanımlanmasını sağladı. Ayrıca, bu çalışma kapsamında ilk kez bildirilen patojenik *ZEB2* varyantları ile hastalığın moleküler spektrumu da genişletilmiş oldu.

Anahtar Kelimeler: Mowat-Wilson sendromu, *ZEB2*, zihinsel yetersizlik, CHARGE sendromu, Aicardi sendromu

INTRODUCTION

Mowat-Wilson syndrome (MOWS, MIM #235730) is a rare, dominantly inherited malformation syndrome characterised by recognisable facial features, moderate-to-severe intellectual deficit, and variable multisystem involvement comprising corpus callosum anomalies, ocular features, genital anomalies, congenital heart defects, and Hirschsprung's disease (1). MOWS is caused by heterozygous loss-of-function variants in the "zinc finger E-box binding homeobox 2 (*ZEB2*)" gene (MIM# 605802) (2, 3). The majority of causative *ZEB2* variants identified to date are truncating variants or large deletions resulting in haploinsufficiency (4). Although nearly all cases arise sporadically due to *de novo* variants, familial recurrence has been described in four families, suggesting low-level somatic or putative germline mosaicism (5-8). The prevalence of MOWS is estimated to be 1 in 50,000 to 70,000 live births, although some authors propose that the prevalence may be higher because milder cases may remain undiagnosed (9, 10).

The phenotypic spectrum of MOWS is well characterised through genetically confirmed patient series (9, 11-17). A comprehensive review of 344 molecularly confirmed cases by Ivanovski et al. revealed that the most common clinical features observed in patients with MOWS are seizures, microcephaly, callosal anomalies, hypospadias, and congenital heart defects (4). Although Hirschsprung's disease and postnatal-onset short stature were initially considered cardinal features, they have been reported in less than half of the affected individuals (4, 18, 19).

ZEB2 encodes the 1214-amino acid-long Smad-interacting protein 1 (SIP1), an evolutionarily conserved member of the two-handed zinc finger and homeodomain-containing protein family. It functions as a transcriptional repressor by interacting with activated SMAD proteins (20). SIP1 plays a critical role in various stages of vertebrate embryogenesis, with knockout mouse model studies revealing several functions, including signal regulation during corticogenesis and the modulation of BMP-Smad and Wnt-β-catenin pathways involved in central nervous system (CNS) myelination (21-23). In humans, *ZEB2* is localised to the 2q21-q23 region, and the canonical transcript (NM_014795.4) consists of 10 exons spanning 136 kb. Nearly half of the known *ZEB2* pathogenic variants are found in exon 8. The N-terminal region of the ZEB2 protein (NP_055610.1) contains five C2H2-type zinc finger domains (ZnF_C2H2), four of which are encoded by exon 6 and one by exon 8. These are followed by a homeobox domain also encoded by exon 8 and a cluster of three ZnF_C2H2 domains encoded by exons 9 and 10 (24).

No standardised diagnostic criteria have been established for MOWS to date. The recognisable facial appearance is the most reliable handle for the clinical diagnosis, except in mildly affected patients harbouring missense or specific splice-site (5'UTR) variants (25-27). The distinctive facial features associated with MOWS include hypertelorism, medial flaring of the eyebrows, low-hanging columella, deeply-set eyes, long and pointed chin, an open mouth, and centrally depressed-uplifted earlobes. While facial appearance tends to evolve with age, the unique ear configuration, likened to orecchiette pasta or red blood cells, remains a constant finding throughout life (15).

In this study, we investigated a cohort of 12 MOWS patients with deep phenotyping and *ZEB2* sequencing to

further expand the clinical and molecular spectrum of MOWS.

MATERIAL AND METHODS

Twelve patients with MOWS were included in the study based on clinical evaluations by medical geneticists or paediatric geneticists from four national centres. Growth centiles were assessed following both the national guidelines and MOWS growth charts (28, 29). In all cases, high-resolution chromosome analysis was performed to exclude gross chromosomal abnormalities.

DNA isolation for the sequencing of *ZEB2* (NM_014795.4) was performed using a commercial kit according to the instructions of the manufacturer (DNA Isolation Kit for Mammalian Blood, Roche). Nine coding exons (exon 2-10) of *ZEB2* and the flanking intronic regions were amplified by PCR and sequenced using the Sanger technique (ABI3500). The human genome assembly GRCh38/hg38 was used as the reference genome.

Ethical approval for this study was provided by the Istanbul Faculty of Medicine, Clinical Research Ethics Committee (Date: 07.10.2022, No: 18), in accordance with the Helsinki Declaration. Informed written consent for genetic testing and the use of clinical photographs was obtained from the legal guardians.

RESULTS

This cohort included 12 unrelated patients diagnosed with MOWS, comprising eight males and four females. The age at the last clinical assessment ranged between 12 months and 10 years 3 months. The clinical data are described below and summarised in Table 1.

Antenatal and natal findings

The prenatal period was unremarkable in the majority, except for abortus imminens in two cases, severe hyperemesis gravidarum in one case, and polyhydramnios of unknown aetiology in another. One individual was born at the late preterm period, 36 weeks of gestation, and the remaining were born at an average of 38^{+3} weeks. The standard deviations (SDs) corresponding to the mean birth weight and birth length of the cohort were 0.20±0.6 and 0.47±0.31, respectively. Occipitofrontal circumference (OFC) measurements at birth were within normal limits in the three patients where it was available. One patient developed prolonged neonatal jaundice. No neonatal complications requiring neonatal intensive care unit support were reported in any of the patients.

Growth

At the last clinical evaluation between 12 months and 10 years 3 months (mean: 47.8 months), the mean SD values for weight, length/height, and OFC according to the national growth charts were -1.37±0.46 (range -2.43 to 0.53), -1.31±0.59 (range -3.49 to 0.32), and -2.89±0.59 (range -0.60 to -4.58), respectively. Two patients had OFC values within the normal range, and the remaining patients developed postnatal microcephaly, borderline (between -2/-3 SD) in four and true (below -3 SD) in six. Short stature was observed in two patients.

Craniofacial features

The dysmorphic features observed in all patients included a long face, medially flared and broad eyebrows, and a long chin (Figure 1). Other consistent facial features were characteristic ear shape (n=11/12), hypertelorism (n=10/12), deeply-set eyes (n=10/12), wide nasal root (n=10/12), low-hanging columella (n=10/12), prominent or bulbous nasal tip (n=7/12), open-mouth appearance (n=7/12), thick or prominent lower lip vermilion (n=7/12), and short philtrum (n=7/12).

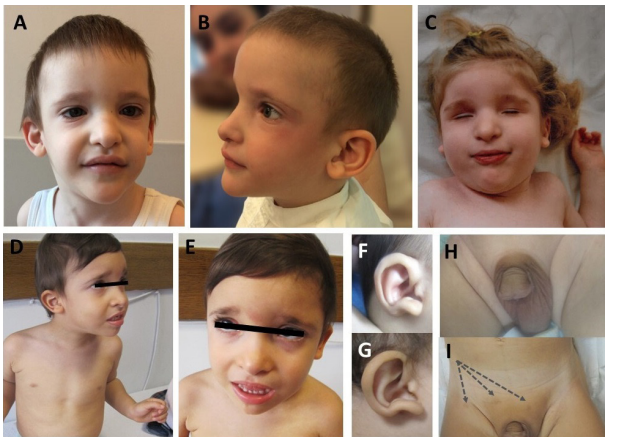

Figure 1: Clinical photographs of individuals with Mowat-Wilson syndrome. Facial features of Patient 8 at 2 years 10 months (A, B), Patient 9 at 3 years 1 month (C), and Patient 4 at 3 years 7 months (D, E). All three patients exhibited an elongated face, broad and medially flared eyebrows, deeply set eyes, low-hanging columella, full lips, short philtrum, and prominent chin. Notably, Patient 8 also displayed a prominent forehead (B), and both Patient 8 and Patient 9 had ears with uplifted lobules (B, C). Ear photographs of Patient 12 taken at 2 months (F) and 19 months (G) illustrate the characteristic ear configuration, likened to orecchiette pasta or red blood cells. Atypical findings observed in Patient 12 included hyperpigmented shawl scrotum (H), and hyperpigmented spots along with wide hypopigmented patches of skin (dashed arrows) (I). Note that the shawl scrotum appearance regressed over time, while the pigmentation anomalies became more pronounced

Neurological findings, development, and behavioural features

All 12 patients exhibited global developmental delay and/or intellectual deficit, severe in five patients, moderate to severe in four, and moderate in three. The mean

Table 1: Clinical and molecular overview of the cohort

Patient	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Antenatal and natal history												
Prenatal findings	-	-	abortus imminens	-	abortus imminens	hyperemesis gravidarum	-	polyhydramnios	-	-	-	-
Gestational week at birth	39	40	37	40	39	39	38	38	40	36	39	37
Length at birth [SD]	50 cm [-0.05]	NA	49 cm [0.35]	51 cm [0.28]	50 cm [-0.05]	NA	50 cm [0.21]	51 cm [0.74]	NA	50 cm [1.1]	NA	51 cm [1.15]
Weight at birth [SD]	3450 g [0.28]	3000 g [-0.87]	3150 g [0.72]	3150 g [-0.84]	3350 g [0.02]	3000 g [-0.82]	3700 g [1.24]	3250 g [0.15]	2750 g [-1.58]	3350 g [1.68]	3400 g [0.43]	3820 g [2.02]
OFC at birth [SD]	NA	NA	NA	33 cm [-1.68]	34 cm [-0.73]	NA	NA	NA	NA	NA	NA	34 cm [-0.08]
Neurological and behavioural features												
Epilepsy (onset)	+ (2 yrs 6 mos)	-	-	-	+ (2 yrs)	-	-	-	+ (2 yrs)	+ (9 mos)	-	-
Pathological EEG pattern	epileptiform activity	epilep-tiform activity	diffuse spike and slow wave discharges	NA	bilateral central spike discharges with a normal background	-	multifocal spike-and-wave discharge	-	bilateral frontotemporal spike and wave discharges	slowing of background activity with frequent generalised bursts	-	-
Callosal anomalies	ACC	-	DCC	-	-	ACC	-	-	CH	-	NA	CH
Additional structural anom-alies on MRI	-	-	-	-	-	-	CSP	benign tonsillar ectopia	-	Reduced hippocampal volume on the left, deep pa-rieto-occipital white matter hyperinten-sities on T2 and FLAIR sequences	NA	-

Table 1: Continued

Patient	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Neurological and behavioural features												
Additional neurological anomalies	-	ataxic gait	-	-	-	-	-	-	-	ataxic gait	-	Oral motor dysfunction
Abnormal behavioural features	oral behaviours, motor stereotypies (hand biting, chin slapping)	stereotypic hand movements, hyperorality	-	happy demeanour	happy demeanour	lack of eye contact	-	-	oral behaviours, motor stereotypies (hand biting), episodic laughter	-	-	-
Gastrointestinal findings												
Hirschprung disease (HSCR)	+	-	-	-	+	+	+	-	-	-	-	-
Chronic constipation without HSCR	-	+	-	+	-	-	-	-	-	+	-	-
Congenital cardiac defects	PPS	-	ASD	-	small perimembranous VSD	PPS	ASD	PDA, VSD, PLSVC, PHT	-	bicuspid aorta	-	bicuspid aorta PDA, PFO
Genitourinary findings												
Renal abnormalities	-	NA	-	-	-	-	-	-	-	transient pelviectasis	grade I pelviectasis (L)	pelvicalicial ectasia (L)

Table 1: Continued

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Genitourinary findings												
Genital abnormalities	hypospadias, cryptorchidism (R)	-	-	hypospadias, cryptorchidism (blt)	hypospadias	Cryptorchidism (blt)	-	hypospadias	-	hypospadias	-	hypospadias, cryptorchidism (R), minimal hydrocele (L), chordee, shawl scrotum with severe hyperpigmentation
Ophthalmologic abnormalities	microphthalmia (L), microcornea	-	-	-	astigmatism, myopia	-	-	peripapillery atrophy	microphthalmia and aniridia (blt), congenital cataracts (L), retinal atrophy	esotropia (L), congenital retinal atrophy	-	iris coloboma (R), nasolacrimal duct obstruction
History of recurrent infections	frequent otitis media	-	-	frequent upper respiratory tract infections and otitis media		-	transient hypogammaglobulinemia of infancy	-	-		-	-
Other	fibroma in the occipital subcutaneous region	hypersalivation	prolonged neonatal jaundice	hypopigmented spots, low-set ears, delayed eruption, genu valgum	precocius puberty, accessory nipple (R), hypopigmented skin lesions	nail hypoplasia, positional anomalies of toes	hypopigmented macules around the abdomen	broad and deep philtrum, pes valgus	-	torticollis	-	1 CAL spot, choanal stenosis, hypopigmented macules
Age at last examination	3 yrs 6 mos	5 yrs 6 mos	3 yrs 5 mos	3 yrs 7 mos	10 yrs 3 mos	24 mos	22 mos	2 yrs 10 mos	3 yrs	9 yrs 4 mos	12 mos	19 mos
Height (cm) [SD]	97.5 [-0.73]	103 [-1.93]	99 [0.14]	98 [-0.74]	118 [-3.49]	81 [-1.86]	83 [-0.93]	92 [-0.89]	85 [-2.60]	125 [-1.58]	76 [0.32]	79 [-1.37]
Weight [SD]	14 [-1.0]	14.5 [-2.07]	16 [0.53]	14.5 [-0.79]	28 [-1.03]	9.55 [-2.43]	10.5 [-1.32]	13.2 [-0.74]	11.4 [-1.74]	20.9 [-2.39]	8.05 [-1.26]	9.02 [-2.14]
Occipitofrontal circumference [SD]	47 cm [-2.49]	45 cm [-4.03]	47 cm [-1.64]	47 cm [-2.52]	47 cm [-4.58]	45 cm [-2.81]	45.7 cm [-2.19]	49 cm [-0.60]	44 cm [-3.11]	48 cm [-3.60]	41.5 cm [-3.35]	43 cm [-3.7]

Table 1: Continued

Patient	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Genitourinary findings												
Height, weight and OFC centiles according to MOWS charts (Ivanovski et al., 2020)	75p, 50-75p, 25-50p	25-50p, 25p, 5-25p	75-95p, 75-95p, 50-75p	50-75p, 50-75p, 25-50p	5-25p, 50-75p, 25-50p	25-50p, 5-25p, 25-50p	50-75p, 25-50p, 50p	75p, 50-75p, >95p	5-25p, 25p, 5p	50-75p, 25p, 25p	75-95p, 25-50p, 5-25p	25-50p, 5-25p, 5-25p
Achievement of developmental milestones												
Unsupported sitting	21 mos	NA	9 mos	11 mos	11 mos	20 mos	10 mos	9 mos	25 mos	NA	8 mos	14 mos
Unsupported walking	-	4 yrs	-	2 yrs	3 yrs	-	-	18 mos	-	4 yrs	-	-
Speech and language skills	none	vocalises random sounds	2-3 words	babbles	builds 2-3 word sentences	none	babbles	3-4 words in total	babbles	none	none	none
Bowel/bladder control	-	-	-	-	-	-	-	-	-	-	-	-
Intellectual Disability/Global Developmental Delay	severe	moderate to severe	moderate to severe	severe	moderate	severe	moderate to severe	moderate	severe	moderate	moderate to severe	severe
Dysmorphic facial features												
Elongated face	+	+	+	+	+	+	+	+	+	+	+	+
Broad or medially flared eyebrows	+	+	+	+	+	+	+	+	+	+	+	+
Hypertelorism	+	+	+	+	-	+	+	+	-	+	+	+
Deeply set eyes	+	+	-	+	+	+	-	+	+	+	+	+
Downslanting palpebral fissures	-	-	-	-	-	+	-	-	-	-	+	-
Wide nasal bridge	+	+	+	+	-	+	+	+	+	+	-	+
Prominent or bulbous nasal tip	+	+	-	+	+	+	-	+	-	+	-	-
Columella extending below the ala nasi	+	-	+	+	+	-	+	+	+	+	+	+
Open-mouthed expression	+	+	+	+	+	+	-	-	-	+	-	-

Table 1: Continued

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Gender	**male**	**female**	**female**	**male**	**male**	**male**	**male**	**male**	**female**	**male**	**female**	**male**
Dysmorphic facial features												
Thick or everted lower lip vermillion	+	+	-	+	+	-	+	+	-	-	-	+
Short philtrum	+	-	-	+	+	-	+	+	+	+	-	-
Prominent/ pointed chin	+	+	+	+	+	+	+	+	+	+	+	+
Typical ear configuration	+	+	+	-	+	+	+	+	+	+	+	+
ZEB2 variant [NM_014795.4]	c.2908 C>T	c.575 dupA	c.930 C>A	c.1337_ 1340delCTTT	c.2254 dupA	c.2083 C>T	c.2585 T>A	c.2266 delC	c.1027 C>T	c.310 C>T	c.540 delT	c.2335 delA
Exon	exon 9	exon 5	exon 8	exon 8	exon 8	exon 8	exon 8	exon 8	exon 8	exon 3	exon 5	exon 8
Amino acid change (NP_055610.1)	p.(Gln 970*)	p.(Asn192 Lysfs*7)	p.(Tyr 310*)	p.(Pro-446Hisfs*7)	p.(Thr752 Asnfs*4)	p.(Arg 695*)	p.(Leu 862*)	p.(Pro756 Leufs*5)	p.(Arg 343*)	p.(Gln 104*)	p.(Glu181 Argfs*31)	p.(Arg779 Glyfs*8)
Genomic position [hg38] (NC_000002.12)	g.144396 571G>A	g.14440 4852 A>AT	g.144400 257G>T	g.144399847 delAAAG	g.1443989 32G>GT	g.144399 104G>A	g.144398 602A>T	g.144398 920delG	g.144400 160G>A	g.144429 790G>A	g.144404 888delA	g.144398 852delT
Novelty	VCV00037 3064.3 (Clin-Var)	novel	VCV00016 7857.4 (ClinVar)	novel	VCV0026 87506.1 (ClinVar)	PMID: 33510600	novel	novel	PMID: 31376723	PMID: 19006215	VCV00268 7499.1 (ClinVar)	novel

SD: Standard deviation scores, NA: not applicable, OFC: occipitofrontal circumference, yrs: years, mos: months, ACC: agenesis of the corpus callosum, DCC: dysgenesis of the corpus callosum, CH: callosal hypoplasia, CSP: cavum septum pellucidum, HSCR: Hirschprung disease, PPS: peripheral pulmonary stenosis, blt: bilateral, ASD: atrial septal defect, VSD: ventricular septal defect, PDA: patent ductus arteriosus, PLSVC: persistent left superior vena cava, PHT: pulmonary hypertension, PFO: patent foramen ovale, L: left, R: right, CAL: café au lait, p: percentile

age at unsupported sitting was 13.6 months (range 5-25 months). When patients who were over 18 months of age were considered, five out of eleven could walk unsupported at a mean age of 34.8 months (range 18-48 months). Of the five patients who could walk, two had a wide-based, ataxic-like gait. The remaining patients who could not walk were younger, with the age at the last examination varying between 19 and 42 months. Language deficit was particularly pronounced: patients had absent or severely restricted speech, except a 10-year 3-month-old patient who could build short sentences. No patient achieved bowel or bladder control.

All patients except one underwent brain MRI. The most common CNS anomaly was callosal abnormalities (n=5/11). Additional structural brain abnormalities were cavum septum pellucidum, benign tonsillar ectopia, and decreased volume of the left hippocampus with signal alterations in the parietooccipital white matter.

Four patients experienced seizures (n=4/12), with onset between 6 and 30 months, which were successfully controlled with anti-seizure medicine (ASM). In patient 10, an electroencephalogram (EEG) performed after seizure presentation showed mild slowing of background activity with frequent generalised bursts, suggestive of epileptic encephalopathy. His seizures were severe and frequently necessitated hospitalisation in the beginning, but he responded well to ASMs and showed amelioration with time. Sleep EEG demonstrated abnormal brain activity including diffuse spike and slow wave discharges in three of seven patients without seizures.

Behavioural features included repetitive oral-motor behaviour (n=3/12), self-injurious behaviour (n=2/12), happy demeanour (n=2/12), hyperorality (n=1/12), stereotypic hand movements (n=1/12), paroxysmal laughter (n=1/12), and poor eye contact (n=1/12).

Gastrointestinal anomalies
Gastrointestinal issues were present in the form of biopsy-proven Hirschsprung disease in four patients (n=4/12) and chronic constipation without an observable defect in ganglion function in three patients (n=3/12). One individual had swallowing difficulties due to neurological deficits.

Cardiac anomalies
Eight patients showed cardiac abnormalities in the form of peripheral pulmonary stenosis (n=2/12), atrial septal defect (n=2/12), ventricular septal defect (n=2/12), patent ductus arteriosus (n=2/12), bicuspid aorta (n=2/12), persistent left superior vena cava (n=1/12), and pulmonary hypertension (n=1/12).

Genitourinary abnormalities
The genitourinary abnormalities observed in eight patients included hypospadias (n=6/8), cryptorchidism (n=4/8), transient pelviectasis (n=3/12), shawl scrotum (n=1/8), hydrocele (n=1/8), and chordee (n=1/8).

Ophthalmological anomalies
Ocular abnormalities were observed in six patients as microphthalmia (n=2/12), retinal atrophy (n=2/12), microcornea (n=1/12), aniridia (n=1/12), congenital cataracts (n=1/12), peripapillary atrophy (PPA) (n=1/12), strabismus (n=1/12), iris coloboma (n=1/12), nasolacrimal duct obstruction (n=1/12), and astigmatism with myopia (n=1/12).

Immunological problems
Immunological findings were observed in the form of transient hypogammaglobulinemia of infancy (n=1/12), recurrent otitis media (n=2/12), and upper respiratory infections (n=1/12) in three patients.

Additional findings
Additional findings comprised precocious puberty (n=1/12), hypersalivation (n=1/12), occipital fibroma (n=1/12), torticollis (n=1/12), hypopigmented macules (n=3/12), café au lait spot (n=1/12), genu valgum (n=1/12), accessory nipple (n=1/12), nail hypoplasia (n=1/12), position abnormalities of the toes (1/12), pes valgus (n=1/12), choanal stenosis (n=1/12), and delayed teeth eruption (n=1/12).

***ZEB2* variants**
We identified 12 heterozygous truncating variants in *ZEB2*. These include six nonsense variants and six small indels leading to the disruption of the reading frame. Five variants were previously unreported in the literature. Eight variants were located within exon 8, two in exon 5, and one each in exons 3 and 9. Parental studies were only pursued for Patient 9 and Patient 12 by Sanger sequencing of the relevant exon and did not reveal any pathogenic variants. The molecular results are detailed in Table 1.

DISCUSSION

Here we describe a further 12 patients with a definitive clinical and molecular diagnosis of MOWS due to five novel and seven previously reported pathogenic *ZEB2* alterations.

Because all patients of the cohort were recruited through a gestalt diagnosis approach, they all shared the characteristic facial appearance comprising an elongated face with medially flared and broad eyebrows and a long chin (Figure 1). Characteristic ear shape with uplifted lobules, hypertelorism, deeply-set eyes, wide nasal root, and low-hanging columella were other consistent features observed in more than 80% of the cohort. These findings are consistent with previous, larger cohorts that elaborated the facial findings of the MOWS phenotypic spectrum (15). The facial characteristics of MOWS have been shown to temporally evolve from childhood to adulthood

(15). In infancy, the face is square shaped with a broad nasal bridge. As affected individuals get older, the face tends to elongate as the jaw becomes more prominent, eyebrows become heavier, the nasal tip lengthens and becomes depressed, and the columella overhangs the philtrum. Uplifted earlobes remain observable at all ages. Since our cohort included only three patients over the age of five, with the oldest patient being ten years of age, we were unable to evaluate the temporal evolution of the facial phenotype.

The mean gestational age at delivery of the cohort was similar to that of the general population. Except for one patient with a birth weight of -2.02 SD, all patients had a birth weight appropriate for gestational age with no significant differences between males and females. Length and OFC at birth were also within normal ranges. In the postnatal period, two patients developed short stature, and 10 out of 12 were microcephalic according to the national growth curves. However, when growth charts specifically designed for MOWS were applied, height, weight, and OFC measurements were generally within the normal range, except for one patient whose OFC was above the 95th percentile (29). In general, our growth findings are consistent with the existing notion that children with MOWS typically exhibit normal birth length and weight with a slightly smaller OFC compared to age peers and experience delayed postnatal growth, particularly in OFC, after the age of one (29).

Seizures have been reported in 78.5% of individuals with MOWS, with 25.9% exhibiting resistance to ASMs (4). Four patients in this study experienced seizures, and none were refractory to treatment with ASMs. The lower frequency of epilepsy observed in the cohort may be attributed to the small sample size and the relatively young age of the patients, limiting our ability to draw definitive conclusions. Of note, Patient 9, who presented with infantile spasms at six months of age, displayed features reminiscent of Aicardi syndrome, including dysgenetic corpus callosum and retinal anomalies. However, upon further evaluation, we concluded that these manifestations represented a severe presentation within the phenotypic spectrum of MOWS.

Callosal defects were the most prevalent form of CNS involvement in the cohort, consistent with previous reports (30). Additional anomalies included cavum septum pellucidum, benign tonsillar ectopia, decreased volume of the left hippocampus, and signal alterations in the parieto-occipital white matter in individual cases. A comprehensive study on neuroimaging findings in MOWS revealed that hippocampal abnormalities are the second most common type of CNS involvement, occurring in 79.6% of cases, followed by reduced white matter thickness in 40.7% and localised signal alterations in 22.2% (30). The low frequency of these specific brain findings in our cohort may be attributed to the lack of targeted imaging protocols because most MRI scans were performed before the definite diagnosis of MOWS and did not focus on its characteristic abnormalities.

Global developmental delay and/or cognitive impairment was a universal finding in this study, and speech was particularly affected. A range of behavioural phenotypes observed in the cohort was found to be consistent with the literature data, including oral behaviours, a happy demeanour/laughter for no apparent reason, and stereotypic hand movements (4, 31). Two patients exhibited self-injurious behaviour, which may partially be attributed to underreaction to pain, another behavioural characteristic of MOWS.

Various rare ophthalmologic findings were observed in the cohort. These include aniridia and peripapiller atrophy (PPA), which have been reported only once, and the hitherto undescribed iridodialysis and nasolacrimal duct obstruction (32, 33). Although microphthalmia is generally considered to be very rare in MOWS, two patients in our study exhibited microphthalmia (4). A similar cohort of 15 Chinese patients also had two cases of microphthalmia, while three additional cases were reported in a cohort of 28 (6, 34). Furthermore, one patient in our study had PPA, which was also reported in a previously published case of MOWS that lacked molecular confirmation and detailed clinical description (33). Collectively, these observations suggest that the frequency of microphthalmia may be higher than previously anticipated and that aniridia and PPA could be rare ocular anomalies associated with MOWS.

Patient 12 presented with a constellation of clinical features that extended beyond the core gestalt of MOWS, suggesting an overlap with CHARGE syndrome. These features included choanal stenosis, iris coloboma, congenital heart defects (patent ductus arteriosus, patent foramen ovale, bicuspid aorta), developmental delay, hypospadias, cryptorchidism, and renal pelvis dilation. To the best of our knowledge, patient 12 represents the third documented patient with a CHARGE-mimicking MOWS phenotype (14). Notably, this patient also exhibited nasolacrimal duct obstruction and a shawl scrotum, findings previously undescribed in either disorder.

Four patients exhibited skin pigmentation defects, including patchy hypopigmentation, café-au-lait spots, and scrotal hyperpigmentation during infancy. Although depigmentation has previously been associated with MOWS, it is considered rare among affected individuals (10). The high frequency observed in this study suggests that skin involvement, particularly hypopigmented patches, may be an overlooked finding that is more common in MOWS than previously thought.

All patients reported here were found to harbour nonsense or frameshift variants, the two most common intragenic alterations underlying MOWS. A milder clinical phenotype has been associated with missense variants in MOWS; however, no such cases were identified in this cohort to allow further exploration of this genotype-phenotype correlation (14, 25, 27). Eight of the 12 variants identified in our study were located in exon 8, consistent with previous reports. Parental segregation studies were not performed, as almost all reported MOWS cases to date are due to *de novo* mutations with nearly complete penetrance, except for very rare instances of low-level somatic or presumed germline mosaicism (5-8).

Our study has some limitations. The relatively small sample size, due to the rarity of MOWS, may limit the generalizability of our findings. Additionally, molecular analyses were limited to *ZEB2* sequencing, and further genetic studies in patients with atypical findings could have provided additional insights into other genetic contributors or modifiers.

CONCLUSION

This study increases the number of patients with *ZEB2* mutations in the literature. Our deep phenotyping data enabled us to identify previously uncertain clinical associations and novel or potentially underrepresented features, including several ocular anomalies (colobomas, PPA, microphthalmia), choanal atresia, skin pigmentation defects, and shawl scrotum, most of which likely constitute an expansion of the MOWS phenotype. Further cohorts may provide additional insights into MOWS by revealing new specific phenotypes associated with the syndrome and expand the *ZEB2* molecular landscape, allowing for more precise genotype-phenotype correlations.

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