A case of Mowat-Wilson syndrome caused by a truncating mutation within exon 8 of the *ZEB2* gene

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Mowat-Wilson syndrome (MWS) is characterized by severe mental retardation with seizures, specific facial dysmorphism, Hirschsprung disease, anomalies of the corpus callosum, and genitourinary and cardiac malformations. The cause of MWS is a *de novo* mutation in the *ZEB2* gene. This report describes a Turkish boy who was clinically diagnosed with MWS and had his diagnosis confirmed by molecular analysis of the *ZEB2* gene. The investigation identified a heterozygous complex rearrangement in exon 8 of *ZEB2*, specifically a 48-nucleotide deletion and a 44-nucleotide insertion that caused a frameshift. MWS is a relatively newly identified disorder, and even MWS patients without Hirschsprung disease can be diagnosed easily based on clinical findings alone.

Key words: Mowat-Wilson syndrome, facial dysmorphism, Hirschsprung disease, mental retardation, ZEB2 gene.

Mowat-Wilson syndrome (MWS) is a relatively newly identified multiple congenital anomaly/mental retardation syndrome that is characterized by specific facial dysmorphism, Hirschsprung disease (HRSC), seizures, and anomalies of the corpus callosum. Genitourinary and cardiac anomalies have also been noted in these patients[1-3]. The facial features of MWS are ocular hypertelorism, medially flared and broad eyebrows, prominent columella, pointed chin, uplifted earlobes with a central depression, and an open-mouthed expression[3].

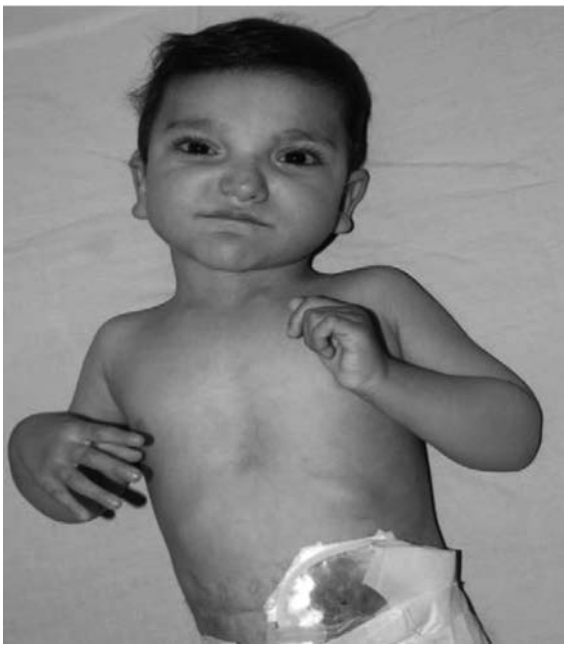
Mowat et al.[1] published the first description of this syndrome in six patients in 1998. In 2001, other researchers identified the cause as mutations of the ZFHX1B gene, also known as zinc finger E-box protein 2 (ZEB2) (or Smad-interacting protein 1 [SIP1]) gene, on chromosome 2q21-q23[4,5]. The incidence of MWS is unknown but the syndrome is thought to be under-reported[6].

Case Report

A 22-month-old boy with mental-motor retardation, epilepsy, HRSC, and multiple congenital anomalies was referred to our center for assessment. He was the third child of healthy non-consanguineous parents and there was no family history of genetic disorders. The patient was born at 38 weeks' gestation by spontaneous vaginal delivery after an uncomplicated pregnancy. His birth weight, height and head circumference were 3.8 kg, 49 cm and 34 cm, respectively. He had been diagnosed with HRSC as a neonate based on delayed passage of meconium and abdominal distension, and had undergone four operations for HRSC. The parents had noticed developmental delay and hypotonia, and the patient started to experience epileptic seizures at 13 months of age. Antiepileptic therapy (valproic acid) had been initiated at that time but this had not controlled the seizures.

Neurological examination on admission revealed severe developmental delay, truncal hypotonia, and increased leg muscle tone. The patient had a happy disposition, smiled frequently, and was affectionate. He was unable to walk and his speech was limited to a few words. Physical examination revealed body weight 11.5 kg (between the 25th and 50th percentile for age),

body length 84 cm (between the 25th and 50th percentile for age), and head circumference 43 cm (below the 3rd percentile for age). The patient had a colostomy bag as a result of HRSC operations. He had many dysmorphic features. The craniofacial dysmorphic findings were microcephaly, square-shaped face, sparse and fine hair, medially flaring and sparse eyebrows, deep-set eyes, epicanthic folds, hypertelorism, prominent columella, overhanging nasal tip, M-shaped upper lip, wide mouth, triangular/prominent chin, and prognathism (Fig. 1A). His ear lobes were uplifted and had a central depressed area that created a "red blood cell" shape (Figs. 1B, 2). The patient also had pectus excavatum deformity, bilateral cryptorchidism and hypospadias.



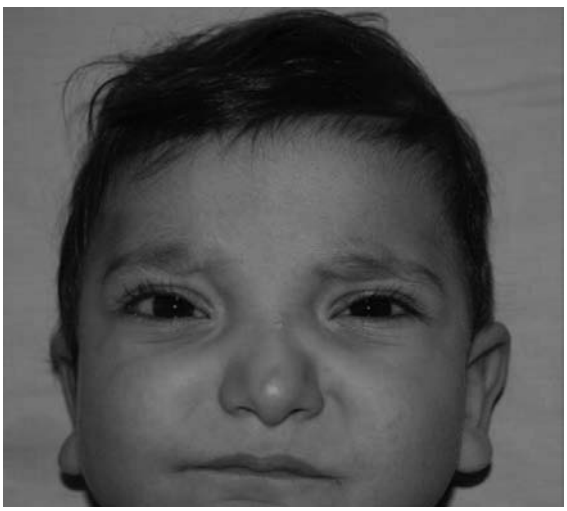


Fig. 1A, B. Photos of the patient with dysmorpic features characteristic of Mowat-Wilson syndrome: ocular hypertelorism, medially flared eyebrows, prominent columella, prominent chin, and uplifted ear lobes.

Laboratory investigations of complete blood count, routine biochemical testing of liver and kidney parameters, and serum electrolyte levels revealed nothing abnormal. Chromosome analysis performed on a peripheral blood sample revealed normal karyotype (46, XY). Brain magnetic resonance imaging demonstrated hypoplasia of the corpus callosum and mildly dilated lateral ventricles. The findings on echocardiography and abdominal ultrasonographic examination were normal. Psychometric tests according to age (Denver tests adapted for Turkish children) showed severe retardation.

The patient presented with all the characteristic clinical and dysmorphic features of MWS, and molecular genetic analysis was performed to confirm the diagnosis. Informed consent was obtained from the family and peripheral blood samples were collected from the patient and his parents. These were sent to the Institute of Medical Genetics at A. Gemelli Catholic

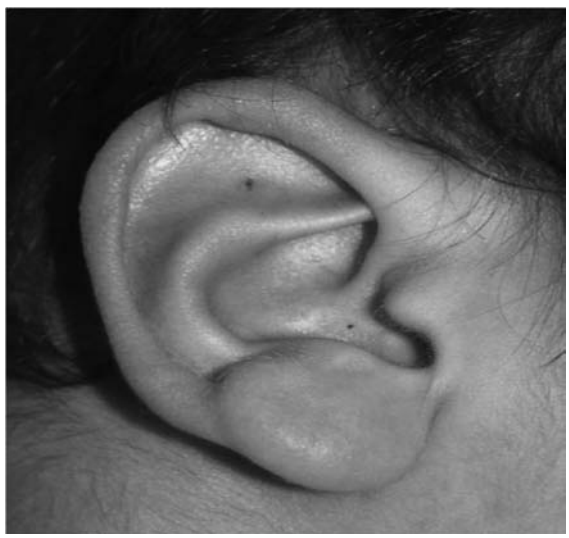


Fig. 2. Characteristic appearance of the ear lobe: uplifted and with central depression ("red blood cell" shaped or "corpuscle-like").

Table I. Clinical Findings in Mowat-Wilson Syndrome Patients with Confirmed *ZEB2* Mutations[2,7,8]

Clinical features	Proportion of cases in the literature	Present patient
Facial dysmorpism	100%	+
Round or square face in infancy, longer face in childhood Hypertelorism, deep-set but large eyes Broad nasal bridge Medially flared and broad eyebrows, heavier and broad eyebrows in adolescence Prominent columella Prominent or pointed chin in infancy, prominent triangular jaw in adolescence Open-mouthed expression Posteriorly rotated ears, large uplifted ear lobes with a central depression		
Modere to severe mental retardation	100% (usually severe)	+
Microcephaly	80%	+
Seizures	74%	+
Hypospadias	56%	+
Hirschsprung disease	54%	+
Congenital heart defects	54%	-
Short stature	50%	-
Hypoplasia or agenesis of corpus callosum	46%	+
Cryptorchidism	37%	+
Constipation	30%	+
Renal anomalies	13%	-
Pyloric stenosis	5%	-
Structural eye anomalies	4%	-
Cleft palate	3%	-
Pulmonary artery sling with/without tracheal stenosis/hypoplasia	3%	-

University of Rome, Italy, where DNA was extracted and the samples were analyzed for mutations of the ZEB2 gene using two methods: multiplex ligation-dependent probe amplification (MLPA) (MRC Holland: SALSA P169-B1 Hirschsprung lot 0808) and sequencing of ZEB2 between amino acid positions 1 and 1214 (Gene Bank access NM_014795.2). The testing revealed a heterozygous complex rearrangement (large indel) within exon 8 of ZEB2, specifically a 48-nucleotide deletion (from position 1073 to 1122 of the coding DNA) and 44-nucleotide insertion that caused a frameshift. At the protein level, a premature stop codon is expected at the 362 amino acid position. Both parents were negative for this mutation. These results are consistent with a diagnosis of MWS.

Discussion

To date, approximately 192 MWS cases with a mutation in the ZEB2 gene have been reported in the literature. Table I summarizes the clinical features of these historical cases, the proportion of patients with each trait, and our patient's clinical findings[2,7,8]. Almost all of our patient's clinical features were consistent with MWS. Further, most MWS patients have a happy demeanor and tend to smile frequently and be affectionate and sociable,[2,7,8] and our patient had these behavioral traits as well.

To diagnose MWS, physicians must be able to recognize the characteristic facies of the

syndrome regardless of the presence/absence of HRSC[2]. Our patient had facial dysmorphism typical of MWS. Diagnosis of this syndrome is based primarily on a distinct pattern of craniofacial anomalies[7]. With the exception of HRSC, the major congenital anomalies that are common in MWS cases (agenesis or hypoplasia of the corpus callosum, hypospadias, and cryptorchidism, for example) are nonspecific in MWS[7]. Particular craniofacial symptoms can facilitate the identification of MWS, notably the shape of the eyebrows, fleshy and uplifted ear lobes, pointed chin, and microcephaly[7].

In patients with MWS, the facial dysmorphism is more subtle in childhood and changes over time[7,8]. The characteristic face of an affected young child is round or square, with eyes deep- set and large, and eyebrows sparse but broad. With increasing age, the face lengthens to resemble the general population, but the jaw becomes more prominent and triangular and the columella becomes prominent, giving the impression of a short philtrum. The eyebrows may become even heavier and broad, with a diffuse and extended medial portion[7,8]. Becoming familiar with the clinical manifestations of MWS should improve the likelihood of early diagnosis[7].

All typical MWS cases result from haploinsufficiency of the ZEB2 gene, which is also called the SIP1 or ZFHX1B gene and is located at chromosome 2q22[9-12]. ZEB2 has been implicated in many steps of embryogenesis and functions as a transcriptional repressor, interacting with Smad proteins in the transforming growth factor-beta (TGF-b) signaling pathway[12].

Of all the ZEB2 mutations published to date, approximately 80% were detectable by sequencing and the majority of these were nonsense or frameshift mutations[12]. Approximately 50% of these mutations detectable by sequencing were in exon 8, which comprises roughly half of the coding region[12]. Approximately 15% of ZEB2 mutations were large deletions that were detectable by fluorescence in situ hybridization (FISH)[12]. Findings indicate that 5% of individuals with MWS have a partial gene deletion that would be missed by FISH and sequencing and that requires quantitative polymerase chain reaction (PCR), MLPA, or another technique to detect dosage at the single exon level[12]. In our case, molecular analysis was done using MLPA and ZEB2 gene sequencing techniques. About 10% of the patients, who have a typical clinical phenotype for MWS, have a negative genetic test. In these cases, atypical mutations can be considered, and the diagnosis can be confirmed clinically[13,14].

Genotype-phenotype analysis of the patients with MWS showed that in most cases, the phenotype was similar in both deletions and truncating mutations[14-16]. Developmental delay, particularly of language, and facial dysmorphism are invariable. However, presence of severe congenital malformations, e.g. HRSC and agenesis of the corpus callosum, is variable[14-16]. There is no overt correlation between the phenotype and the size of deletion, except for extremely large deletions (>5 Mb) in patients who were more heavily affected[15,17]. For example, the presence or manifestation of HRSC is not affected by deletion size. Furthermore, ZFHX1B knockout mice do not manifest HRSC; therefore, the manifestation of HRSC may be influenced by a non-allelic modifier[15].

Studies have shown that there is a phenotypic variability for a single mutation in cases with MWS. Furthermore, this variability is evident in the same family[14]. Unusual mutations can lead to an atypical phenotype in a small number of patients (approximately 2.4%)[14,15]. One of them (a de novo 3356A-G transition in exon 10) showed associated unusual malformations; cleft lip and palate, brachytelephalangy, and broad thumbs and halluces[18].

To date, all documented cases of MWS have been sporadic, caused by de novo deletions or new dominant mutations in the ZEB2 gene. In situations where sporadic occurrence is certain, families can be counseled that recurrence risk is low but that the possibility of germline mosaicism cannot be excluded[2,13]. This was how we counseled our patient's family.

Patients with MWS should undergo clinical follow-up involving a multidisciplinary approach and regular physical examinations. Rehabilitation, including physical therapy and psychomotor and speech therapy, should be started as soon as possible after diagnosis[13]. All advised routine vaccinations of childhood are recommended[13]. Congenital heart disease

and HRSC require surgery in the first days or months of life, and constipation may persist after HRSC surgery[13]. Eye problems are frequent in patients with MWS and these require specialist care[13,14]. Orthopedic evaluation is appropriate, as patients may develop musculoskeletal anomalies, such as pes planus, calcaneovalgus deformity, and scoliosis. Audiology is also recommended, though loss of hearing is rare[13,14].

In our case, the patient had many typical features of MWS and was easy to diagnose based on the clinical features alone. We note that MWS can be easily recognized based on typical dysmorphic features. Although the incidence of MWS is unknown, considering the number of cases that have been identified in a short span of time, we believe that MWS is greatly under-reported. This syndrome should be considered in any case where a child presents with severe mental retardation and seizure, and particularly if the patient has typical facial dysmorphism, HRSC, and/or anomalies involving the corpus callosum, heart, or genitourinary system.

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