American Journal of Medical Genetics Part A 146A:3095–3099 (2008)

Research Letter

Recurrence of Mowat–Wilson Syndrome in Siblings With a Novel Mutation in the *ZEB2* Gene

Massimiliano Cecconi,[1] Francesca Forzano,[1] Livia Garavelli,[2] Chiara Pantaleoni,[3] Marina Grasso,[1] Franca Dagna Bricarelli,[1] Lucia Perroni,[1] Emilio Di Maria,[1,4] and Francesca Faravelli[1]*

[1]Laboratory of Genetics, Galliera Hospital, Genova, Italy
[2]Clinical Genetics Unit, Santa Maria Nuova Hospital, Reggio Emilia, Italy
[3]Developmental Neurology Division, Istituto Nazionale Neurologico Besta, Milano, Italy
[4]Department of Neuroscience, Ophthalmology and Genetics, University of Genova, Italy

Received 4 July 2008; Accepted 21 August 2008

How to cite this article: Cecconi M, Forzano F, Garavelli L, Pantaleoni C, Grasso M, Dagna Bricarelli F, Perroni L, Di Maria E, Faravelli F. 2008. Recurrence of Mowat–Wilson syndrome in siblings with a novel mutation in the *ZEB2* Gene. Am J Med Genet Part A 146A:3095–3099.

To the Editor:

Mowat et al. [1998] described a series of six isolated patients with microcephaly, mental retardation, and a peculiar facial phenotype. Five patients had Hirschsprung disease (HSCR) [Mowat et al., 1998]. The disorder, which was designated by the eponym Mowat–Wilson syndrome (MWS, OMIM #235730), was demonstrated to be caused by heterozygous mutations in the Zinc finger E-box-Binding homeobox 2 gene (*ZEB2*, also known as *ZFHX1B* or *SMADIP1*) [Cacheux et al., 2001; Wakamatsu et al., 2001; Yamada et al., 2001]. Molecular analysis helped to delineate the cardinal features of MWS (facial gestalt and delayed psychomotor development) as well as several variably associated congenital anomalies, including HSCR, agenesis of the corpus callosum, seizures, eye anomalies, heart malformations, genital, and urinary tract defects [reviewed by Adam et al., 2006; Garavelli and Mainardi, 2007].

To date, there are approximately 180 mutation-positive patients with MWS in the literature, with 100 different *ZEB2* mutations reported [Zweier et al., 2005]. Three cases of recurrence in siblings have been reported [McGaughran et al., 2005; Zweier et al., 2005; Ohtsuka et al., 2008]. We describe two sisters with clinical features of MWS in whom the same nonsense mutation in the *ZEB2* gene was found.

The older sibling is now 6 years old. She was born by spontaneous delivery at 39 weeks of gestation. Antenatal scanning performed at 20 weeks suggested agenesis of the corpus callosum. Birth weight was 3,670 g (75th centile), length was 52 cm (75th centile). Head circumference at birth was not measured. Hypotonia and feeding difficulties were present in the neonatal period. Growth was normal. Psychomotor development was delayed: she walked at 30 months of age and still pronounces 4–5 words. At 18 months an episode of febrile seizures occurred, followed by afebrile tonic-clonic seizures treated with valproate. Postnatal cerebral MRI confirmed agenesis of the corpus callosum. Ultrasound scans of the heart and abdomen and karyotype were normal. Constipation was never reported. The clinical diagnosis of MWS was first raised when she was 5 years of age by the presence of her facial gestalt (Fig. 1A–C). Length was 112 cm (50–75th centile), weight 18 kg (25th centile), head circumference was 51 cm (50th centile).

The sister was born at 39 weeks of gestation by spontaneous delivery. Again probable agenesis of the corpus callosum was noted on the antenatal ultrasound scan (20th week of gestation). At birth weight was 4,010 g (90th centile), length 52 cm (75th centile), head circumference was not measured. Echocardiography on day 3 revealed a complex heart malformation: aortic coarctation and valvular stenosis, pulmonary valve stenosis, multiple

Massimiliano Cecconi and Francesca Forzano contributed equally to this work.

Grant sponsor: Telethon onlus; Grant number: GTF04003.

*Correspondence to: Dr. Francesca Faravelli, Laboratory of Genetics, Galliera Hospital, Via Volta, 6, 16128 Genova, Italy.
E-mail: francesca.faravelli@galliera.it

Published online 12 November 2008 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/ajmg.a.32568

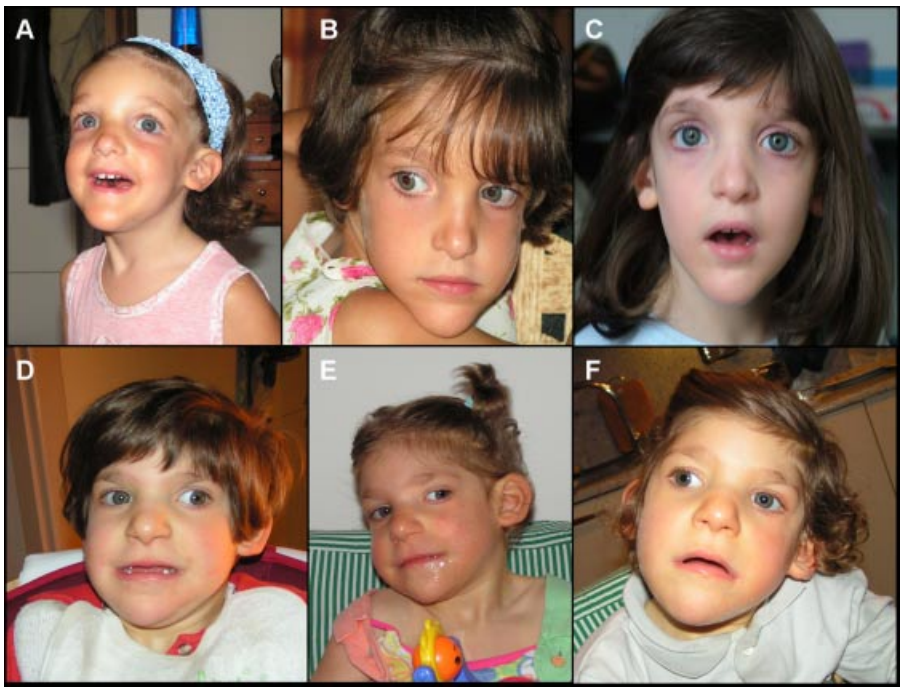

FIG. 1. Phenotypic appearance of the two siblings: older sib, at 2 (**A**), 3 (**B**) and 5 (**C**) years of age; second sib, at 1 (**D**) and 3 (**E**,**F**) years of age. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

interventricular septal defects and atrial septal defect. No renal anomalies were detected at both the antenatal and neonatal examinations. A first surgical intervention on the congenital heart defects was performed on day 27 and was complicated by renal failure. At 11 months afebrile tonic clonic seizures occurred and treatment with valproate was commenced. At 12 months she underwent further heart surgery complicated by heart failure. The outcome was complicated by sepsis and cardiac arrest from which she was resuscitated. At discharge she presented with hypotonic paraplegia and neurological bladder. Cerebral MRI scan was consistent with her having hypoxic ischemic encephalopathy. Karyotype was normal. At 3 years of age length 86 cm (<3rd centile), weight 12.8 kg (10th centile), head circumference was 44 cm (<3rd centile). She showed an open mouth, severe hyperlordosis, upper limb hyperreflexia, and lower limb paraplegia (Fig. 1D–F).

DNA was isolated from peripheral blood, after informed consent. A set of primer pairs was designed to amplify the nine coding exons and intron-exon boundaries of the *ZEB2* gene (genomic contig AY029472 and mRNA sequence NM_014795, GenBank database). Primer sequences and detailed protocols are available on request.

Mutation analysis was performed by direct sequencing, using the BigDye sequencing reagents on a 3130xl capillary sequencer (Applied Biosystems, Foster City, CA).

The presence of somatic mosaicism in probands and their parents was also evaluated using DHPLC analysis. The PCR product spanning the mutation (primer sequences: forward: ttaactaaacaattaggggtggc; reverse: gtgggcgatctgctaggtgg) was resolved on a DHPLC equipment (Transgenomics Ltd., Hillington, Glasgow, UK) at 58.3°C.

The DHPLC elution profiles were used also to estimate the frequency of the c.310C > T substitution in the general population. A cohort of 94 normal unrelated individuals of Italian origin was screened. Exact binomial confidence intervals were calculated from observed frequency using Stata 9 (StataCorp., College Station, TX). The exon 3 PCR product did not generate any aberrant elution profile in controls, resulting in an estimated allele frequency $\ll$1% (observed frequency: 0.0; 95% exact confidence interval: 0.0001–0.0293).

Direct sequencing of *ZEB2* in both patients revealed a heterozygous C > T transition in the exon 3 (c.310C > T), resulting in a premature stop codon (p.Q104X). No other nucleotide variant was detected in either proposita. The nonsense c.310C > T mutation was not present in the parents (Fig. 2).

No evidence of somatic mosaicism was found at the inspection of the electropherograms. To further check for the presence of low-level somatic mosaicism, patients and parents were subsequently examined by DHPLC analysis. While the heterozygous mutation produced a clearly abnormal profile, the chromatograms obtained from both

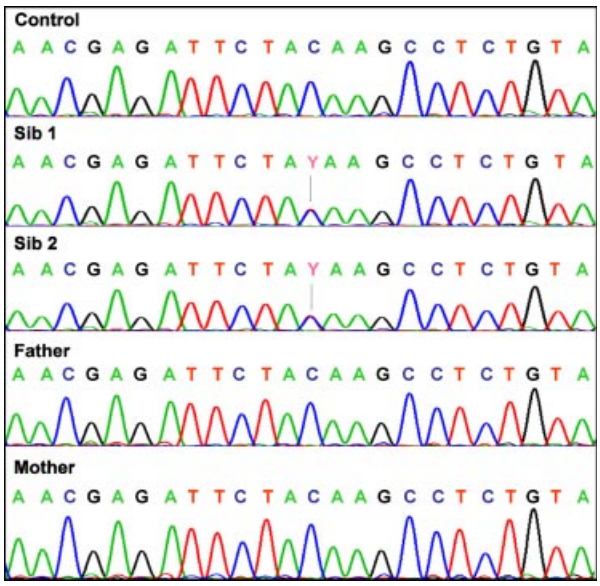

FIG. 2. Sequence analysis in the family: the electropherograms show the *ZEB2* exon 3 region spanning the c.310C > T mutation. Both sibs carry the heterozygous C > T substitution, while relatives show a sequence profile similar to the normal control. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

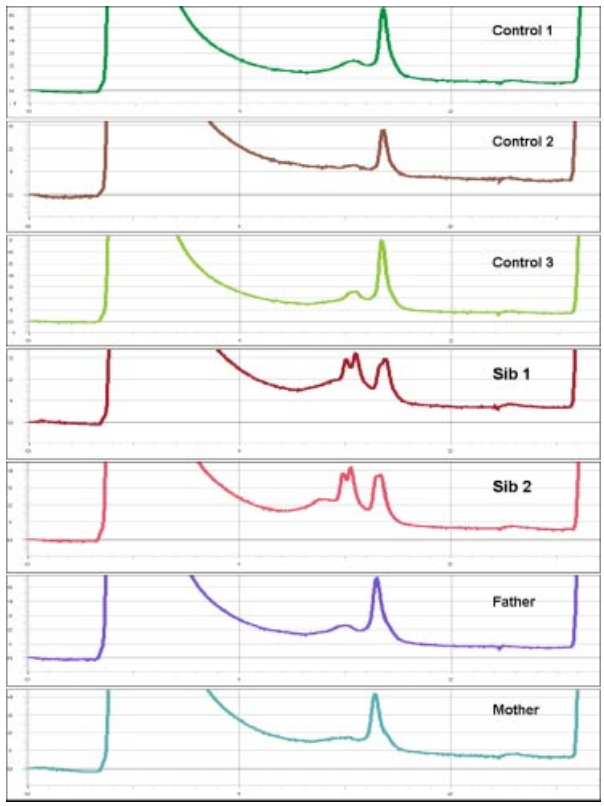

FIG. 3. DHPLC analysis in the family: the chromatograms show the *ZEB2* exon 3 PCR including the c.310C > T mutation. Both patients show an abnormal elution profile, while relatives demonstrate a pattern similar to the normal controls. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

parents' DNA did not reveal any difference with respect to normal controls (Fig. 3).

The nonsense c.310C > T mutation has not been previously described. Deletions and truncating substitutions represent the vast majority of the mutations associated with MWS. The premature stop codon caused by the c.310C > T mutation produces a very short putative truncated protein, as compared to most MWS-associated mutations [Dastot-Le Moal, 2007], consistently with haploinsufficiency. Taken together, our findings allowed us to conclude that c.310C > T is in fact the disease-causing mutation in the siblings.

To our knowledge, the family described herein is the fourth case of recurrent MWS. Zweier et al. [2005] reported two sisters with MWS. Another recurrence was found in a brother and a sister with clinical features of MWS and the same truncating mutation in exon 8. The parents were phenotypically normal, without mutation in the *ZEB2* gene [McGaughran et al., 2005]. Recently, another family with three affected sibs has been reported [Ohtsuka et al., 2008].

Based on these lines of evidence, germ-line mosaicism is the most consistent hypothesis to explain familial recurrence, as already proposed based on analogue mechanism demonstrated in other dominant diseases [McGaughran et al., 2005]. Zweier et al. [2005] demonstrated somatic mosaicism in a parent with mild clinical signs. Our analysis did not replicate this finding. However, the occurrence of somatic mosaicism cannot be definitely excluded as a low-level mosaicism could be restricted to certain cell types or be under the detection threshold.

The clinical presentation of these sisters further underscore the clinical variability and how this influences outcome rather than the specific mutation alone. Table I summarizes the clinical and molecular findings in the siblings, compared with the previously reported cases of recurrent MWS.

Currently, taking into account only published observation, recurrence risk can be estimated as high as 2.3% (4/175), with a 95% confidence interval ranging from 0.6% to 5.7%. According to this estimation, targeted genetic counseling and prenatal diagnosis procedures should be applied in families with an isolated case of MWS. In genetic counseling the clinician should consider a number of uncertainties, deriving from intrinsic clinical variability, risk of complications and still inaccurate empiric risk of recurrence.

ACKNOWLEDGMENTS

Dr. D. Mowat is gratefully acknowledged for critical reading of the manuscript. Biological samples

TABLE I. Clinical Features of the Familial Cases With MWS Reported to Date

Clinical features	Frequency in MWS[a]	Present report		McGaughran et al. [2005]		Zweier et al. [2005]			Ohtsuka et al. [2008]		
		Sib 1	Sib 2	Sib 1	Sib 2	Sib 1	Sib 2	Father	Sib 1	Sib 2	Sib 3
Gender		F	F	F	M	F	F	M	F	F	M
Age at evaluation		6 y	5 y	30 m	1 m	6 y 4 m	4 y	nr	nr	nr	2 y7 m
ZEB2 mutation	100%	Q104X	Q104X	V621AfsX25	V621AfsX25	T285fsX293	T285fsX293	Low level mosaic for T285fsX293	E87X	E87X	E87X
Facial gestalt[b]	100%	MWS	MWS	MWS	MWS	MWS	MWS	Pointed nasal tip, uplifted ear lobe	MWS	MWS	MWS
Walking age	3–4 y	30 m	−	2 y	−	4 y	−	Normal	Delayed	4 y 6 m	−
Limited/absent speech	100%	+	+	+		+	+	−	+	+	+
Seizures	70%	+	+	nr	nr	+	+	−	+	+	Hypsarrhythmia
Microcephaly	83%	−	+	nr	nr	+	+	−	nr	nr	nr
Corpum callosum abnormalities	41%	Agenesis	Agenesis	Agenesis	Agenesis	Hypoplasia	Agenesis	−	−	Not analyzed	Hypoplasia
Other cerebral abnormalities		−	Consistent with anoxia	−	−	−	−	−	Hyppocampal dysplasia	nr	−
Hirschprung disease	57%	−	−	+	+	+	+	−	−	−	−
Constipation	nr	−	−	nr	nr	nr	nr	−	nr	nr	+
Cardiac defects	53%	−	CA, AS, PS, multiple ASD and VSD	−	CA	PAS, PFO	Atypical LPA origin, PAS	−	−	−	−
Urogenital abnormalities	50%	−	Neurological bladder after surgery	Mild renal pelvic dilatation in prenatal scan, normal postnatally	−	−	−	−	−	−	Bilateral cryptorchidism
Ophthalmic abnormalities	nr	Mild strabismus	−	Divergent strabismus	Bilateral chorio–retinal coloboma	nr	nr	nr	nr	nr	nr
Other	Short stature 47%	Mild abnormal. of the V finger term. phalanx	−	−	−	Tracheal hypoplasia	−	Short stature (150 cm)	−	−	Adducted thumb

"+" and "−" indicate presence and absence of the sign, respectively; "nr" indicates that the relevant information was not reported in the literature.
m, month; y, year; AS, Aortic valve Stenosis; ASD, Aortic Septal Defect; CA, Coarctation of the Aorta; LPA, Left Pulmonary Artery; PA, Pulmonary valve Stenosis; PDA, Patent Ductus Arteriosus; PFO, Patent Foramen Ovale; VSD, Ventricular Septal Defect.
[a]According to "GeneReviews" (http://www.geneclinics.org; last revision February 11, 2008; last access April 23, 2008).
[b]MWS facial gestalt includes: broad, medial flared eyebrows, hypertelorism, prominent columella, uplifted earlobes, and pointed chin.

were stored in the Galliera Genetic Bank, funded by Telethon onlus (grant GTF04003).

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