Recurrence of Mowat–Wilson Syndrome in Siblings With a Novel Mutation in the \textit{ZEB2} Gene

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Received 4 July 2008; Accepted 21 August 2008


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 (\textit{ZEB2}, also known as \textit{ZFHX1B} or \textit{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 \textit{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 \textit{ZEB2} gene was found.

The older sibling is now 6 years old. She was born by spontaneous delivery at 39 weeks of gestation. Antenatal ultrasound performed at 20 weeks suggested agenesis of the corpus callosum. Birth weight was 3,670 g (75th centile), length was 52 cm (75th 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 51 cm (50th 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. Ultrasonic 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).

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Massimiliano Cecconi and Francesca Forzano contributed equally to this work.

Grant sponsor: Telethon onlus; Grant number: GTF04003.
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Published online 12 November 2008 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/ajmg.a.32568
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: ttaactaaacattaaggtgcc; reverse: gttggccatctgctaggtgg) 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 ≤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
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 underscores 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

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<tbody>
<tr>
<td>Gender</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Age at evaluation</td>
<td>6 y</td>
<td>5 y</td>
<td>30 m</td>
<td>1 m</td>
<td></td>
</tr>
<tr>
<td>ZEB2 mutation</td>
<td>Q104X</td>
<td>Q104X</td>
<td>V621AfsX25</td>
<td>V621AfsX25</td>
<td></td>
</tr>
<tr>
<td>Facial gestalt&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MWS</td>
<td>MWS</td>
<td>MWS</td>
<td>MWS</td>
<td>Pointed nasal tip, uplifted ear lobe</td>
</tr>
<tr>
<td>Walking age</td>
<td>3–4 y</td>
<td>30 m</td>
<td>2 y</td>
<td>4 y</td>
<td>Normal</td>
</tr>
<tr>
<td>Seizures</td>
<td>70%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Microcephaly</td>
<td>83%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Corpum callosum abnormalities</td>
<td>41%</td>
<td>Agenesis</td>
<td>Agenesis</td>
<td>Hypoplasia</td>
<td>Agenesis</td>
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<tr>
<td>Other cerebral abnormalities</td>
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<tr>
<td>Hirschprung disease</td>
<td>57%</td>
<td>+</td>
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<tr>
<td>Cardiac defects</td>
<td>nr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Urogenital abnormalities</td>
<td>50%</td>
<td>Neurological bladder after surgery</td>
<td>Mild renal pelvic dilatation in prenatal scan, normal postnatally</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ophthalmic abnormalities</td>
<td>nr</td>
<td>Mild strabismus</td>
<td>Divergent strabismus</td>
<td>Bilateral chorioretinal coloboma</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Short stature</td>
<td></td>
<td>Tracheal hypoplasia</td>
<td>Short stature (150 cm)</td>
<td>Adducted thumb</td>
</tr>
</tbody>
</table>

<sup>a</sup> “+” and “−” indicate presence and absence of the sign, respectively; “nr” indicates that the relevant information was not reported in the literature.

<sup>b</sup> According to “GeneReviews” (http://www.geneclinics.org; last revision February 11, 2008; last access April 23, 2008).
were stored in the Galliera Genetic Bank, funded by Telethon onlus (grant GTF04003).

REFERENCES


