A de novo triplication on 2q22.3 including the entire ZEB2 gene associated with global developmental delay, multiple congenital anomalies and behavioral abnormalities

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Abstract

Background: Mowat-Wilson syndrome (MWS) is a genetic condition characterized by distinctive facial features, moderate to severe intellectual disability, developmental delay and multiple congenital anomalies. MWS is caused by heterozygous mutations or deletions of the ZEB2 gene located on chromosome 2q22.3. At present, over 190 cases with mutations and deletions involving the ZEB2 gene have been reported, but triplication or duplication of reciprocal region of Mowat-Wilson syndrome has never been reported.

Case Presentation: Here we report a 2-year-2-month-old boy carrying a de novo 2.9 Mb complex copy number gain at 2q22.3 involving triplication of ZEB2 gene. The boy is characterized by intrauterine growth retardation, hypotonia, cognitive impairment, multiple congenital anomalies and behavioral abnormalities.

Conclusion: This case provides evidence that triplication of ZEB2 gene may be clinical significance and ZEB2 gene is likely to be a dosage sensitive gene.

Keywords: Mowat-Wilson syndrome, Distinctive facial features, Intellectual disability, Developmental delay, Congenital anomalies, Behavioral abnormalities, ZEB2-triplication

Background

Mowat-Wilson syndrome (MWS; OMIM# 235730) is an autosomal dominant genetic syndrome with multiple congenital anomalies. MWS is characterized by distinctive facial features, epilepsy, moderate to severe intellectual disability, global developmental delay, and congenital anomalies including agenesis of the corpus callosum, Hirschsprung disease, genitourinary anomalies, hypospadias, congenital heart disease, short stature and hypotonia [1–6]. MWS individuals display behavior problems including a happy affect and sociable demeanor, repetitive behaviors, pain insensitivity and a high rate of oral behaviors [7]. Eye abnormalities and craniostenosis are rare features of this syndrome [8–10]. Eye abnormalities include iris/retinal colobomas, atrophy or absence of the optic nerve, hyphema, and deep refraction troubles, sometimes leading to severe visual consequences [8]. The syndrome is caused by heterozygous deletions or mutations of ZEB2 (OMIM# 605802) gene located on chromosome 2q22.3. So far, more than 190 individuals with MWS have been described, who result from more than 100 different mutations or deletions of ZEB2 gene. However, no obvious genotype-phenotype correlation was observed unless MWS patients carrying large deletions presented with more severe conditions, which may be the effect of continuous genes deletion [11–14]. Currently, no clinical presentations of patients with ZEB2 copy number gain have been reported. Here, we report the first case of a de novo 2.9 Mb copy number gain at 2q22.3 involving triplication of the entire ZEB2 gene detected by chromosomal microarray analysis (CMA). This case suggests that ZEB2 gene is likely to be a dosage sensitive gene.

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Case presentation
The proband was the first child of healthy unrelated parents and family history was unremarkable. Intrauterine growth retardation was noticed by ultrasound examination at 7 months of pregnancy. He was born by vaginal delivery at 38 weeks of gestation. Birth weight was 3.0 kg (20.3 %), length 48 cm (8.5 %) and head circumference 32 cm (1.7 %). Apgar scores were all 9. He had severe hypotonia. No feeding difficulty was noted at all times. The development milestones were delayed: he raised his head at 4 months of age, sat alone at 8 months and walked without assistance at 1 year 8 months. Language development was significantly delayed.

The patient was 2 years 2 months old at the time of molecular evaluation. His weight was 12.5 kg (39.7 %), height 86.2 cm (17.9 %) and head circumference 48.5 cm (46.3 %). He demonstrated catch-up growth but hypotonia persisted. His voice was low and he cried weakly. His receptive language was relative normal but he used body language to communicate. His cognitive competence was lower than his peers. On physical examination, his distinctive facial features included scaphocephaly, flat facial profile, auricle dysplasia, low-set and asymmetrical ears, small eyes, flat nose bridge, shallow philtrum, small mouth, teeth dysplasia, micrognathia, sparse eyebrows and hair. He had short hands and broad fingers (Fig. 1). Echocardiography revealed a small atrial septal defect. No genitourinary anomalies was noticed except for small testes. He had chronic and mild to moderate constipation, but no intestinal blockage and enlargement of the colon, and was not diagnosed with Hirschsprung disease. He always displayed a smiling, open-mouth expression and a happy, sociable demeanor as well as timid behavior. He never presented with epileptic seizures, and EEG was normal. The brain magnetic resonance imaging (MRI) showed normal corpus callosum and no other brain structural abnormalities. No additional abnormalities was noticed.

Methods
Chromosomal microarray analysis
Chromosomal microarray analysis was performed for the proband and both parents using Affymetrix Cytoscan HD Array (Affymetrix, USA). Genomic DNA was extracted from peripheral blood using a commercial kit (Qiagen). The labeling and hybridization procedures were performed following manufacturer's instructions. The raw data of chromosomal microarray was analyzed by Affymetrix Chromosome Analysis Suite Software.

Results
CMA test revealed a complex gain of copy number at 2q22.2-22.3, which involves a duplication (chr2:143,886,436-144,391,185) and a triplication (chr2:144,391,186-146,831,592) (Fig. 2). Parental tests were normal. Thus, the proband carried a de novo copy number variant.

Discussion
ZEB2 gene mutations or deletions cause Mowat-Wilson syndrome through a haploinsufficiency mechanism, but little is known about the clinical significance of ZEB2 copy number gain. In this study, we report a 2-year-2-month-old boy with global developmental delay, cognitive impairment, multiple congenital anomalies and behavior problems who carried a de novo 2.9 Mb triplication at 2q22.3 involving the entire ZEB2, GTDC1 and TEX41 genes and part of ARHGAPI5 gene. No other clinical significant CNVs were detected. The patient's clinical presentation was compared with the typical features of Mowat-Wilson syndrome (Table 1). Some of our patient's clinical features overlapped with that of Mowat-Wilson syndrome, in particular, severe speech impairment with relative preservation of receptive language, open-mouth appearance and happy demeanor. However, his distinctive facial features were significantly different from that of MWS which included deep-set large and widely spaced eyes, upturned earlobes, saddle nose with rounded nasal tip, pointed chin, flaring eyebrows and elongated face. He had significant intrauterine growth retardation and severe hypotonia whereas he demonstrated postnatal catch-up growth but hypotonia persisted. No triplication at this locus had been reported in literature or described in
database. We identified several cases with duplications of ZEB2 gene in DECIPHER and ISCA databases (Table 2 and Fig. 3). All these duplications were de novo except for ones without parental tests and no copy number gain including ZEB2 gene was reported in the DGV, which strongly suggested a pathogenic nature of these copy number gains.

There are four genes involved in the copy number gain at 2q22.3 of our patient: ZEB2, GTDC1 and TEX41 genes are triplicated, part of ARHGAP15 is duplicated. ARHGAP15, a member of the RHO GTPase-activating proteins (GAPs), regulates RHO GTPases (see ARHA; MIM 165390) which regulates diverse biologic processes [15]. GTDC1 is ubiquitously expressed at relatively high levels in lung, spleen, testis, and peripheral blood leukocytes, suggesting that it may have biochemical functions in these organs [16]. TEX41 is a non-protein coding gene. Currently, none of the three genes are known to have any clinical significance.

The protein encoded by ZEB2 gene is a member of δEF1/Zfh-1 family, containing a Smad-binding domain, a homeodomain-like sequence, and two separate clusters of zinc fingers at the amino and carboxy terminals [17]. The ZEB2 protein interacts with SMAD proteins and acts as a transcriptional repressor in response to TGF-β signaling [17]. The SMAD proteins are cytoplasmic mediators that are tightly controlled and play an important role in relaying TGF-β signals from cell-surface receptors to the nucleus. The TGF-β family exerts a wide range of biological functions in cell growth, differentiation, apoptosis and development of the embryo. ZEB2 gene is highly conserved among different species. The homologous alignment at amino acid levels reveals 97 % similarities between human and mouse, and 88 % between human and Xenopuslaevis. In addition, these proteins share the same amino acids in the zinc finger domain and certain similarities in their Smad binding domain (SBD). These findings suggest that the protein plays a similar role in vivo.

It was important to note that overexpression of Xenopus SIP1 (XSIP1) induced enlargement of neural tissue in anterior region, and some embryos failed to form eye vesicles and normal head phenotypes. Ectopic expression of XSIP1 induced anterior neural markers suggesting that XSIP1 played a role in early neurogenesis [18]. The animal model evidence shows that the ZEB2 gene is dosage sensitive and its precise regulation and expression is vital to embryonic neural and neural crest development.

Currently several genes have been known to be dosage sensitive genes, such as MECP2, NIPBL and NSD1 etc. For example, it is well known that haploinsufficiency of MECP2 gene typically results in Rett syndrome in females and severe neonatal encephalopathy or lethality in males [19]. Duplications overlapping the entire MECP2 gene are associated with MECP2 duplication syndrome
characterized by global developmental delay, intellectual
disability, autistic features, epilepsy and recurrent infec-
tions [20]. Patients with MECP2 triplications have also
been reported with more severe phenotypes [21]. Corne-
lia de Lange syndrome is a multisystem congenital
anomaly disorder and mutations or deletions of NIPBL
gene is a major cause for this condition [22]. Conversely,
NIPBL copy number gain is responsible for 5p13 dupli-
cation syndrome consisting of developmental delay,
developmental delay, learning disability, distinctive facial features and behavior
problems [23–25]. Similarly, haploinsufficiency of the
NSD1 gene located on 5q35 is the major cause of
Sotos syndrome recognized by intellectual disability, over-
growth, typical facial appearance, behavior problems and
seizures [26], whereas reciprocal duplications of Sotos
syndrome region overlapping the entire NSD1 gene
present a reverse phenotype including delayed bone age,
microcephaly, developmental delay and seizures [27, 28].
We believe more dosage sensitive genes exist in the
human genome and are yet to be discovered. Here we pro-
vide the first evidence suggesting that ZEB2 gene is such a
dosage sensitive gene similar to the aforementioned genes.

In conclusion, we first report a patient carrying a tripli-
cation at 2q22.3 involving the entire ZEB2 gene who pre-
sents overlapping features of Mowat-Wilson syndrome.
Based on the clinical evidence from patients with de novo
copy number gain involving the ZEB2 gene and the ex-
perimental evidence from Xenopus ZEB2 overexpression

| Table 1 Comparison of the clinical features of Mowat-Wilson syndrome and our patient with 2q22.3 triplication involving ZEB2 gene |
|---------------------------------------------------------------|
| **Features of MWS**                                            | **Features of our patient** |
| craniofacial features                                         | craniosynostosis-scaphocephaly |
| ▶ craniosynostosis                                            | - |
| ▶ frontal bossing                                             | microcephaly at birth, normal at 2 years 2 months |
| ▶ microcephaly                                                | - (small eyes) |
| ▶ deep-set large and widely spaced eyes                       | auricle dysplasia, low-set and asymmetrical |
| ▶ large uplifted earlobes with a dimple in the middle         | - (flat nose bridge) |
| ▶ a saddle nose with a rounded nasal tip                      | - (micrognathia) |
| ▶ open mouth appearance                                       | - (sparse eyebrows and hair) |
| ▶ M-shaped upper lip                                          | - (flat facial profile) |
| ▶ prominent but narrow chin                                   | mild cognitive impairment |
| ▶ large, flaring eyebrows                                     | moderate to severe intellectual disability |
| ▶ elongated face                                               | developmental delay |
| ▶ growth development                                          | ▶ (mild to moderate constipation) |
| ▶ delayed motor development                                   | ▶ delayed motor development |
| ▶ severe speech impairment with relative preservation of receptive language | ▶ delayed motor development |
| ▶ short stature                                                | ▶ (small atrial septal defect) |
| ▶ hypotonia                                                   | ▶ growth development |
| ▶ heart defects                                               | ▶ (small testes) |
| ▶ corpus callosum agenesis                                     | ▶ (mild to moderate constipation) |
| ▶ epilepsy                                                    | ▶ (mild to moderate constipation) |
| ▶ hirschsprung disease                                        | ▶ (mild to moderate constipation) |
| ▶ friendly and happy personalities                             | ▶ (mild to moderate constipation) |
| ▶ abnormalities of the urinary tract and genitalia            | ▶ (mild to moderate constipation) |
| ▶ hypospadias                                                 | ▶ (mild to moderate constipation) |
| ▶ eye defects                                                 | ▶ (mild to moderate constipation) |
| ▶ hand anomalies                                              | ▶ (mild to moderate constipation) |
| ▶ others (skin pigmentary changes, etc.)                      | ▶ (mild to moderate constipation) |

+ feature present; − feature absent
| Patients | Our patient | Decipher 305834 | Decipher 248386 | Decipher 251363 | Decipher 260771 | ISCA nssv578831 | ISCA nssv581021 | ISCA nssv582654 | ISCA nssv582319 |
|----------|-------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Genomic location (hg19) | chr2:143886436−146831592 | chr2:143871597−151071321 | chr2:143289932−151305504 | chr2:139199740−151305504 | chr2:145219415−151305504 | chr2:144657717−151305504 | chr2:145219415−145425705 | chr2:144657717−159178136 |
| Size | 2.9 Mb | 2.4 Mb | 6.2 Mb | 8.2 Mb | 12.1 Mb | 203Kb | 768Kb | 14.5 Mb |
| Inheritance | De novo | duplication | De novo | duplication | De novo | De novo | duplication | duplication |
| Phenotype | ID, DD, MCA, Behavior problems | Hearing impairment | ID, distinctive facial features, cryptorchidism, macroodontia | ID | ID | ID | DD, MCA and autism | GDD |
| Genes involved | ARHGAP15, GTDC1, ZEB2, TEX41 | ARHGAP15, GTDC1, ZEB2, TEX41 | TEX41, ACVR2A, ORC4, MBDS, EPC2, KIF5C, MMADHC, etc | ARHGAP15, GTDC1, ZEB2, TEX41, ACVR2A, ORC4, MBDS, EPC2, KIF5C, MMADHC, etc | LRPIB1B, KYNU, ARHGAP15, GTDC1, ZEB2, TEX41, ACVR2A, ORC4, MBDS, EPC2, KIF5C, MMADHC, etc | Part of ZEB2 | GTDC1, ZEB2 | GTDC1, ZEB2, TEX41, ACVR2A, ORC4, MBDS, EPC2, KIF5C, MMADHC, NEB, CACNB4, NR4A2, GP02, ACVR1, etc |

Abbreviations: ID intellectual disability; DD developmental delay; GDD Global developmental delay; MCA multiple congenital anomalies
model, we propose that ZEB2 copy number gain is functionally and clinically significant.

Consent
Written informed consent was obtained from the patient for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests
The authors declare that they have no competing interest.

Authors’ contributions
HF carried out the cytogenetic studies and wrote the manuscript. LL made the clinical evaluation and collected clinical information of the patient in detail. LZ and ZM coordinated the clinical evaluation. MC and JZ carried out the cytogenetic studies. All the authors have read and approved the manuscript.

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