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Case Report

Mowat-Wilson syndrome: clinical and molecular report of the first case in mainland China

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Abstract: Mowat-Wilson syndrome (MWS, MIM #235730) is a rare genetic disorder characterized by moderate-to-severe mental retardation, a recognizable facial gestalt and multiple congenital anomalies. The striking facial phenotype in addition to other features such as microcephaly, congenital heart defects, Hirschsprung disease (HSCR), severely delayed motor/speech development, seizures, short stature, corpus callosum agenesis and hypospadias are particularly important clues for the initial clinical diagnosis. All molecularly confirmed cases with typical MWS have a heterozygous loss-of-function mutation in the *ZEB2* (zinc finger E-box binding homeobox 2) gene, suggesting that haploinsufficiency of the protein is the main pathological mechanism. Here, we report the first individual with MWS in mainland China confirmed by molecular genetic testing. A 1-day-old girl was referred to the department of surgery for abdominal distension and failure to pass meconium. Targeted exome sequencing revealed a *de novo* heterozygous nonsense mutation (p.Arg302X) in *ZEB2* in the patient. Medical record review revealed mild facial gestalt, HSCR and severe congenital heart defects supporting the diagnosis of MWS. We concluded that facial dysmorphism in newborn babies might be atypical; doctors should pay more attention during physical examination and be aware of MWS if multiple congenital defects were discovered. *ZEB2* gene mutation screening would be an effective manner to clarify the diagnosis.

Keywords: Mowat-Wilson syndrome, targeted exome sequencing, *ZEB2* gene, nonsense mutation, Chinese

Introduction

Mowat-Wilson syndrome (MWS, MIM #235730) is a complex developmental disorder first described by Mowat et al. [1, 2] in 1998 in 6 children with distinct facial appearance, microcephaly, mental retardation and short stature. Five of the children also had Hirschsprung disease (HSCR, MIM #142623) and hence the disorder was initially thought to be a novel syndromic form of HSCR. Numerous studies since then have demonstrated that HSCR is not an obligatory feature of the disease and patients both with and without HSCR can be recognized by their characteristic facial gestalt including hypertelorism, prominent columella, broad nasal bridge, pointed chin, broad, medial flared eyebrows and uplifted earlobes [3, 4]. In addition, several other clinical findings were often associated with MWS such as congenital heart disease, epilepsy, agenesis of the corpus callosum (ACC) and hypospadias, suggesting that the underlying pathology may affect multiple organs during embryogenesis.

Since 2001, deletions and truncating mutations (nonsense or frameshift mutations) in the zinc finger E-box binding homeobox 2 gene (*ZEB2*, also known as *ZFHX1B* and *SIP1*) have been successively identified to underlie this syndrome [5, 6]. To date, about 256 molecularly proven MWS cases with over 100 different

ZEB2 mutations have been reported [7]. Among which, most mutations were considered to be *de novo* whereas only four families with MWS in siblings have been reported to be likely caused by germ-line mosaicism [8-11]. ZEB2 is a member of the Zfh1 family of 2-handed zinc finger/homeodomain proteins. These proteins generally consist of multiple functional domains, and are characterized by two widely separated clusters of C2H2-type zinc-finger domains and a centrally located homeodomain. As a DNA-binding transcriptional repressor, ZEB2 functions at the crossroads of multiple developmental pathways and is essential for the construction of the normal architecture of organisms. Recently, several Zeb2 functions in neuronal development and maturation have been identified by analyzing conditional knockout mice and its role has been expanded from central nervous system (CNS) to peripheral nervous system (PNS) and enteric nervous system (ENS) primodria [12].

Herewith, we report the first genetically diagnosed patient with MWS in mainland China. We also analyzed the failure of early diagnosis with the aim of raising awareness of this rare syndrome in China, especially for surgeons.

Material and methods

Patient and genomic DNA extraction

Written informed consent was obtained from the participants (patient and her parents) and our study was reviewed and approved by the Ethical Committee of the Capital Institute of Pediatrics (Ethical Number: SHERLL 2013039). Venous blood (1~2 ml) was obtained from the study participants in EDTA vials. Genomic DNA of peripheral blood leukocytes was extracted using salt-precipitation method [13].

Targeted exome sequencing

A minimum of 3 μg patient genomic DNA was used for the indexed Illumina libraries construction following the manufacturer's protocol. A final library size of 350-400 bp, including adapter sequences, was selected. 172 HSCR disease candidate genes (selected from an exome sequencing project, data not published) were enriched by a gene capture strategy using a GenCap Custom Enrichment Kit (MyGenostics, Beijing, China) according to the previously described technologies [14, 15]. Briefly, 1 μg DNA library was mixed with Buffer BL and a GenCap hypercholesterolemia probe (MyGenostics) and heated in a polymerase chain reaction (PCR) machine at 95°C for 7 min and 65°C for 2 min. 23 μl of the 65°C pre-warmed Buffer HY (MyGenostics) was added; the mixture was held at 65°C with the PCR lid heat on for 22 h for hybridization. 50 μl MyOne beads (Life Technology, Carlsbad, CA) were washed in 500 μl 1 × binding buffer thrice and re-suspended in 80 μl 1 × binding buffer. 64 μl 2 × binding buffer was added, the mixture was transferred into a tube containing 80 μl MyOne beads, and spun for 1 h on a rotator. We then washed the beads once with WB1 buffer at room temperature for 15 min and WB3 buffer thrice at 65°C for 15 min. Elution buffer was used to elute the bound DNA, which was amplified as follows: 98°C for 30 s; 98 for 25 s, 65°C for 30 s, 72°C for 30 s (15 cycles); 72°C for 5 min. We purified the PCR product using SPRI beads (Beckman Coulter) following the manufacturer's protocol. Enrichment libraries were sequenced on an Illumina HiSeq 2000 sequencer (Illumina, San Diego, CA) for 100-bp paired reads.

Bioinformatics analysis

After sequencing, we retrieved high-quality reads from the raw reads by filtering out the low-quality reads and adaptor sequences using the Solexa QA package [16] and cutadapt program (http://code.google.com/p/cutadapt/), respectively. We used the SOAPaligner program [17] to align the clean read sequences to the human reference genome (UCSC Genome Browser hg19). After removing duplicates with Picard software [18], single-nucleotide polymorphisms (SNPs) were identified using the SOAPsnp program [17] (http://soap.genomics.org.cn/soapsnp.html). Subsequently, reads were realigned to the reference genome using the Burrows-Wheeler alignment program [19], and insertions or deletions (InDels) were identified with the Genome Analysis Toolkit [20] (http://www.broadinstitute.org/gsa/wiki/index.php/Home_Page). We annotated identified SNPs and InDels using the Exome-assistant program (http://122.228.158. 106/exomeassistant). Short read alignment and candidate SNP and InDel validation was performed using MagicViewer [21].

Sanger sequencing validation

Sanger sequencing was applied to validate the nonsense mutation detected in the patient

Figure 1. Characteristic facial appearance of our patient with Mowat-Wilson syndrome. Note the flat nasal bridge, beaked nose, micrognathia and upswept ear lobules.

(study ID: HSCR0038) by TES. The genomic DNA reference sequence was NM 014795.3. PCR of exon seven was performed using primers 5'-AAGCATGCACTCAAACCTTTT-3' (forward)

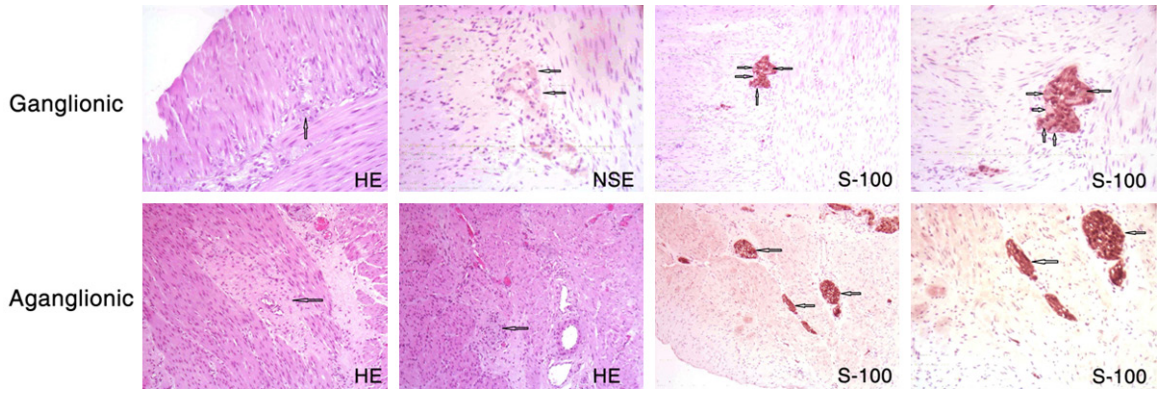

Figure 2. Pathological photomicrographs of ganglionic and aganglionic colon tissue by H&E, NSE and S-100 staining of our patient. Arrows indicate the ganglionic cells and fibrous tissue of hyperplasia in ganglionic (the first row) and aganglionic (the second row) tissue respectively.

and 5'-CAGGCACACAGAGTTGATGA-3' (reverse). One hundred and fifty nanograms of DNA were added into PCR mixture, which contained 5 μl of 10 × PCR buffer, 5 μl dNTP mixture (2.5 mM each), 2 μl of each primer working solution (20 μM), and 0.5 μl Takara Taq (Takara R001B) to a final volume of 50 μl. Amplification was performed using PCR System 9700 (Applied Biosystem) with the following protocol: 1 min denaturation at 94°C, followed by 35 cycles (94°C 30 s, 55°C 30 s, 68°C 30 s) and a final elongation step at 72°C for 1 min. The result was analyzed on ABI 3730 analyzer (Applied Biosystem).

Results

Clinical presentation

The patient is a 1-day-old girl (study ID: HSCR-0038), born at full term by cesarean section with a birth weight of 3050 g (50th centile) and length of 49 cm (50th centile) with no obvious postnatal complications. She was referred to our outpatient department because of abdominal distension and failure to pass meconium. The antenatal course was unremarkable. Fetal movement and all prenatal ultrasound examinations were normal. The parents denied consanguineous marriage and there was no family history of neurologic or neuro-developmental anomalies, specifically no history of Hirschsprung disease or other congenital malformations or genetic abnormalities. On general examination, she had microcephaly with a head circumference of 31 cm (<1st centile) and mild facial dysmorphism including flat nasal bridge, beaked nose, micrognathia, and upswept ear lobules (**Figure 1**). Routine physical examination also found III/6 grade systolic bruit. Per rectal digital examination, and abdominal radiographs were diagnostic of Hirschsprung disease. Trans-anal pull-through operation was conducted at the age of 23 days. Intra-operative multipoint seromuscular colon biopsies confirmed absence of ganglion cells up to the level of transverse colon. Post-operative immunostaining further confirmed the diagnosis of long segment HSCR (**Figure 2**). Severe congenital heart defect was diagnosed with echocardiogram and she underwent additional cardiac operation three month later. However, the child succumbed at the age of 4 month and 10 days due to heart failure and pneumonia. Karyotype analysis showed a normal 46, XX karyotype (Supplementary Figure 1). Continuous recording of early aEEG (amplitude-integrated electroencephalogram) was conducted for 4-6 h within 12-h after delivery and no abnormal values and patterns were noticed (**Figure 3**). All the abnormal laboratory studies are outlined in **Table 1**.

Molecular diagnosis and inheritance determination

Targeted exome sequencing was conducted on the patient with the purpose of determining the underlying pathology. Altogether 17 genetic variants were found including 2 non-frameshift deletions, 14 non-synonymous substitutions and 1 stop-gain mutation (Supplementary Table 1). Among which, the nonsense mutation in *ZEB2*, denoted as p.Arg302Stop at the protein level and c.904 C>T at the cDNA level was the most conspicuous finding. This substitution

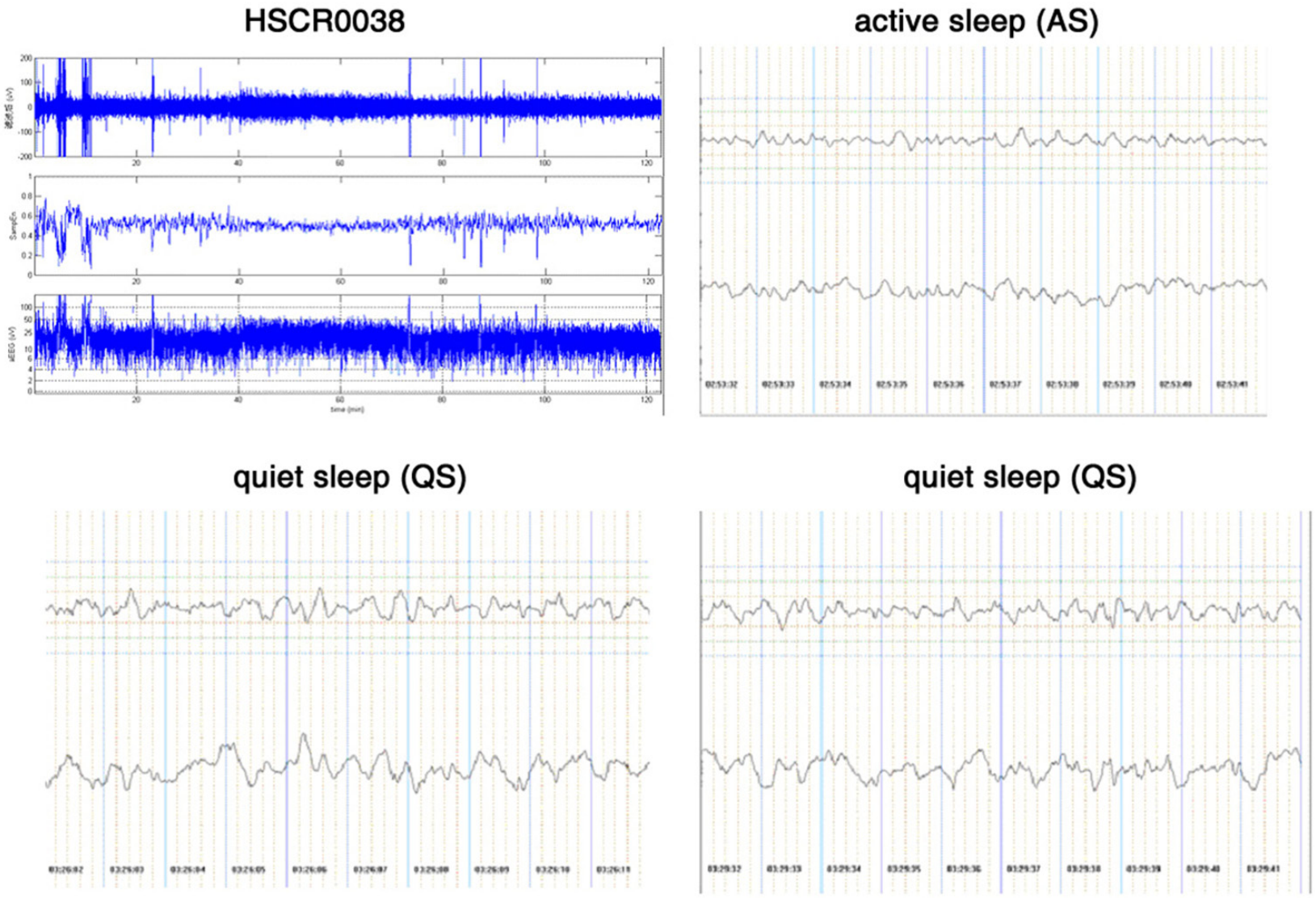

Figure 3. The patient underwent continuous recording of early aEEG (amplitude-integrated electroencephalogram) for 4-6 h within 12-h after delivery and no abnormal values and patterns were noticed. Representative photographs during both active sleep (AS) and quiet sleep (QS) were displayed here.

Table 1. Abnormal clinical and laboratory findings of our MWS patient

Investigations	Findings
Contrast enema	●Narrow rectum and left colon with distension from the splenic flexure
Echocardiography	●Pulmonary stenosis (PS), moderate to severe
	●Persistent ductus arteriosus (PDA)
	●Ventricular septal defect (VSD), multiple muscular
	●Atrial septal defect (ASD), multiple ostium secundum
Intra-operative multipoint seromuscular layer biopsy (H&E staining)	●Lack of ganglion cells beyond the transverse colon
Post-operative Immunohistochemistry staining on colon tissue	●Lack of ganglion cells beyond the transverse colon

creates a nonsense mutation, changing an *Arginine* codon to a premature stop codon (CGA>TGA) in exon seven of the *ZEB2* gene (NM_014795.3). This Arg302Stop nonsense mutation in the *ZEB2* gene has been reported previously in a patient with Mowat-Wilson syndrome [22]. This mutation is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay, and its presence is consistent with the diagnosis of Mowat-Wilson syndrome, an autosomal dominant disorder. On the basis of the TES results, the child's records were reviewed and clinical features of MWS were identified as mild facial dysmorphism, severe congenital heart defect and moderate Hirschsprung disease (described under case report). Later on, genetic examination verified both parents to be wild-type, and hence the patient's mutation was *de novo* (**Figure 4**).

Discussion

This is the first patient with MWS in mainland China confirmed by molecular genetic testing. Mowat-Wilson syndrome is a genetic disorder first described by Mowat et al. in 1998, and since then about 256 molecularly confirmed cases with this syndrome have been reported. The incidence in mainland China is unknown, on the basis of the data from the Department of Health in Hong Kong, the prevalence is estimated to be 1 in 130,000 [23]; lower than the prevalence in Japan as of 1:90,000 [24]. Mowat-Wilson syndrome exhibits a wide spectrum of clinical features, varying from the typical facial appearance and developmental delay to that with Hirschsprung disease, congenital heart defects, and a variety of other congenital anomalies, including hypoplasia or agenesis of the corpus callosum [22, 25]. Our patient here exhibits mild facial gestalt, HSCR and severe congenital heart defects. Brain imaging was not performed as the child had passed away from severe heart failure and pneumonia before the diagnosis of MWS was established.

Up to now, a consensus clinical diagnostic criteria for Mowat-Wilson syndrome have not been established. Facial appearance would provide the first clue of the diagnosis and it warrants a *ZEB2* mutation screening [26]. All the *ZEB2* gene mutations described to date in classic Mowat-Wilson syndrome are either large deletions or frame shift or nonsense mutations. Altogether 17 nonsense mutations have been reported which scattered throughout the gene (**Figure 4B**), leading to null function of the protein. Among these, there are four recurrent mutations (R302X, R343X, R695X, R921X), which, as Yamada et al. [3] already suspected, always derived from CpG doublets known to be a frequent target of C to T transitions [27]. The German reported patient with *de novo* R302X mutation was a 16-year-old girl with limited speech, microcephaly, constipation and agenesis of corpus callosum [9]. While a Japanese patient harboring the same mutation, a 7-year-old boy, presented with HSCR, epilepsy, mental retardation, microcephaly, cryptorchism and cavum septi pellucidi on brain imaging examination [22]. Our patient passed away at a very early age from severe heart failure and so we have no chance to know if she would develop mental retardation, epilepsy and/or delayed motor/speech development, but studies have indeed revealed the nature of *ZEB2* mutations as remarkable phenotypic variability, even for the same mutation.

In addition to MWS, mutations in the ZEB2 were also demonstrated in patients with microcephaly, mental retardation, epilepsy, and distinct facial appearance [5]. *ZEB2* is a zinc finger E-box-binding homeobox 2 gene, also known as *SIP1* (Smad interacting protein 1) or *ZFHX1b* (zinc finger homeobox gene 1b) that is expressed in the nervous system throughout its devel-

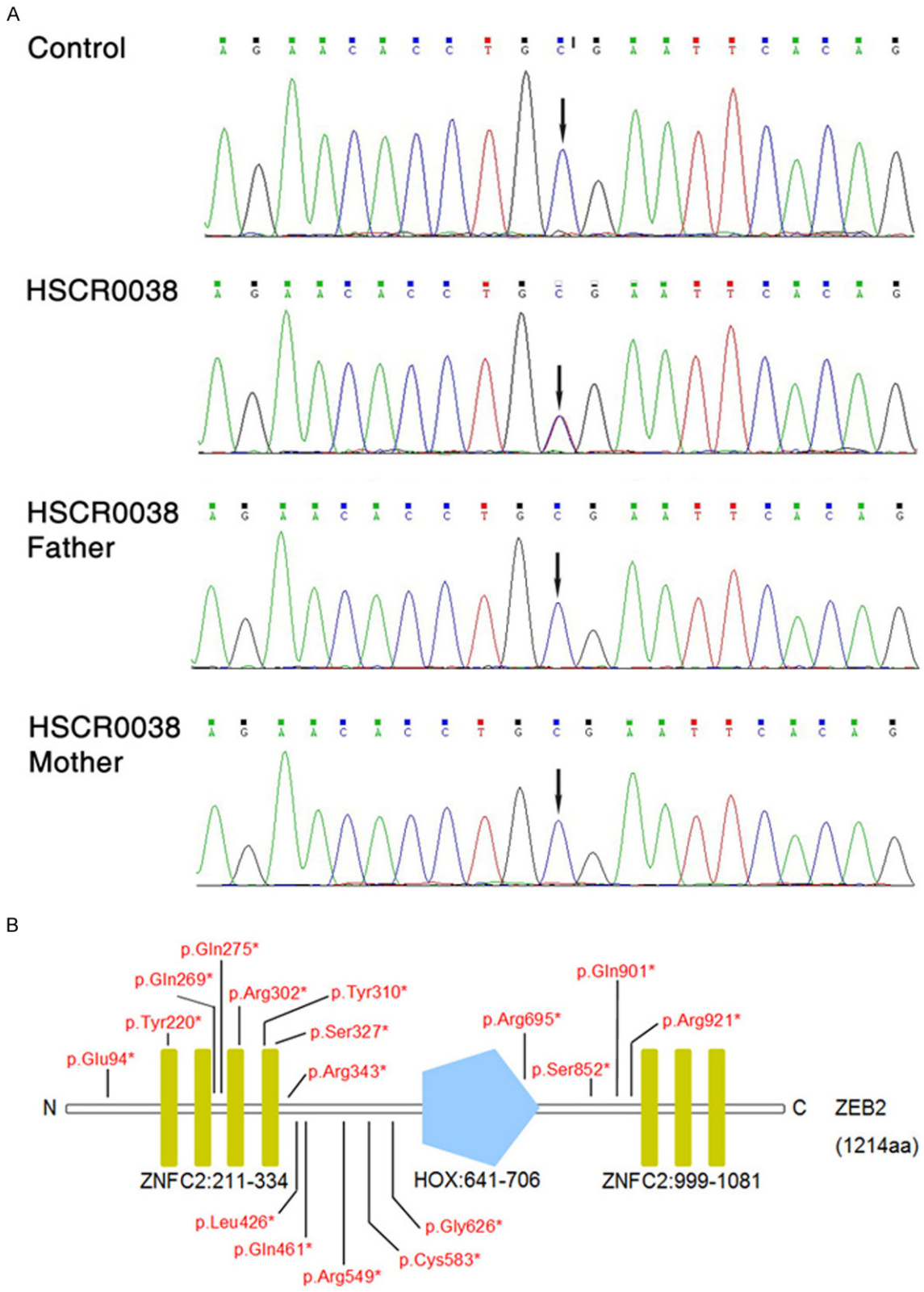

Figure 4. A. Electropherograms from sequencing of exon seven in patient, her parents and 1 control. The heterozygous c.904C>T (p.R302X) mutation was confirmed and both parents were demonstrated to be wild-type. B. Schematic representation of the functional domains in the 1214 amino acid-long human ZEB2 and distribution of all the 17 nonsense mutations reported in Mowat-Wilson syndrome affected patients up to date.

opment. To date, Zeb2 has been shown to control the generation of the neocortex, hippocampus, corpus callosum, and spinal cord, as well as autonomic and enteric nervous systems. In addition to this, gliogenesis and myelinogenesis are also regulated by Zeb2 in the CNS. The multifarious actions of Zeb2 in both neurogenic and gliogenic processes reflect the wide range of target genes and of binding partners of Zeb2, as well as the complex regulation of Zeb2 at the genetic level [12].

In conclusion, we report a patient with Mowat-Wilson syndrome. We hope that it will raise awareness of this unusual syndrome, especially for surgeons in the mainland China. As HSCR is a common association, patients are often referred to the surgeons before we take notice of their facial dysmorphisms. Establishing a molecular diagnosis is important for the patients and their families as it allows reliable genetic counseling for their families and a better clinical management of the patients.

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Disclosure of conflict of interest

None.

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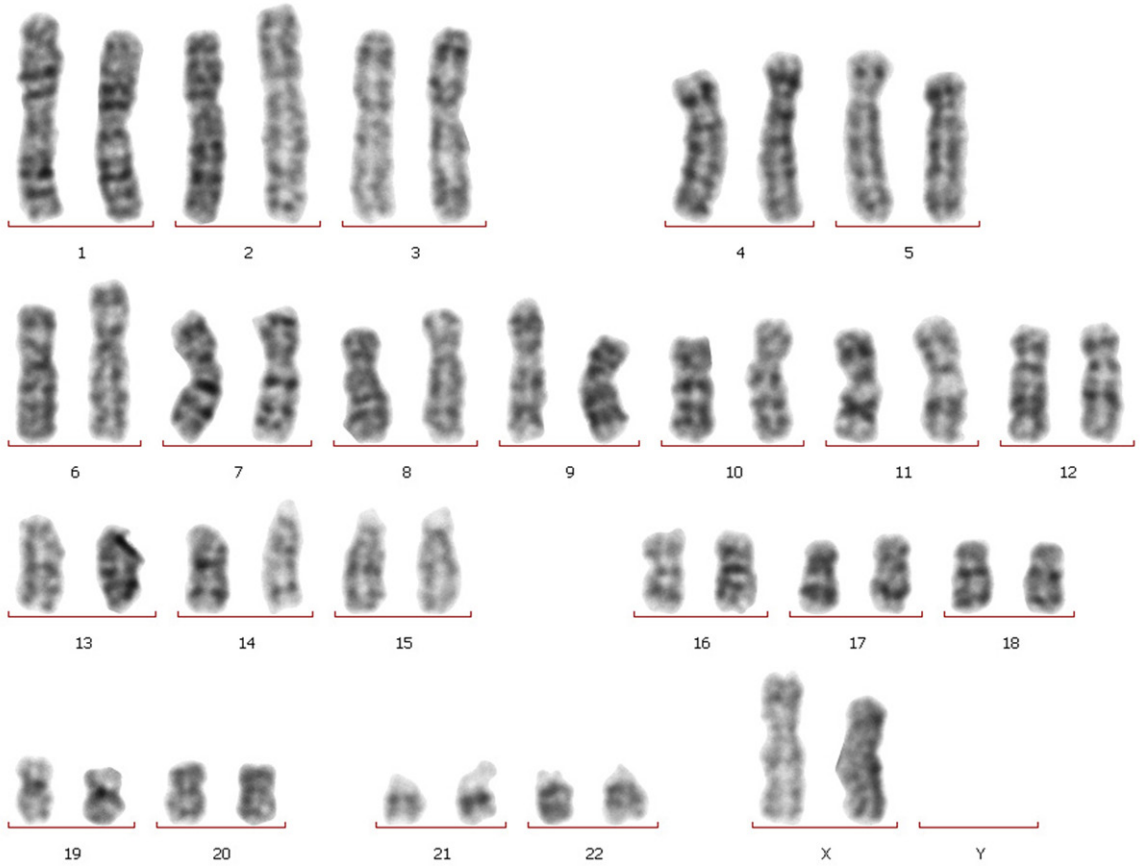

Supplementary Figure 1. G-banding karyotype analysis revealed a normal 46, XX karyotype.