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Novel Zeb2 gene variation in the Mowat Wilson syndrome (MWS)

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ABSTRACT

Background: Mowat Wilson syndrome (MWS) is an uncommon association of Hirschsprung's disease (HSCR). Phenotypic features may develop with time, causing initial difficulties in diagnosis. MWS results from haploinsufficiency of the Zinc finger E-box-binding homeobox 2 (ZEB2) gene, and molecular diagnosis of ZEB2 mutation is required to confirm the diagnosis. We report the first confirmed cases of MWS in three children with the typical facial features, mental retardation, absent corpus callosum, epilepsy, and HSCR and novel Zeb2 variations on DNA analysis.

Methodology: Clinical features were monitored. DNA extracted from peripheral blood was subjected to bidirectional sequencing analysis following PCR DNA amplification. ZEB2 gene results were compared to the ZEB2 reference sequence (ENS00000169554) for variation. Bioinformatic investigation of novel gene variants was via the "*Blastx*" *program* function available via the National Center for Biotechnology Information (http://www.bioinfo.org/NPInter/blast/blast_link.cgi).

Results: Clinical follow-up showed that the phenotypic features were not all present at birth but developed with time in 2 surviving patients. Several Zeb2 variations were detected in the promoter region of the ZEB2 gene of which 2 were novel (−56A/T 1174 11A/12A). In addition, a novel heterozygous single nucleotide insertion in exon 2 of ZEB2 in one patient results in a frameshift causing deletion of the first 8 amino acids of the ZEB2 protein and an alteration of amino acids 9 (G9A), 11 (R11G), and 12 (C12A). In the third patient, a novel single nucleotide deletion exon 8 (1784delC Het) results in a frameshift at amino acid 595 of translated protein. This shortens protein from 1214 to 594 amino acids and affects the functionality of the critical ZEB2 protein.

Conclusions: MWS is an important link to recognise clinically. It underlines the functionality of the Zeb2 gene in certain syndromic Hirschsprung's disease. These variations probably contribute to the clinical features of the Mowat Wilson phenotype in Hirschsprung's disease but should be confirmed in further research.

Since its initial description by Mowat et al. [1], the Mowat–Wilson syndrome (MWS) has been reported in countries mainly Europe, Australia and the USA. More recently, more than 179 cases from countries all over the world have been reported [2] with large series reported from countries like Japan [3] as well as a number of isolated cases from different countries.

Clinically, Mowat–Wilson syndrome is a rare condition representing a spectrum of congenital dysmorphic features of the brain, head and face (microcephaly, corpus callosal agenesis, hypertelorism, prominent columella, pointed chin, and uplifted earlobes) as well as GI motility disturbances (including Hirschsprung's disease(>50%) [1] and/or severe constipation) [4,5]. In addition, genitourinary anomalies (especially hypospadias and renal tract anomalies), congenital heart defects, short stature and eye defects are not uncommon [6]. Focal epilepsy appears to be common in these children particularly during sleep [7]. More recently, MWS has also been associated with CNS tumours [8].

It has a genetic basis and has been reported to recur in families with an approximately 1% autosomal dominant recurrence risk [9–11]. As the clinical diagnosis of Mowat–Wilson syndrome (MWS) may prove difficult because of phenotypic variation, and the progression of clinical features with time, molecular diagnosis of ZEB2 mutation is required to confirm the diagnosis of MWS.

The association between MWS and Hirschsprung's disease (HSCR) is interesting as this association includes brain and facial development in addition to the congenital absence of ganglion cells in intermyenteric plexuses of the intestinal wall. HSCR is known to be a complex multigenic disorder, resulting from the effects of genetic variations of at least 12 known susceptibility genes which affect a number of stages of the normal process of Enteric Nervous system (ENS) development (including cell migration, differentiation, and survival) [12]. Although many of these genetic pathways influence the major susceptibility

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RErranged during Transfection (RET) and Endothelin B receptor (EDNRB) gene pathways, the mechanism of the ZEB2 gene in HSCR remains unclear.

We report the first confirmed three cases of MWS in Africa with ZEB2 and an associated novel ZEB2 gene variation.

1. Methods

Clinical features were documented from hospital records. Congenital dysmorphic features of the head and face were monitored. Neurological and brain anomalies were documented both clinically and radiologically. GI motility function was evaluated on clinical follow-up. Other anomalies were documented.

1.1. DNA evaluation and the ZEB2 gene

The blood sample received was subjected to DNA extraction followed by PCR amplification of the promoter and exons 1 to 10 of the *ZEB2* gene. Successful PCR amplifications were subjected to semiautomated bidirectional sequencing analysis. Sequencing results were analysed using ClustalW and BioEdit Sequence Alignment Software [13]. The results were compared to the ZEB2 reference sequence on Ensembl (ENSG00000169554).

Bioinformatic investigation of novel gene variants was via the "*Blastx*" *program* function available on the National Center for Biotechnology Information website http://www.bioinfo.org/NPInter/blast/blast_link.cgi.

2. Results

In addition to sharing the common feature of Hirschsprung's disease, the clinical features of 3 Caucasian suspected cases were in keeping with a diagnosis of MWS (Table 1). Clinical follow up showed that other phenotypic facial features were not all present at birth but developed with time in the 2 surviving patients (Fig. 1). The presence of brain mediated developmental delay was constant in all three and early severe apnoeic attacks with subsequent epilepsy were observed in one patient. CT scanning of the brain showed an absent corpus callosum in 2 patients.

3. DNA results

Heterozygous single nucleotide variations in exon 10 (case 1) as well as an insertion in exon 2 (Case 2) and an exon 8 deletion (case 3) of the ZEB2 gene was noted. The latter 2 appeared to be functional, resulting in a frameshift which altered the translated protein. (Table 2) The frameshift resulting from the exon 2 insertion resulted in a deletion of the first 8 amino acids of the ZEB2 protein and an alteration of amino acids 9 (G9A), 11 (R11G) and 12 (C12A). The exon 8 deletion resulted in a frameshift affecting amino acid 595 of the translated ZEB2 protein (Table 2) and shortens the protein from 1214 to 594 amino acids.

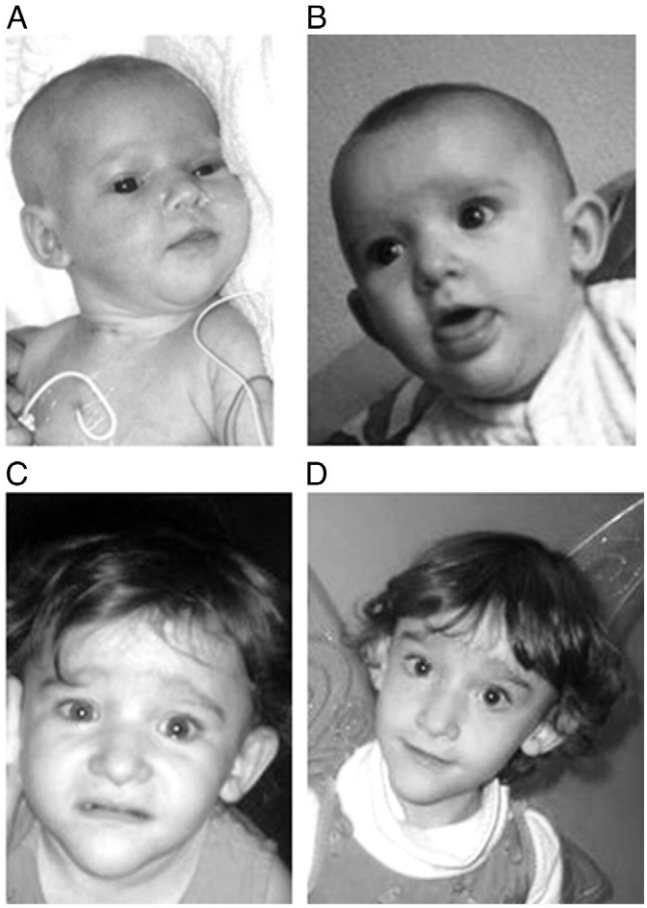

Fig. 1. Mowat Wilson syndrome progression of facial features with age (case 3) as neonate (A), at 7 months (B), at 2 years (C) and at 6 years (D) (*all photographs used with permission*).

These changes affect the functionality of the critical ZEB2 protein. In addition, several Zeb2 variations were detected in the promoter region of the ZEB2 gene of which 2 were novel (−56A/T; −1174 11A/12A;). Of these the −56A/T Promoter variant appears to be potentially functional.

4. Discussion

The diagnosis of MWS may be clinically difficult because of the variability of the phenotype and the widespread nature of the phenotypic features such as microcephaly and facial features which may not be obvious at birth, The clinical features and facial features of Mowat Wilson syndrome have been shown to develop with time [15] (as shown in Fig. 1). However, the presence of HSCR associated with abnormalities

Table 1
Clinical feature of Mowat–Wilson syndrome (MWS).

No.	Gender	Facial features	Brain	Development	Clinical	Hirschsprung's	Other
Case 1	Male	Open mouth smile; dysplastic ears with a lifted tragus	Absent corpus callosum	Developmental delay	Epilepsy DAY 2; Brachycephaly; open anterior fontanelle;	Hirschsprung's diagnosed @ 2 months	Undescended testes Bilateral colobomas Residual hypomotile bowel
Case 2	Female	Hypertelorism; prominent columella; pointed chin; uplifted earlobes	Corpus callosum agenesis	Developmental delay	microcephaly; Developed Epilepsy	Hirschsprung's disease	Bilateral colobomas
Case 3	Female	Hypertelorism, prominent columella, pointed chin, and uplifted earlobes	No CT	Developmental delay	Developed Epilepsy	Hirschsprung's disease	