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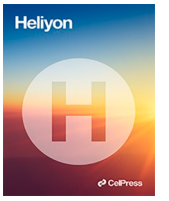

Novel *STAMBP* mutations in a Chinese girl with rare symptoms of microcephaly-capillary malformation syndrome and Mowat-Wilson syndrome

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ABSTRACT

Microcephaly-capillary malformation syndrome (MIC-CAP) and Mowat-Wilson syndrome (MWS) are both rare hereditary diseases with several overlapping symptoms. We here report a Chinese patient simultaneously affected by MIC-CAP and MWS, presenting with moderate anaemia because of repeated, unilateral refractory epistaxis. The girl was initially diagnosed with MWS after discovery of a pathogenic nonsense mutation in *ZEB2*. Starting from the age of 3 years old, the child experienced repeated epistaxis on the right side without obvious incentive or trauma. The bleeding was quite difficult to stop and her hemoglobin dropped from 124 g/L to 64 g/L in three months. Both coagulation disorders and allergic rhinitis were excluded by extensive workup and experimental therapeutics. Retrospective genetic analysis revealed that she carried two novel compound heterozygous mutations in *STAMBP* (c.610T > C: p.Ser204Pro and c.945C > G: p.Asn315Lys). This case report demonstrates a rare presentation of MIC-CAP in the pediatric population and enriches the variant spectrum of *STAMBP*.

1. Introduction

Microcephaly-capillary malformation syndrome (MIC-CAP) [1] is caused by biallelic *STAMBP* variants [2,3]. Only 24 patients with *STAMBP* mutations have been reported so far [4–6]. Microcephaly, generalized capillary malformations on the skin, global developmental delay and intractable epilepsy are the typical clinical manifestations. Previously reported vascular anomalies are not restricted to the skin but have been very rare: one presented with cerebellar angioma [7], and another presented with vascular malformations in the liver [1]. Repeated bleeding due to vascular malformation in this syndrome has not been reported before. The genetic basis of Mowat-Wilson syndrome (MWS) is heterozygous deletion or loss-of-function variant of the *ZEB2* gene [8], with substantial overlapping symptoms with MIC-CAP. To date, there have been no reports of people suffering from these two genetic

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diseases, or whether there is any correlation between the two diseases. We here reported a 3-year-and-9-month-old Chinese girl affected by both MIC-CAP and MWS.

2. Materials and methods

2.1. Patient

The patient was the first child of healthy nonconsanguineous parents of Han Chinese ethnicity (31-year-old mother and 54-year-old father). Except for moderate maternal hypothyroidism and gestational diabetes, the pregnancy was uneventful. At term, the weight, length and occipitofrontal circumference (OFC) of the girl were 3130 g (10th-50th), 50 cm (mean) and 34 cm (mean), respectively. Asphyxia was not reported at birth. She presented with neonatal feeding difficulties that did not require enteral nutrition. Simian line and erythema on the forehead and right eyelid were noted. She had a very low sloping forehead, wide nasal bridge, hypertelorism, thin upper lip and micrognathia (Fig. 1A). Physical examination revealed multiple small capillary malformations on her trunk, which became hyperpigmented with age (Fig. 1B). Limb examination showed short, tapered fingers and clinodactyly of the second toe bilaterally (Fig. 1C and D).

The developmental milestones were comprehensively delayed in this child. She developed head control at the age of 4 months and learned to roll over at 7 months. To date, she is still unable to walk and can be supported to stand for only a brief period. Her speech and language acquisition were considerably delayed, and for the record, she could not say "mama" and "papa". At the age of 3-year-and-2-month, her weight, height and OFC were 10.5 kg (<3rd), 92 cm (3rd-10th) and 45 cm (<3rd), respectively. The Children Neuropsychological and Behavioral Scale-Revision 2016 was applied to evaluate her mental development at the age of 3-year-and-6-month with a mental age of 5.7 months and a developmental quotient (DQ) of 14.

The girl was affected by complicated congenital heart disease (CHD, including an atrial septal defect, patent ductus arteriosus, pulmonary hypertension) and pulmonary artery sling, both of which are common phenotypes of MWS. She underwent corrective surgery for her CHD at 54 days. She first developed febrile seizures at the age of 2-year-and-7-month. Electroencephalographies (EEGs) presented the slowing of background rhythm (θ-δ) and epileptiform discharges mainly occurred in the posterior region of the brain. No obvious abnormalities were found on cranial computed tomography (CT) at 2 years. Cerebral MRI examination was not applied because of the steel wire placed in her chest. Blood and urinary metabolic screening indicated normal results. Valproic acid (VPA) was first started to treat her seizures but was soon demonstrated to have low efficacy. Lamotrigine, levetiracetam and lacoxamide were then gradually added while the dose of VPA was reduced. The seizure frequency during optimum control has been approximately 1–3 times per month thus far.

Starting from the age of 3 years old, the child experienced repeated epistaxis on the right side without obvious incentive or trauma (Fig. 1E). The bleeding was quite difficult to stop (each bleeding duration varied from 1.5 to 16 hours). The platelet count (116-166 $\times$ 10^9/L), coagulation function and platelet function were all within the normal ranges in multiple tests. The platelet antibody and autoantibody tests were demonstrated to be negative. No signs of bleeding were observed in other organs, and abdominal ultrasound revealed no definite vascular malformation. Allergic rhinitis was first suspected to be the cause of epistaxis, but experimental therapeutics with loratadine and cetirizine did not alleviate her symptoms. Due to repeated massive bleeding in a short period of time, her hemoglobin dropped from 124 g/L to 64 g/L in three months.

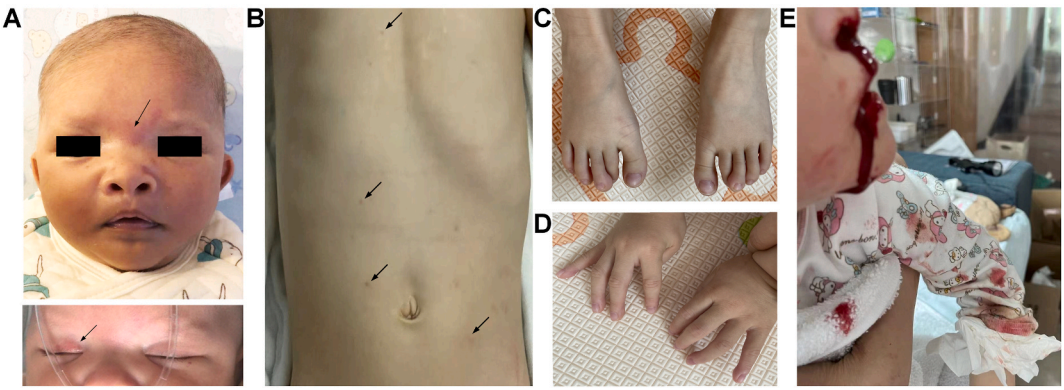

Fig. 1. Clinical features of the 3-year-and-9-month-old Chinese girl simultaneously affected by MIC-CAP and MWS. (A) Facial appearance of the patient with protrusion of the forehead, flat nasal bridge, beaked nose, hypertelorism, thin upper lip, micrognathia and multiple small capillary malformations on the face from birth (black arrows). (B) Multiple small capillary malformations on the trunk at the age of 3-year-and-8-month (black arrows). These changes became hyperpigmented with age. (C) Images of the feet. (D) Images of the hands. The short and tapered fingers and clinodactyly of the second toe were noted bilaterally. (E) Photograph of her hemorrhinia status.

2.2. Whole-exome sequencing

This study was approved by the Ethics Committee of Capital Institute of Pediatrics (SHERLL 2013039). With the informed consent, the peripheral blood samples of the proband and her parents were captured using the Agilent Sure-Select Human All Exon Kit (Agilent Technologies, Santa Clara, CA) and sequenced on an MGISEQ-2000 sequencer (MGI Tech, China) for whole-exome sequencing (WES) at the AmCare Genomic Laboratory (Guangzhou, China) and Sanger sequencing.

3. Results

WES revealed a *de novo* pathogenic heterozygous mutation in *ZEB2* (c.2083C > T: p.Arg695Ter) and two novel compound heterozygous mutations in *STAMBP* (c.610T > C: p.Ser204Pro and c.945C > G: p.Asn315Lys). All were validated with Sanger sequencing (Fig. 2A, Table 1). The nonsense mutation in *ZEB2* had been previously described in other MWS patients [9,10] and was categorized as clinically pathogenic according to ACMG guidelines. The two missense variants in *STAMBP*, in contrast, were both defined as variants of uncertain significance. The mean coverage of the *STAMBP* gene was 170 × and no other pathogenic variants were detected. In addition, no other clinically relevant variants were detected in any vascular anomalies' associated genes. After searching the PubMed, HGMD, ClinVar and CNKI databases as of November 2022, we found that both variants in *STAMBP* had not been reported. Multiple sequence alignment showed that the paternal inherited variant converts a conserved serine into proline (p.Ser204Pro), while the maternal inherited variant converts a conserved asparagine into lysine (p.Asn315Lys, Fig. 2B). Three functional prediction programs were used to determine the effects of the mutations: p.Ser204Pro was predicted to be "Disease_causing" by MutationTaster, and p.Asn315Lys was predicted to be "Damaging", "Possibly_damaging" and "Disease_causing" by SIFT, PolyPhen2 and MutationTaster. The former variant is not documented in 1000G and our in-house datasets, while 1 heterozygous carrier is reported in the gnomAD database giving an allele frequency of 0.00003186. For p.Asn315Lys, 7 heterozygous carriers are reported in the gnomAD database giving an allele frequency of 2×10^{-5}.

Contrast-enhanced paranasal sinus spiral CT revealed abnormal enhancement of the right inferior turbinate mucosa (Fig. 2C), with CT values of 133 HU and 159 HU in the arterial and venous phases, respectively (the values of the contralateral turbinate mucosa were 48 HU and 79 HU). Further nasal endoscopic examination confirmed the patient's vascular malformation with capillary dilation (Fig. 2D and E), and electrocoagulation under a nasal endoscope was conducted to treat her epistaxis. The patient had no further

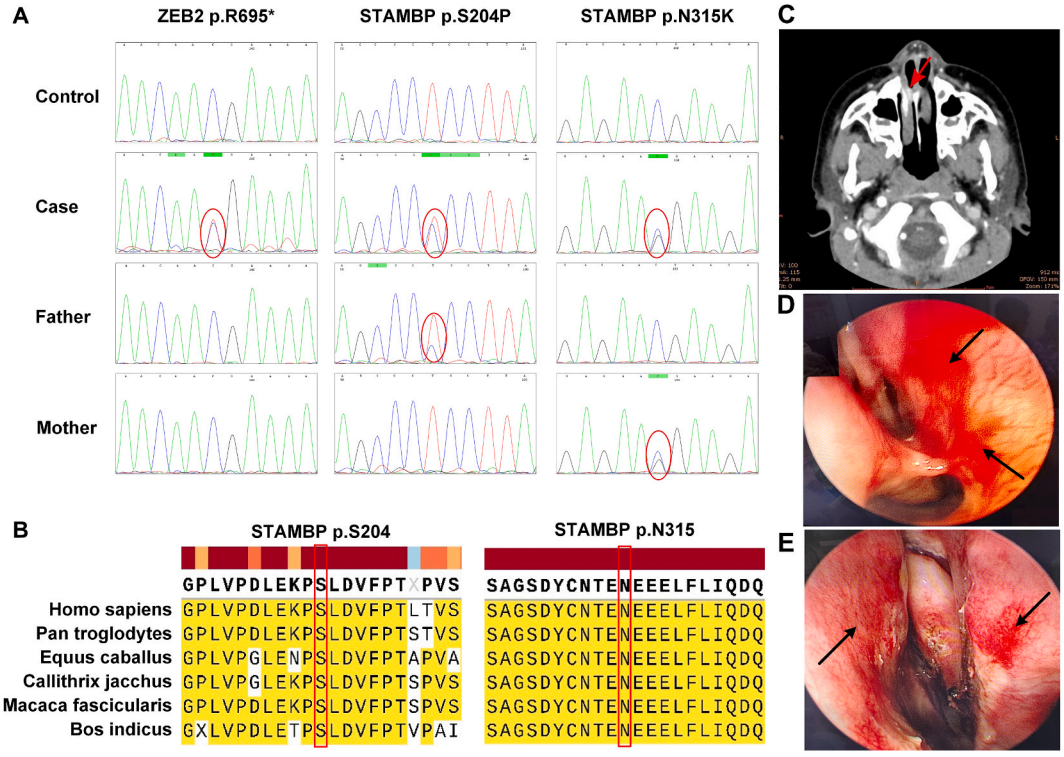

Fig. 2. Genetic and clinical examinations of the proband. (A) Electropherograms from the sequencing of *ZEB2* and *STAMBP* in one control, the patient and her parents are shown. The heterozygous c.2083 C > T (p.Arg695Ter) mutation in *ZEB2* and the compound heterozygous mutation c.610 T > C (p.Ser204Pro) and c.945 C > G (p.Asn315Lys) in *STAMBP* was confirmed. (B) Evolutionary conservation of the *STAMBP* sequence flanking the altered Ser204 and Asn315 amino acids. (C) Contrast-enhanced paranasal sinus spiral CT revealed abnormal enhancement of the right inferior turbinate mucosa. (D, E) Nasal endoscopic examination discovered vascular malformation with capillary dilation.

Table 1
The *ZEB2* and *STAMBP* mutations discovered in our patient.

Gene	Chromosome position (hg19)	Nucleotide change	amino-acid change	Exon	Frequency			Inheritance
					1000G (2015Aug all)	gnomAD	In-house	
ZEB2 (NM_014795)	chr2:145156671	c.2083 C > T	p.Arg695Ter	Exon 8	–	–	–	*de novo*
STAMBP (NM_006463)	chr2:74074748	c.610 T > C	p.Ser204Pro	Exon 6	–	0.00003186	–	Paternal
	chr2:74077580	c.945 C > G	p.Asn315Lys	Exon 8	–	0.0000279	–	Maternal

"-", not reported.

bleeding episodes through 3 months after hospital discharge.

4. Discussion

MIC-CAP is a rare neurodevelopmental disorder characterized by congenital microcephaly, early-onset intractable epilepsy, profound intellectual disability and diffuse cutaneous capillary malformations. Altogether 22 pathogenic mutations in the STAM binding protein gene have been reported in 24 patients from 8 ethnic populations [4–6]. The patient presented here met most of the characteristics of this novel syndrome just described in 2013 (Table 2). Reduced STAMBP expression has been demonstrated to be associated with accumulation of ubiquitin-conjugated protein aggregates, elevated apoptosis and insensitive activation of the RAS-MAPK and PI3K-AKT-mTOR pathways in patient-derived lymphoblastoid cell lines, possibly contributing to the vasculature and growth defects of this syndrome [1].

Multiple generalized capillary malformations were observed in 22 of 24 (92 %) patients studied in previous reports, with only two (8.3 %, 2/24) involving organs other than the skin [4,5]. Here, we present a challenging case of a girl affected by both MIC-CAP and MWS who experienced repeated refractory epistaxis over nine months and failed multiple conventional therapies. Defects in multiple organ systems also dramatically increased the difficulty of treatment, such as tracheal stenosis and CHD. Coagulation disorders and autoimmune disease were initially suspected to be the major causes of her bleeding, but the subsequent extensive workup excluded these diagnoses. Later, allergic rhinitis was also excluded due to a negative allergen test result and the failure of experimental therapeutics with anti-allergic drugs. Detailed physical examination, medical history review and evaluation of the genetic analysis provided new clues for the real underlying mechanism. Contrast-enhanced paranasal sinus spiral CT and nasal endoscopic examination finally confirmed our suspicion and showed how a vascular malformation caused by single gene mutations could produce severe clinical symptoms and unpredictable consequences without appropriate and timely interventions. Our study indicates how uncertain significance variants in compound heterozygosity could suggest a diagnosis when the clinical manifestation is highly specific for a certain disease. Further experiments can be conducted to test the functional effects of these *STAMBP* novel variants on protein expression, PI3K-AKT-mTOR pathway activation and cell apoptosis.

MWS is a rare autosomal dominant disorder that shares several clinical manifestations with MIC-CAP [5,8]. Our patient showed a dysmorphic appearance, global developmental delay, progressive microcephaly, and epilepsy and was only initially diagnosed with

Table 2
Comparison of clinical features of the patient reported here with those of previously reported individuals with MIC-CAP and MWS patients.

Clinical feature	This paper	Previously-reported individuals with MIC-CAP	Previously-reported individuals with MWS (n = 87)[a]
Gender	Female	10 female/14 male	42 female/45 male
EEG anomalies/seizures	Yes	24/24	73/87
Global developmental delay	Yes	24/24	87/87
Microcephaly	Yes	24/24	55/81
Multiple generalized capillary malformations	Yes	22/24	0/87
Infantile onset epilepsy	No	22/23	NA
Small for gestational age	No	12/19	0/87
Hypoplastic distal phalanges	Yes	16/22	NA
Feeding intolerance	Yes	9/9	NA
Abnormal brain imaging	No	19/21	NA
Sensorineural deafness	No	2/24	4/77
Duplicated collecting system and vesicoureteral reflux	No	1/24	NA
Optic nerve hypoplasia	No	3/24	NA
Hypothyroidism	No	3/24	0/87
Distinctive facial appearance	Yes	0/24	87/87
Hirschsprung disease	No	0/24	26/85

EEG, electroencephalography; NA, data not available.
[a] Data derived from reference [8]: Genet Med. 2018. 20(9): 965–975.

MWS after the discovery of a pathogenic nonsense mutation in *ZEB2*. It is worth noting that her epilepsy, neither infantile onset nor intractable, is different from typical MIC-CAP. An increased extra-axial space and diffuse cerebral atrophy were also not observed at the age of two years old. This rare case re-emphasized the importance of dual diagnoses in clinical practice and the importance of sufficient clinical characterization of a patient in order to accurately analyze her/his genetic data and to avoid missing diagnoses. Considering the role of both *STAMBP* and *ZEB2* in the normal development of the central nervous system, an interesting question will be to study the interaction between these two proteins and their effects on human brain development in the future.

In conclusion, this case report demonstrates a rare presentation of MIC-CAP in the pediatric population and enriches the variant spectrum of *STAMBP*. The bleeding tendency of corresponding organs caused by vascular malformations could be a serious potential risk and should be appropriately treated in clinical practice.

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Data availability statement

Most data generated or analyzed during this study are included in this published article. The raw sequencing data are not publicly available due to concerns regarding patient anonymity. Requests to access the datasets could be directed to the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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