Rare hypomagnesemia, seizures, and mental retardation in a 4-month-old patient caused by novel CNNM2 mutation Tyr189Cys: Genetic analysis and review

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Abstract

Background: Hypomagnesemia, seizures, and mental retardation (HSMR) syndrome is a rare genetic disease. Presently, only 24 cases have been reported and the clinical features of the disease are yet to be fully described, thereby making diagnosis challenging.

Methods: Trio-whole-exome sequencing was used for the patient and her parents, and the structure of the variant protein was analyzed by molecular dynamics. Finally, the characteristics of HSMR were summarized by reviewing the previous literature.

Results: The main disease manifestations in the patient were seizures, liver function damage, hypomagnesemia, atrial septal defect, and sinus arrhythmia. A novel mutation in CNNM2 (c.566A>G/p.Tyr189Cys) was identified by genetic detection. The parents were wild type, and the mutation was rated as pathogenic by American College of Medical Genetics and Genomics guidelines. Ab initio modeling and molecular dynamics simulation show that the mutation destroys the surrounding hydrogen bonds, which may reduce the local stability of the protein structure. In the previous literature, only 24 children with HSMR have been reported, mainly manifested as hypomagnesemia, mental retardation, seizures, and language and motor impairment.

Conclusion: We have reported the second case of HSMR in the Chinese population, which further expands the phenotypic spectrum of congenital heart disease and the variation spectrum of CNNM2.

Keywords
CHD, CNNM2, HSMR, hypomagnesemia, mental retardation, seizures
INTRODUCTION

The cystathionine-β-synthase (CBS)-pair domain divalent metal cation transport mediator (CNNM) family includes four proteins (CNNM1–4), which maintain Mg²⁺ homeostasis in vivo. These proteins are mainly expressed in the basolateral membrane of the loop of Henle and other tissues such as the brain, lung, liver, spleen, and heart (Chen et al., 2020; Li et al., 2021; Stuiver et al., 2011). CNNM2 heterozygous mutations lead to hypomagnesemia, seizures, and mental retardation syndrome (HSMR, OMIM#616418), first reported in 2011 (Stuiver et al., 2011). To date, only 24 cases of HSMR caused by CNNM2 mutations have been reported worldwide; therefore, the phenotypic characteristics of the syndrome are still unclear (Accogli et al., 2019; Arjona et al., 2014; Bamhraz et al., 2020; Franken, Müller, et al., 2021; García-Castaño et al., 2021; Stuiver et al., 2011). We have identified a novel heterozygous variant of CNNM2, c.566A>G/p.Tyr189Cys, resulting in hypomagnesemia, hypocalcemia, seizures, mental retardation, and other typical features of HSMR accompanied by congenital heart disease (CHD) and liver enzyme abnormalities in a Chinese child. This is the second report of an association between a CNNM2 variant and HSMR in the Chinese population. Revealing variant associations with the syndrome may lead to improved differential diagnoses.

MATERIALS AND METHODS

CASE PRESENTATION

The patient was a 4-month and 9-day-old female infant who was delivered naturally and had normal physical development. She experienced one episode of uninduced focal epilepsy at 4 months of age, during which she held her hands tightly. The upper limb tremor was relieved after several seconds. Biochemical examination indicated abnormal serum magnesium and calcium levels, alanine, and aspartate aminotransferases, suggesting aberrant liver function. A cardiac function examination revealed high levels of creatine kinase. While a Doppler ultrasound showed characteristics of CHD, including atrial septal defect (ASD), an electrocardiogram (ECG) suggested sinus arrhythmia (Table 1). Magnetic resonance imaging (MRI) and electroencephalography revealed no abnormalities. The parents were healthy and denied the marriage of close relatives. There was no family history of genetic disorders.

GENETIC TESTING AND VARIANT ANALYSIS (INCLUDING ETHICAL CONSIDERATIONS)

The work described in this case report has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (http://www.wma.net/e/policy/b3.htm). The Hospital Ethics Committee approved the study and publication of the report (PJ2020-08-21). After obtaining informed consent from the parents, the patient and parents underwent trio-whole-exome sequencing (trio-WES). Peripheral blood (3 ml, in an ethylenediaminetetraacetic acid-coated tube to prevent coagulation) was collected, and the white blood cell DNA was extracted as per the manufacturer’s instructions for the genomic extraction kit (CWBO, Beijing, China). After constructing the library, the sequence was captured using an Illumina Noveseq 6000 high-throughput sequencer (Illumina, San Diego, CA, USA). Normal population distribution databases, including dbSNP, ExAC, and 1000 Genomes, were screened for suspicious variants, and bioinformatic prediction analysis (SIFT, Polyphen2, and MutationTaster) was carried out. The amino acid sequencing homology was analyzed using Mega7.0, and their pathogenicity was evaluated based on the American College of Medical Genetics and Genomics (ACMG) guidelines.

MOLECULAR DYNAMICS (MD) SIMULATION

Using the QUARK ab initio calculation (1–430aa is ab initio construction, 430–823aa is the crystal structure, PDB Code, 6N7E) (QUARK, https://zhanglab.ccmb.med.umich.edu/QUARK/), a three-dimensional (3D) model of the CNNM2 protein was constructed. Using Modeller10.1 (https://salilab.org/modeller/) multi-template modeling, model_multi.py, and other scripts for multi-template modeling and 3D structure simulation, the tertiary structure of the full-length protein was obtained (Supporting Information 1). The entire simulation process included system construction, energy minimization, NVT and NPT ensemble simulation, and finished product simulation. Finally, the interaction between the variant and the surrounding residues was analyzed with Chimera 1.15 (http://www.cgl.ucsf.edu/chimera/).

RESULTS

GENETIC TESTING

A CNNM2 heterozygous variant (NM_017649: c.566A>G/p.Tyr189Cys) was detected in the patient but not in the parents or the normal population database. A search of the ClinVar database and Human Gene Mutation Database (HGMD) revealed the variant to be a novel CNNM2 mutation, not yet reported. The results of the SIFT, Polyphen2, and MutationTaster analyses were...
0.01 (damaging), 0.99 (probably damaging), and 0.99999 (disease-causing), respectively. The variant was rated as pathogenic (PS2 + PM1 + PM2 + PP2 + PP3) as per the ACMG guidelines (Richards et al., 2015). Homology analysis found Tyr189 to be highly conserved among the different species (Figure 1).

### 3.2 | MD results

The root mean square deviation (RMSD) results showed that the mutant and wild type were balanced at around 2000 ps, and their conformational overlap map suggested that the variant may not affect the secondary protein structure. However, further interaction analysis indicated that the wild-type Tyr189 formed a hydrogen bond with Asn83 and a water bridge with Glu637 and Ser205, whereas the Cys189 in the mutant had no polar interaction with the surrounding residues due to its small side chain. As a result, compared with Tyr189, Cys189 had weaker interactions with the surrounding residues (Figure 2).

### 4 | DISCUSSION

HSMR is a rare genetic disease characterized by hypomagnesemia, seizures, and intellectual disability due to renal Mg\(^{2+}\) consumption. In some cases, additional symptoms, such as obesity or autism spectrum disorder, have been reported (Arjona et al., 2014). To date, only 24

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**TABLE 1** Clinical characteristics of the patient with CNNM2 variant c.566A>G/p.Tyr189Cys

| Essential information                  | Patient | Reference values |
|----------------------------------------|---------|-----------------|
| Gender                                 | Female  | –               |
| Age of onset                           | 4 months| –               |
| Age of diagnosis                       | 6 months| –               |
| Seizure type                           | Focal   | –               |
| Motor development                      | Unstable vertical head | –         |
| Intellectual development               | Mild mental retardation | –         |
| Language development                   | No abnormality | –         |
| Biochemical examination                |         |                 |
| Serum K\(^{+}\) (mmol/L)               | 4.59    | 3.50–5.30       |
| Serum Na\(^{+}\) (mmol/L)              | 138.59  | 137–147.00      |
| Serum Mg\(^{2+}\) (mmol/L)             | 0.62\(\downarrow\) | 0.75–1.02     |
| Serum Ca\(^{2+}\) (mmol/L)             | 1.92\(\downarrow\) | 2.11–2.52     |
| Serum P (mmol/L)                       | 1.88\(\uparrow\) | 0.85–1.51     |
| Urea (mmol/L)                          | 1.65\(\downarrow\) | 3.10–8.00     |
| Glucose (mmol/L)                       | 5.99    | 3.89–6.11       |
| Alanine transaminase (U/L)             | 99.50\(\uparrow\) | 9.00–50.00    |
| Aspartate aminotransferase (U/L)       | 138.5\(\uparrow\) | 15.0–40.0     |
| Alkaline phosphatase (U/L)             | 201.7\(\uparrow\) | 45.0–125.0    |
| Creatine kinase isoenzyme MB (U/L)     | 56\(\uparrow\) | 0–24          |
| Creatine kinase (U/L)                  | 197     | 50–310          |
| Lactate dehydrogenase (U/L)            | 471.00\(\uparrow\) | 120.00–250.00 |
| Lactic acid (mmol/L)                   | 7.8\(\uparrow\) | 0.4–1.8        |
| Ceruloplasmin (g/L)                    | 0.19\(\downarrow\) | 0.30–0.65     |
| Imaging examination                    |         |                 |
| EEG                                    | No abnormality | –         |
| Head MRI                               | No abnormality | –         |
| Echocardiography                       | Atrial septal defect | –         |
| ECG                                    | Sinus arrhythmia, P–P interval difference > 0.12 s | –         |

Abbreviations: ECG, electrocardiogram; EEG, electroencephalogram; MRI, magnetic resonance imaging.
HSMR cases have been reported worldwide and the characteristic clinical spectrum has not been well described, making its differential diagnosis challenging (Accogli et al., 2019; Arjona et al., 2014; Bamhraz et al., 2021; Franken, Müller, et al., 2021; García-Castaño et al., 2020; Stuiver et al., 2011). We retrospectively analyzed the reported cases and found that in addition to the HSMR triad, language retardation, motor development disorder, and obesity also occur in the high incidence phenotypes of the syndrome. A few patients will also have ASD, microcephaly, and hypocalciuria (Figure 3a). The infant in this study had typical HSMR features, such as seizures, hypomagnesemia, and mild intellectual impairment at 4 months of age. It is worth noting that the infant also showed evidence of CHD with elevated myocardial enzymes and atrial septal defect. ECG showed sinus arrhythmia; however, few previously reported HSMR cases have shown congenital heart disease symptoms. Accogli et al. (2019) reported that a fourth male newborn in a family member of an HSMR patient showed severe phenotypes, including severe cardiac structural abnormalities, ECG abnormalities, and craniofacial malformations, and died of cardiogenic shock on the third day after birth. Arrhythmia in HSMR patients may be caused by early afterdepolarization. Since Mg\(^{2+}\) affects myocardial contraction mainly by antagonizing Ca\(^{2+}\) regulation, hypomagnesemia can increase the risk of arrhythmia (Tangvoraphonkchai & Davenport, 2018). Although aberrant CNNM2 activity generally leads to hypomagnesemia, reports of arrhythmia are rare, which could be due to the lack of relevant cohort studies and case reports. In addition, a direct correlation between cardiac structural abnormalities and CNNM2 function is not yet clear. However, in a mouse model of magnesium deficiency, various cardiovascular structural abnormalities, including aortic wall thinning, tissue disorder, elastic fiber rupture, and collagen abnormality, were observed. This collection of symptoms may be related to increased matrix metalloproteinase (MMP)-2 and MMP-9 activities.
Therefore, **CNNM2** defects may indirectly affect the cardiovascular system.

The majority of **CNNM2** mutations leading to HSMR are de novo mutations, followed by dominant inheritance, and a few patients exist with recessive inheritance. Additionally, in previously reported cases, **CNNM2** variations tend to be missense mutations. Other types, such as nonsense mutations, frameshift mutations, and copy number variations, have also been reported (Figure 3a).

The severity of the HSMR phenotype may be related to the mutation site and the genetic pattern of the **CNNM2** gene mutation. If the pathogenic mutation occurs at a key site and is homozygous, it may have a more serious nervous system phenotype. **CNNM2** is located on chromosome 10q24.32 and encodes a protein of 875 amino acids, which features an N-terminal extracellular domain, four transmembrane α-helix transmembrane domains, and the cyclic nucleotide-binding homology (CNBH) domain (Chen et al., 2020). There is a glycosylation site (Asn112) in the N-terminal extracellular domain, which can recruit signal recognition particles to perform the signal peptide cleavage process and plays a role in key functions such as membrane stability and protein localization (de Baaij et al., 2012; Vagin et al., 2009). A mutation near the locus can result in severe nervous system-related phenotypes, such as intractable epilepsy, microcephaly, severe intellectual impairment, and abnormal brain MRI (Arjona et al., 2014). Additionally, CBS-pair dimerization (CBS1, CBS2) mediated by the CNBH domain has important biological functions. This domain significantly affects the physiological role of Mg\(^{2+}\) efflux (Hardy et al., 2019). Patients with **CNNM2** mutations affecting the CBS2 structure can also have severe nervous system-related phenotypes such as refractory epilepsy, severe intellectual impairment, and even death (Accogli et al., 2019) (Figure 3b).

The novel mutation (c.566A>G/p.Tyr189Cys) in this case appears in the N-terminal extracellular domain of the **CNNM2** protein. Through ab initio modeling and molecular dynamic simulation, we determined that the mutation may not affect the secondary structure of the protein. However, the ectopic point destroys the polar interaction with the surrounding residues, thus reducing the local stability.
bond of the surrounding residues, which may affect the local stability of the N-terminal structure.

The mechanism by which the loss of function of CNNM2 leads to abnormal Mg\(^{2+}\) metabolism and affects the function of the nervous system remains controversial, but studies have shown that the gene is located in the midbrain–hindbrain boundary, which can affect the development and differentiation of the central nervous system (CNS). Therefore, the CNNM2 protein function is very important for the development of CNS constituent regions (Arjona et al., 2014; Franken, Seker, et al., 2021). In the study of model organisms, both Cnnm2\(^{-/-}\) mouse and zebrafish models showed the absence of lethal brain structure in the early stage of the embryo. For example, around 36% of Cnnm2\(^{-/-}\) mouse embryos had anencephaly, showing that neural tube development defects led to a brain phenotype (Franken, Seker, et al., 2021). In the phenotype of an electrolyte disorder, the Cnnm2\(^{+/+}\)/\(^{-/-}\) mouse model is consistent with the observations of the human phenotype. Both Cnnm2\(^{+/+}\)/\(^{-/-}\) mouse models show that the level of Mg\(^{2+}\) in hemorrhage is reduced; however, the excretion of Mg\(^{2+}\) in the urine is not significantly increased. Mice exposed to a low-magnesium diet may even show a reduction of Mg\(^{2+}\) excretion in the urine, which suggests the ability to compensate for Mg\(^{2+}\) reabsorption. However,
5 | CONCLUSION

In conclusion, we performed a genetic analysis and provided a clinical case description of a Chinese infant with HSMR through trio-WES. The infant showed epilepsy and mild intellectual impairment without inducement. Biochemical examination showed electrolyte disorders such as hypomagnesemia and hypocalcemia. In addition, the infant had rare HSMR phenotypes such as abnormal myocardial enzymes caused by CHD and abnormal liver enzymes caused by impaired liver function. However, there are still some limitations to this study. For example, there is a lack of long-term follow-up records and in-depth analysis of treatment for HSMR patients. In conclusion, this report enriches the knowledge of the phenotypic and variation spectrum of HSMR. Clinicians should consider the possibility of CNNM2 mutations in infants with hypomagnesemia, seizures, mental retardation, and CHD symptoms. The early identification of HSMR is of positive significance for clinical hierarchical management of the disease.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of the First Affiliated Hospital of Anhui Medical University (PJ2020-08-21). Written informed consent was provided by the participant.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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