Abstract. Angelman syndrome (AS) is an autosomal dominant neurodevelopmental genetic disease with maternal imprint, which is associated with the presence of the abnormal chromosome 15q11-q13, and the loss of maternal specific expression of ubiquitin-protein ligase E3A (UBE3A). The expression levels of UBE3A depend on the parental origin and exhibit tissue specificity. In normal brain tissues, the maternal UBE3A gene is actively expressed, whereas the paternal UBE3A gene is not. In total, ~85% of pediatric patients with AS present with epilepsy within their 3rd year of life. This condition is usually difficult to control with medical treatment. An 8-year-old female visited the Affiliated Hospital of Jining Medical University due to frequent epilepsy. Her clinical manifestations included specific facial features, moderate mental retardation and frequent seizures. It was interesting to note that her 15-year-old sister exhibited similar clinical manifestations to those of AS. The results of the electroencephalogram and the imaging examinations were also in line with the characteristics of AS. In order to further clarify the diagnosis, all the suspected genes in her sister and in their parents were sequenced. The multiplex ligation-dependent probe amplification project of the Angel/chubby and copy number variation (CNV) sequencing were assessed concomitantly to identify the pathogenic genes responsible for the development of AS. The latter occurs due to the missense mutation c.1146T>G, which results in asparagine replacement by lysine at position 382 (p.Asn382Lys) in exon 7. This amino acid change affects the normal expression of UBE3A; the mutation is a novel mutation, which, to the best of our knowledge, has not been previously reported. Relevant large fragments of mutations and methylation abnormalities were not found in the associated genes. The data further revealed absence of 25-bp repeat mutations at the shear mutation site of exon 1 of the small nuclear ribonucleoprotein polypeptide N gene in the subjects examined. No suspected CNV was found following analysis.

Introduction

Among the various genetic metabolic disorders, Angelman syndrome (AS) has attracted considerable attention due to the abnormal expression of the ubiquitin-protein ligase E3A (UBE3A) gene (1). In 1965, the British Doctor Harry Angelman first described AS and named it after his surname. The frequency of this condition is estimated to be 1 in 15,000 individuals (2). AS is a maternally inherited neurodevelopmental genetic disease associated with chromosomal abnormality at the 15q11-q13 genetic region (3). The loss of the expression of the maternal allele of the UBE3A gene is typically associated with the four following mechanisms: Deletion at the 15q11.2-q13 locus, UBE3A functional loss variation, presence of paternal parthenogenetic double chromosome or genomic imprinting defect (4).

The various characteristics of AS are primarily caused by maternal allele dysfunction of the UBE3A gene and paternal imprinting (5). UBE3A is the only gene in the 15q11-q13 region that indicates biased expression from the maternal allele (6). The expression levels of these genes are tissue-specific and depend on the origin of the parent. In normal brain tissues, the maternally inherited UBE3A allele is actively expressed (7), while the paternally inherited UBE3A gene is not. The UBE3A gene is located on the 15q11.2-q13 locus, UBE3A functional loss variation, presence of paternal parthenogenetic double chromosome or genomic imprinting defect (4).
function of UBE3A (including neuronal excitability) may be associated with the balance of the mTOR signals in the developing neurons (10). Although AS presents with typical characteristics, genetic diagnosis may be hindered by the change in disease presentation and the presence of different molecular mechanisms. Therefore, ~10-15% of patients, which are suspected to have AS, are not diagnosed at an early stage of the disease.

In the present study, two sisters of a Chinese family with clinical features and genetic variations of AS were tested. The electroencephalograms (EEGs) of the children in this family were assessed in combination with the expression of the associated genes in the sisters in order to fully understand AS and provide additional evidence for its diagnosis.

Materials and methods

Patient consent and approval. This study was approved by the Ethics Committee of Affiliated Hospital of Jining Medical College (Jining, China). The parents of the patients provided written informed consent for their participation. The parents of the children provided written informed consent for publication of the data and images.

Genetic screening and sample collection. A total of 5 ml peripheral blood was collected from both the proband and her sister. A total of 2 ml peripheral blood was collected from their parents and their grandmother. The blood samples were sent to Beijing Kangso Medical Inspection for panel testing (a total of 324 genes). Total exon screening was performed for the two sisters and copy number variation (CNV) sequencing and multiplex ligation-dependent probe amplification testing for the proband.

Genomic DNA extraction. The Qiagen FlexiGene DNA kit (Qiagen GmbH) was used to extract genomic DNA from blood samples according to the procedure described by the manufacturer.

DNA library construction. To construct the DNA library, genomic DNA samples were fragmented into 150-300 bp DNA fragments using an ultrasonic processor. The adaptors used for both ends of these DNA fragments were ligated and the cohesive ends of the DNA fragments were trimmed. Subsequently, the DNA library was amplified using PrimeStar HS DNA Polymerase with the following thermocycling conditions: Initial denaturation at 95˚C for 10 min, followed by 6 cycles of 30 sec at 95˚C, 30 sec at 60˚C and 45 sec at 72˚C. A final extension was performed at 72˚C for 5 min. The primer sequences used were as follows: Forward primer, 5’-TGT CAG CTC GCT GAA AAC TG-3’ and reverse primer, 5’-TTG CAG CCCCAAG GAAA ACTG-3’. The PCR products were purified using a nucleic acid purification kit according to the manufacturer’s protocol.

Hybrid capture. The target DNA fragments from the amplified DNA library were hybridized and captured by specific probes. Subsequently, the fragments were amplified using the SureSelect target enrichment system according to the manufacturer's protocol (Agilent Technologies, Inc.). Finally, a customized gene panel for inherited metabolic diseases was designed, which consisted of a total of 324 genes. The DNA fragments were hybridized, isolated and then amplified using the SureSelect Target Enrichment System (Herculase ii Fusion enzyme dNTP combo; Agilent Technologies, Inc.) with the following thermocycling conditions: Initial denaturation at 98˚C for 2 min; followed by 15 cycles of 30 sec at 98˚C, 30 sec at 62˚C and 1 min at 72˚C; with a final extension at 72˚C for 10 min.

Sequencing. Single-read sequencing was performed by NextSeq500 (Illumina, Inc.). Raw data were obtained in the FastQ format.

Data analysis. The raw data were transformed into identifiable base sequences using CASAVA (version 1.8.2; Illumina, inc.). Sequences were aligned to Grch37 (as known as hg19) using Burrow-Wheeler aligner version 0.7.15-r1140, and single nucleotide and deletion/insertion polymorphisms analyses were performed to obtain mutation information within the targeted regions using GaTK version 3.6. Finally, protein damage analysis was performed to qualitatively predict the probability of the results using PolyPhen2 (Version 2; http://genetics.bwh.harvard.edu/pph2/). The mutation sites, which were obtained, were further validated.

First-generation sequencing verification. The gene sequences of the aforementioned mutation sites were obtained from GenBank. The primers were designed by the website Primer Z (http://genepipe.ncgm.sinica.edu.tw/primerz/primerz4.do) and subsequently synthesized. The mutation sites were amplified by PCR and sequenced using first-generation sequencing by Kangso Medical Inspection (Beijing, Chain). The obtained sequences were aligned with the previous results and the false positive sites obtained by next generation sequencing were ruled out.

Bioinformatics analysis. To investigate the effects of the detected variants, bioinformatics analyses were performed. RaptorX (http://raptorx.uchicago.edu) (11) can predict protein tertiary structures (5). Following sequence input, the 3D structure of the protein sequence was predicted from the protein database (Fig. 1). The patient's UBE3A gene did not fold completely in its spatial structure compared with the wild-type gene, thus affecting its protein function.

Case report

The present study investigated a Chinese family with five members (Fig. 2). The second child in the family was a proband, an 8-year-old female who was delivered via cesarean section at full-term. The proband revealed no apparent abnormalities at birth, no perinatal problems or hypotonia, and no apparent abnormality in prenatal examination. Initially, no apparent difference was noted in diet and sleep compared with those of healthy infants. The abnormality was initially discovered when the child was 7-8 months old. She could not sit alone and could not interact with her parents. The child was admitted to the Children's Health and Genetics Clinic of the Affiliated Hospital of Jining Medical University and was initially...
diagnosed with ‘stunting’. Due to the similar medical history of her sister, she did not receive the corresponding examination and treatment. The child exhibited her first epileptic seizure at the age of 15 months in the absence of fever. The seizure condition was unknown and the pediatric patient was treated in the Epilepsy Clinic of Affiliated Hospital of Jining Medical University with no apparent abnormality in routine EEG. The dynamic EEG indicated no epileptic discharge compared with the EEG obtained when the patient was 16 months and awake. When the patient was asleep, a medium-high amplitude peak wave and a steeple slow coincidence wave were noted in the EEG. The central, right parietal, frontal, left middle temporal and posterior temporal regions were the primary regions that were monitored. Craniocerebral MRI indicated that the anterior longitudinal cisterna and the bilateral frontotemporal extracephalic space were widened. At that time, the patient was administered oral sodium valproate. The pediatric patient was on sodium valproate treatment for >2 years and her seizures did not show significant improvements. Therefore, the family members of the child terminated drug administration.

When the child was 5 years old, she suddenly exhibited frequent epileptic seizures, including limb paralysis, falling to the ground and shaking of the limbs. These conditions were relieved after a few seconds. The number of seizures increased from 3-5 times a day to >10 times a day, and shock and stimulation were prone to occur. When the frequency of the epileptic attacks increased, the child was unable to walk alone (the child could walk alone prior to the onset of the disease) and was admitted to Peking University First Hospital. At that time, the child suffered from poor sleep quality, could not take care of herself during defecation or urination, and walked unsteadily. Specialist examination showed intelligence and reaction ability were low. The child would not take the initiative to speak and pronounced unconsciously the words ‘mama’ and ‘babar’, could understand simple sentences and exhibited serious salivation and no mouth angle skew. The patient also presented with binocular strabismus, coarse test vision and normal hearing. The pediatric patient demonstrated the ability to chase an object and had thick upper limbs, mobility, and a physical examination revealed a lack of cooperation. The long-range video EEG detection report indicated that during waking, the full conduction medium, high radiation frequency (2.5-3.0 Hz rate) and slow composite wave burst were observed. The normal background was restored for ~5 sec with left and right synchronization. The high and slow complex wave distributions were occasionally noted in the left frontal, central, parietal regions and the left middle-posterior temporal regions. At the onset stage, the spine rhythm was observed in the bilateral frontal pole and anterior temporal region at the beginning of the onset. The spine rhythm was mixed with movement and electromyogenic pseudo difference in the middle and later stages of the onset, which lasted for ~15 sec. During the hospitalization, the patient was treated with nerve pulse fusion therapy and transcranial magnetic stimulation therapy, which demonstrated aggravation rather than apparent improvement of the condition. In view of the frequent seizures, intramuscular injection of diazepam was provided. However, the seizure intensity nor frequency were not reduced compared with before diazepam administration. The family refused to continue the treatment. Oral administration of levetiracetam and topiramate was provided, which is not a regular regimen.

The facial features of the children exhibited slightly wider eye spacing, lack of downward sloping eye fissure, large mouth, thin upper lip, wide mouth, small teeth and wide spacing, occasionally protruding tongue, serious salivation, microcephaly and normal skin and hair color. The behaviors of the proband included the following: Frequent laughing, cheerful mood, ease of excitement, playing with water, looking at the phone, tearing paper and being able to identify the WeChat application on their father's phone. The patient was not obtaining
sufficient sleep. Moderate mental retardation was noted, which resulted in spelling of the words ‘mom’ and ‘dad’ in an unconscious way. The female pediatric patient could not speak >2 words, but could understand simple commands and sentences. She also used gestures to express her intentions. The upper limb muscle tension was normal, whereas lower limb muscle tension was decreased. Solitary sitting was stable and the patient could walk several steps alone, without pointing her feet. In addition, she failed to walk actively and her upper arm was flexed when descending, resulting in a slightly disordered gait. She was uncooperative upon physical examination and had epileptic seizures ≥10 times a day. Following the induction of shock stimulation, the patient experienced seizures during which her limbs were shaking and her eyes rolled up. This state occurred for no apparent reason and lasted >20 sec prior to returning to their original condition.

The 15-year-old sister of the proband exhibited similar clinical manifestations with those of the proband. She had a natural birth at term, with no apparent abnormalities at birth and no associated perinatal problems. She demonstrated similar developmental delays to those of her sister. The initial report of her condition was at the age of 8 months, when she was found to sit backwards. At that time, she was admitted to the pediatric health clinic of Affiliated Hospital of Jining Medical University and was preliminarily diagnosed as a patient experiencing ‘developmental delays’. The seizures initially occurred at the age of 1.5 years in the absence of fever. The specific onset was unknown. The failure to capture an informative EEG and the insufficient diagnostic evidence resulted in the lack of the diagnosis of epilepsy. Therefore, the patient was not treated with the corresponding treatment required for this condition. At the age of 2, the patient underwent the electrical evoked potential test. The brainstem auditory evoked potential test indicated lack of apparent abnormality. The detection of somatosensory-evoked potential indicated bilateral cortical potential abnormalities. A medical practitioner examined the patient when she was 3 years old in Affiliated Hospital of Jining Medical University due to mental and motor retardation symptoms. At that time, she could not stand or walk alone and referred to her mother and father unconsciously. She was able to walk alone for >10 days when she was 30 months old. However, she could not walk alone after falling to the ground due to epilepsy. In addition, she could not control her urination and her sleep was normal. The muscle strength of both lower limbs was low, resulting in an inability to stand and walk. The results of the intelligence test indicated that the speech intelligence quality was 54 (based on the parent’s account as there was no report card), suggesting moderate mental retardation. At the age of 5, EEG data indicated that a large number of multi-focal low-high amplitude spinous waves and slow spinous waves were emitted, which were clustered or continuously distributed in the occipital and temporal regions in each sleeping stage. The following patterns were observed: Multi-wide, high to very high amplitude, irregular slow-spined wave, slow wave inclusions and short-range spined waves. The patient exhibited increased EEG discharge following examination when she was awake, asleep and with her eyes closed (interonset). No significant improvement was noted following oral administration of valproate for >2 years resulting in the termination of the drug treatment by the patient’s family members. At the age of 8, a gene deletion was noted at the chromosomal location 15q11-13, which was methylated. No gene copy number changes or methylation abnormalities were detected at the 15q11-13 region of the tested
Figure 5. Electroencephalogram of proband: (A) More frequent full lead 2.5-3.5 Hz sharp slow wave or slow wave rhythmic release, sometimes 4-6 Hz after the head rhythm, (B) Generalized tonic-clonic seizure, (C) Atypical absence seizure.
samples. The facial features of the patient included slightly wider eye spacing, lack of downward sloping palpebral fissure, large mouth, thin upper lip, wide mouth, normal tooth spacing with protruding tongue, substantial salivation and microcephaly. No apparent abnormalities were noted in the skin and hair color of the patient. Her behavioral expression included happy behavior and excitable mood, which were accompanied by watching TV and tearing papers. Her sleep time was reduced. Moderate mental retardation was also noted and the patient unconsciously pronounced the words 'dad', 'mom', 'grandpa' and 'grandma'. Furthermore, she was only able to understand simple commands and sentences, and used gestures to express her wishes. The upper limb muscle strength was normal and the lower limb muscle tone was decreased. The patient could sit alone but could not walk actively, which resulted in a disordered gait. She could stand and walk with support and flex the upper arm upon descending. She was uncooperative upon physical examination. The epileptic seizures occurred at a frequency of >10 times a day and following their stimulation loss of consciousness was observed, which was confirmed by visual observation of the eyes, mouth salivation, extended tongue and limb shaking. The attack was relieved within 5-10 sec.

Figure 6. Electroencephalogram of the proband's sister. In the bilateral frontal region, a 2.5-3.5 Hz medium and high amplitude cusp slow composite wave or slow wave rhythmic release was more frequently observed.

Figure 7. Electroencephalogram of the proband's sister Ankylosing-atypical absence seizures complicated with Myoclonic seizures.
The epileptic seizures of the elder sister were slightly less severe compared with that of the proband. The latter was an introvert, and the stress associated with contact with a stranger could trigger an epileptic attack. By contrast, the patient's sister had an extrovert personality. The two sisters exhibited unexplained seizures and stimulation of coexisting seizures in combination with pathological EEG manifestations. However, the EEG of the sister, which provided the diagnostic data, appeared subsequently.

Since the two sisters did not receive medical treatment, they did not have regular follow-ups, which resulted in poor control of the epileptic onset, and this seriously affected their quality of life. The proband could walk several steps alone and exhibited mild ataxia. Her sister could not walk alone and exhibited an abnormal gait when her walk was assisted.

In September 2020, both of the sisters underwent positron emission computed tomography (PET) and video-EEG in the brain. Due to the uncooperative nature of the sister of the proband, only the proband underwent cranial MRI.

The cerebral MRI (Fig. 3) results of the 8-year-old proband were combined with the PET/CT findings (Fig. 4) of the proband's 15-year-old sister. The results indicated lack of apparent abnormalities in the occipital lobe.

The EEG results of the proband suggested an abnormal EEG for a child of that age. The full conduction was more frequent when a sharp slow wave was noted with a frequency range of 2.5-3.5 Hz, or during the rhythmical release of the slow wave. Occasionally, the posterior head exhibited a 4-6 Hz rhythm and was accompanied by a multi-focal sharp wave (apparent in the left and right occipital areas) and comprehensive tonic-clonic and atypical absence seizure patterns (Fig. 5). The EEG results of the proband's sister suggested abnormal EEG in the pediatric patients examined. The EEG activity was mainly distributed in the bilateral frontal region with a frequency of 2.5-3.5 Hz and was associated with a high amplitude cusp slow composite wave or slow wave rhythmic release, which occurred more frequently (Fig. 6). A sharper slow wave emission was noted in the occipital region. A limited number of slow sharp waves with a frequency of 4 Hz were noted in the right posterior temporal region. Several ankylosing-atypical absence seizures were noted, which were accompanied with myoclonic seizures (Fig. 7). The sleep cycle of the proband's sister was disturbed and therefore she did not sleep during video EEG.

The UBE3A gene of the proband and his sister was found to possess a C.1146T>G (p.Asn382Lys) mutation. This mutation has not been previously reported in the HGMD Pro, PubMed and Clinvar databases, and, to the best of our knowledge, had not been previously reported in the literature. The Exome Aggregation Consortium, ESP6500, 1000 Genomes, 1000 Genomes and 1000 Genomes databases were not included. SIFT (Version 2; https://sift.bii.a-star.edu.sg/), Polyphen2 (Version 2; http://genetics.bwh.harvard.edu/pph2/) and MutationTaster software were used to predict the protein damage and the results were classified as follows: Harmful, benign and possibly harmful. The mutation was inherited from the mother as determined by family source verification. The missense mutation resulted in a lysine residue replacing an asparagine residue at position 382 (p.Asn382Lys). Genomic coordinates, chr15:25616184.

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exon 1 of the small nuclear ribonucleoprotein polypeptide N gene in the subjects examined. The suspected CNV was not found following analysis. To determine the pathogenicity of the locus, the proband's grandmother was tested by genetic analysis and she was found to be free of the mutation (Fig. 8). The discovery of the UBE3A mutation was consistent with the clinical manifestations of the patients, the diagnosis of AS and its familial transmission.

Discussion

AS is an autosomal dominant genetic disease secondary to maternal imprinting, which is primarily characterized by dysfunction of the maternal allele of the UBE3A gene (5). UBE3A encodes the E3 ubiquitin ligase, and its deletion or replication leads to various neurodevelopmental disorders. However, how changes in the copy number of ubiquitin ligase genes affect brain development remains unclear (10). UBE3A has six functional domains as follows: The homologous to the E6-AP carboxyl terminus domain (12), the E6 binding domain, the p53 binding domain and three nuclear receptor interaction and activation domains (13). The expression of UBE3A is widespread in various tissues, including the human fetal brain and adult frontal cortex. The UBE3A gene is primarily expressed by maternal imprinting, but not by paternal methylation (10). UBE3A is the only gene that demonstrates partial expression from maternal alleles in the 15q11-q13 region (6). The UBE3A gene encodes two proteins with known functions, of which one is an E6-AP ubiquitin-protein ligase, and this catalyzes the combination of ubiquitin and substrate proteins, which are indispensable in the process of protein catabolism. The other protein is a steroid hormone receptor coactivator (1). The synthetic function of UBE3A (including neuronal excitability) may be associated with the balance of mTOR signaling in the developing neurons (10). Although the cellular functions of UBE3A are incompletely understood, it is known that it plays a role in neurodevelopmental disorders (1).

The novel heterozygous mutation C.1146T>G (p.Asn382Lys) of the UBE3A gene found in this family was considered the cause of the phenotype of these two children with AS. The missense mutation C.1146T>G resulted in asparagine replacement by lysine at position 382 (p.Asn382Lys), which affected the normal expression of UBE3A (Figs. 8 and 9) and reduced ubiquitin ligase synthesis. The mother of the patient carried the same mutation, but did not exhibit the symptoms of AS. Following verification of the mother's pedigree, this locus was not inherited from the grandmother of the patient. Since the grandfather of the proband had passed away, it was impossible to assess whether this locus was inherited from the grandfather of the proband or whether it originated as a novel mutation in the mother. It could only be assessed by referral to the clinical evidence of the proband. Since the locus of the proband was inherited from the mother, the pathogenic characteristics of AS were caused by to the UBE3A gene mutation. The clinical manifestations of the 2 patients reported were highly consistent with AS, suggesting that the comprehensively inferred locus was the pathogenic cause of the proband and her sister. Since a heterozygous mutation of UBE3A was identified by genetic testing of the proband's mother, it was considered that the two mutations were inherited by the sisters via maternal imprinting.

The imaging results of the two sisters indicated a slightly blurred and increased signal intensity of periventricular white matter on T2 weighted and FLAIR image sequences (14). The data were consistent with the results from Harding et al (15) and Castro-Gago et al (16), suggesting that certain infants with AS may exhibit delayed myelination. However, the imaging findings do not usually exhibit substantial specificity, suggesting that this type of imaging examination may not be sufficient for diagnosis of AS.

In contrast to these findings, video EEG examination of children without sedatives usually produce more reliable data, which can be used to establish a more accurate clinical suspicion index prior to the final molecular biology-based diagnosis (14).

It has been previously reported that 80% of patients with AS may demonstrate characteristic EEG seizures and epileptic discharges with notch δ and rhythmic θ activity (14). The seizures usually begin prior to the age of 3 and last until adulthood (15). The EEG results of the two siblings were consistent with the EEG results noted in the literature (16).

AS is characterized by severe stunting and dyskinesia, including ataxia and motor spasms (17). Noticeable behavioral activities include a cheery manner, excitement, frequent smiling, unexplained laughter, and no or limited use of words (18). Other common features of AS include dystonia, tongue protrusion, an abnormal sleep-wake cycle, decreased sleep demand and salivation (19).

The clinical manifestations of the pediatric patients with the 15q11-q13 mutation are similar to those of the clinical phenotype of patients with large deletions in the chromosomal 15q11.2 region, and to those with single diploid and imprint deletion. The clinical phenotype of the former includes severe mental retardation, early epilepsy, microcephaly and severe ataxia, whereas the clinical phenotype of the latter is less severe and is characterized by a lower incidence of epilepsy, microcephaly and special facial deformity, light ataxia and optimal cognitive ability (3).

In a study conducted by Valente et al (20) it was reported that sodium valproic acid could improve the seizures of 19 patients who received monotherapy or multidrug therapy, notably when combined with clonazepam or phenobarbital. In the present study, the seizures of the two sisters were not significantly controlled following treatment with sodium valproic acid. Moreover, the proband had taken levetiracetam combined with topiramate, but this did not control the seizures. At present, lamotrigine combined with sodium valproate is used for the treatment of seizures and this has resulted in a slight reduction in their frequency. However, this effect may not be directly observed.

In conclusion, studies have shown that UBE3A is a multifunctional protein, which has important nuclear and cytoplasmic regulatory functions, and affects proteasome function, Wnt signaling, circadian rhythm, imprinted gene network and chromatin. The synthetic function of UBE3A interacts with light GABA mTOR signals, which is the most critical signal amongst GABAergic neurons (10). The two pediatric patients examined in the present study exhibited AS and carried novel UBE3A mutations that had not been previously reported.
Moreover, the identification of specific pathogenic genes can aid genetic counseling and prenatal testing for families and may be used for early diagnosis of AS in pediatrics. The latter process can improve the control of epilepsy in children and reduce the incidence of brain injury. These pediatric patients must receive specialized education at an early stage in order to improve their quality of life.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors’ contributions

QXK and QBL designed the study. RHL, GFS, LY, QLZ, SYW, and CL collected the clinical data. CL analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript. QXK, QBL, GFS and RHL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Affiliated Hospital of Jining Medical College (Jining, China). The parents of the patients provided written informed consent for their participation.

Patient consent for publication

The parents of the children provided written informed consent for publication of the data and images.

Competing interests

The authors declare that they have no competing interests.

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