Case Study and Literature Review of Phelan-McDermid Syndrome Caused By a Pathogenic Mutation in The SHANK3 Gene

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Abstract

Background: Phelan-McDermid syndrome is a rare genetic disorder resulting from heterozygous deletion of 22q13.3 with the involvement of at least part of *SHANK3* or a heterozygous pathogenic variant in *SHANK3*. We would like to explore the possible pathogenesis and therapeutic direction of PMS.

Methods: We identified a child who harbored a *de novo* *SHANK3* gene mutation and had a speech delay and mental retardation. This pathogenic mutation (c.3679dup, p.A1227Gfs*69) may lead to early termination of protein transcription and is speculated to cause changes in protein function. The gene expression profiles of family members were evaluated by RT-PCR, which showed that *SHANK3* expression was significantly lower in the child than in the other family members. The sites of pathogenic and likely pathogenic mutations in the *SHANK3* gene reported by ClinVar were searched, and the mutation sites were summarized and marked.

Results: The relevant literature was reviewed to understand the current state of research progress and possible future treatment directions. Researchers have found that CDPPB is a potent and selective positive allosteric modulator of mGluR5. In addition, it is important to further study whether NMD is involved in the expression of *SHANK3* and to further investigate PTCs.

Conclusions: Genetic testing is essential for children with development delay. It's necessary to do further research about the therapeutic direction.

Background

Phelan-McDermid syndrome (PMS, OMIM 606232), also called 22q13.3 deletion syndrome, is a rare genetic disorder resulting from heterozygous deletion of 22q13.3 with the involvement of at least part of *SHANK3* or a heterozygous pathogenic variant in *SHANK3*. PMS is characterized by neonatal hypotonia, speech delay, developmental delay, and mildly dysmorphic facial features^[1–4]. Most patients have moderate to profound mental retardation. Other features include large fleshy hands, dysplastic toenails, and decreased perspiration that results in a tendency to overheat. Normal stature and normal head size distinguish patients with PMS from those with other autosomal chromosome disorders. Behavioral characteristics include mouthing or chewing of nonfood items, decreased perception of pain, and the presence of autism spectrum disorder (ASD) or autistic features.

The *SHANK3* gene (also called *ProSAP2*) is located on chromosome 22q13.3 and encodes scaffolding proteins localized at the postsynaptic density (PSD) of nearly all excitatory glutamatergic synapses. Shank is among the most abundant binding partners of Homer in the PSD^[5]. The synapse is the key structure mediating the transmission of signals and/or neurotransmitters between nerve cells and forms an important structural basis for whole-brain learning, memory, and cognition. The PSD is a specialized electron-dense structure underneath the postsynaptic plasma membrane of excitatory synapses and consists of neurotransmitter receptors, signaling molecules, and scaffolding proteins. The PSD has been shown to play a role in synaptic function and structure. Genotype analysis showed that the size and

number of *SHANK3* gene deletions were positively correlated with the severity of clinical symptoms [6–10]. The *SHANK3* gene spans 58 kb and contains 22 exons. The protein product translated from the main mRNA isoform of *SHANK3* contains 1730 amino acids.

Here, we first generally summarize the clinical characteristics and gene sequencing results of a patient with PMS caused by *SHANK3* gene mutation and evaluate gene expression to understand the effect of this gene mutation on *SHANK3* expression. Second, we summarize the characteristics of discovered pathogenic and likely pathogenic mutations in the *SHANK3* gene, including the currently known mutation types and positions. Finally, we discuss the possible pathological mechanisms of these mutations and possible treatments for PMS.

Case Summary

A 4-year-old boy who had a speech delay was brought to our clinic on 7/12/2019. He had a severe speech delay and mild intellectual impairment. He had no dysmorphic facial features. He could understand simple language, could execute some instructions, and produced a copious amount of saliva. His performance on intelligence tests lagged behind that of his peers, and his intellectual age was equivalent to approximately 2 years. He had normal hearing and enjoyed playing with his sister. His birth history was G2P2; he was delivered at term with a birth weight of 4500 grams. There was no history of suffocation at birth or problems during the pregnancy. The parents were not close relatives and had no family history of genetic disease. There was no history of seizures. The patient's development history was as follows: could sit alone at 7 months old, could stand at 9–10 months old, and could walk by himself at 14 months old. At presentation, his fine motor skills, such as pitching, were not very good. His weight was 16 kg, his height was 104 cm, and his muscle tension was normal. A head MRI in 2015 had normal results. The results of other laboratory tests, such as liver and kidney function tests and measurements of blood sugar, complete blood count, serum amino acids, lactic acid, pyruvate, ceruloplasmin, creatine kinase, and urine amino and organic acids, were within normal limits.

Materials And Methods

Exome sequencing

Genomic deoxyribonucleic acid (DNA) from the patient's nuclear family members was sent for exome sequencing. The experimental protocols used were similar to those described in a previous study [11]. In brief, genomic DNA was isolated from the blood of the patient, his parents, and his older sister and was sheared using a Covaris ultrasonicator. Exome capture was carried out using an IDT xGen Exome Research Panel (IDT, USA), and paired-end sequencing was performed on the HiSeq 2500 platform (Illumina, Inc., CA, USA) to obtain 150-bp reads. Burrows-Wheeler Aligner (BWA, version 0.7.15) software was used to align paired-end reads to the University of California Santa Cruz (UCSC) and National Center for Biotechnology Information (NCBI) human reference genome (hg19/GRCh37).

To remove the irrelevant variables, the following procedures were followed. First, only genotypes with quality and size distribution scores ≥ 20 were included for further data analysis. Then, the exon regions (missense mutations, frameshift insertions, frameshift deletions, termination codon acquisition/loss, and splicing mutations) were hybridized for library amplification. The 1000 Genomes project (1000G, <http://www.1000genomes.org>) was used to assess enrichment and quality. Common variants were filtered based on the dbSNP database. The functional impact of the mutations was predicted. The candidate missense, nonsense, splicing, and frameshift mutations were confirmed by traditional Sanger sequencing.

The selected *SHANK3* reference sequences were NM_033517.1 for the mRNA sequence and NP_277052.1 for the protein sequence. The mutation was predicted and categorized as deleterious according to the interpretation of sequence variation by the American College of Medical Genetics and Genomics (ACMG) standards ^[12].

Reverse transcription quantitative PCR (RT-PCR) analysis of the transcription levels of the SHANK3 gene

Total RNA from the patient, his parents, and older sister was extracted using a Qiagen RNA Preparation Kit (QIAGEN Inc. Co., Germany). cDNA was synthesized from these samples by reverse transcription using PrimeScript™ Strand cDNA Synthesis Kit/RT Master Mix (Takara Shuzo Co., Ltd.). Primers were designed to amplify the *SHANK3* gene, and quantitative PCR (qPCR) was conducted using SYBR Premix Ex Taq II (Tli RNase H Plus) (Takara Shuzo Co., Ltd.) in a LightCycler® 96 instrument (Roche Inc., AG Schweiz). Based on the sequencing results for the child, primers were designed to amplify the *SHANK3* sequence: forward, AAAGCTTCACACAAAGGCGAAC; and reverse, TCATGGAAGTTGGGGTCCAG. After initial denaturation at 95.0°C for 3 min, 35 cycles at 95.0°C for 30 s and 60.0°C for 45 s were performed. Each dataset for the transcript level was generated from triplicate experiments, and the data are presented as the mean $\pm$ standard error of the mean (SEM) values. Statistical analyses of the quantitative RT-PCR data were performed to compare the means of the samples using the 2- $\Delta\Delta C_t$ method. Asterisks denote statistically significant differences (Student's t-test, **P < 0.05, ***P < 0.01).

Review Of Previously Reported Shank3 Mutations

We searched the literature in ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>) for pathogenic or likely pathogenic mutations in *SHANK3* and identified 298 mutations. We analyzed the mutations, classified all mutation types and determined the genomic locations of pathogenic and likely pathogenic mutations.

Results

Whole-exome and Sanger sequencing

The patient's genomic DNA was sent for whole-exome sequencing. After removing the low-quality variants, the exon regions (missense mutations, frameshift insertions, frameshift deletions, termination codon acquisition/loss, and splicing mutations) were hybridized for library amplification. The 1000G

project was used to assess enrichment and quality. Common variants were filtered out based on their frequencies in the Exome Aggregation Consortium (ExAC) databases. After genetic diagnosis, analysis was performed with the UCSC Genome Browser and the NCBI human reference genome (hg19/GRCh37).

The c.3679dup (p.A1227Gfs*69) mutation in the *SHANK3* gene (OMIM: 606230) (NM_033517.1) was detected in the whole-blood genomic DNA of the patient and was confirmed by Sanger sequencing. The DNA of the parents showed no variation at this site. According to the ACMG guidelines, the mutation type was pathogenic.

Transcription level of SHANK3 in the patient

The cDNA product obtained by reverse transcription of whole-blood RNA extracted from members of the patient's nuclear family was used for qPCR. Primers to amplify the *SHANK3* gene were designed, and RT-PCR was performed in accordance with a previously described protocol. The patient had significantly lower levels of *SHANK3* expression than his parents and older sister (Fig. 2).

Pathogenic and likely pathogenic mutations in SHANK3

The search in ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>) revealed 298 mutations. A total of 59 pathogenic and likely pathogenic mutations were screened out, among which frameshift mutations were the most prevalent (56%), and the percentages of missense mutations and nonsense mutations were similar (both 22%).

Discussion

The *SHANK* gene encodes SHANK1, SHANK2, and SHANK3, multidomain proteins that contain an ankyrin repeat domain (ANK), a Src homology 3 domain (SH3), a postsynaptic density 95 (PSD-95)/Discs large/zona occludens-1 homology domain (PDZ), a proline-rich region and a sterile α motif domain (SAM) ^[13], another proline-rich region, a Homer-binding site (Hbs), and a cortactin-binding site (Cbs) ^[14].

PMS has the characteristics of an ASD. Some children first visit pediatric departments for symptoms of autism. In a cohort study of 221 patients (133 from the United States, 88 from Italy), the frequency of *SHANK3* mutations was approximately 1% in patients with ASD ^[15], revealing a role for this gene as a possible major contributor to autism. In a Chinese cohort of 539 patients with ASD, pathogenic and likely pathogenic variants were detected in 9.5% of individuals. Variants of *SHANK3* and *SHANK2* had the highest frequencies, especially in females, and occurred in 1.2% of patients ^[16].

In this study, the child visited the outpatient department of neurology due to developmental retardation. The whole-exome sequencing results suggested that the newly identified *SHANK3* gene mutation (OMIM: 606230) (NM_033517.1) c.3679dup (p.A1227Gfs*69) is a *de novo* heterozygous frameshift mutation. Figure 1a shows the family pedigree; Fig. 1b shows verification of the mutation by Sanger sequencing.

The mutation site is marked with a red arrow. The child was heterozygous for the mutation, and the parents and older sister of the child harbored wild-type alleles.

We further performed quantitative RT-PCR analysis of the *SHANK3* gene (Fig. 2) and found that the gene expression level in the patient was significantly lower than that in the general population. This finding suggests that this mutation can cause a decrease in gene expression. Gene mutation is affected by genetic, environmental, and other factors. Humans have regulatory systems to minimize the damage caused by deleterious mutations. One such surveillance system is the translation-dependent nonsense-mediated mRNA decay (NMD) pathway, which exists in eukaryotic cells^[17–19]. NMD recognizes and eliminates mRNAs with suboptimally positioned translation termination codons. NMD-triggering translation termination codons are generically termed premature termination codons (PTCs). PTCs can arise as a result of nonsense or frameshift mutations in the coding DNA; mutations causing errors in splicing or the selection of the translation start site may also introduce PTCs. NMD degrades mRNAs containing PTCs and shortens the half-life of mRNAs^[20, 21].

β -Thalassemia is the earliest identified and most classic example of a disease caused by NMD. The disease is caused by mutation of the β -globin gene, which consists of three exons. Nonsense mutations occur in the first two exons, and NMD results in loss or very low levels of the corresponding mRNA transcripts. Individuals homozygous for this mutation cannot synthesize β -globin, resulting in severe erythropoiesis disorders. Individuals heterozygous for this mutation exhibit only mild anemia; thus, the disease has an autosomal recessive (AR) inheritance pattern^[22, 23]. However, nonsense mutations in exon 3 do not trigger NMD. The mRNA is stably translated into a truncated protein, resulting in abnormal β -globin. Individuals heterozygous for these mutations have severe anemia; thus, these mutations are associated with an autosomal dominant (AD) inheritance pattern^[24].

PTCs at the 3' end of the gene encoding the muscular dystrophy protein cannot be identified by the NMD pathway in patients with Duchenne muscular dystrophy (DMD), and affected transcripts have been found to be translated into truncated proteins with some functionality. The clinical phenotypes are mild, resulting in Becker muscular dystrophy (BMD). In contrast, when PTCs are located distally upstream, they can be identified and degraded via NMD, resulting in the inability to synthesize partially functional dystrophin, leading to severe phenotypes^[25]. Therefore, NMD can either ameliorate or exacerbate diseases. The specific function of NMD in all genetic diseases is unclear.

NMD is a quality control pathway that maintains the normal function of eukaryotic cells. However, not all PTCs trigger NMD. For example, PTCs located less than 50 nucleotides (nt) upstream of the last exon-exon junction typically do not trigger NMD (the 50 nt rule)^[26]. In addition, PTCs located in the last exon of a gene do not trigger NMD (the last exon rule)^[27, 28]. These two canonical rules of NMD have been widely validated^[29].

Figure 5 shows the possible action of NMD in heterozygotes containing PTCs. The child in this case study was heterozygous for a mutation in the *SHANK3* gene and exhibited a clinical phenotype. We found

by RT-PCR that *SHANK3* gene expression was reduced in this patient. The *SHANK3* gene contains 22 exons. The mutation site in *SHANK3* reported here is located in exon 21, which is more than 50 nt from the last exon. This mutation is speculated to trigger NMD, resulting in degradation of the mutated mRNA transcript and subsequent haploinsufficiency and clinical symptoms.

Figure 3 shows the proportions of known pathogenic and likely pathogenic mutations. Frameshift mutations are the most common mutations, accounting for 56% of all known mutations, and other missense mutations and nonsense mutations accounted for 22%. Figure 4 summarizes the specific locations of the known pathogenic and likely pathogenic mutation sites identified in ClinVar. Most mutations occurred at sites upstream of the SAM. If NMD is not triggered, mutations cause changes in protein structure and function. The red arrow in Fig. 3 indicates the mutation site of the patient in this paper. A frameshift mutation at this site in exon 21 leads to premature termination of protein transcription, resulting in a truncated protein with deletion of the Homer-binding site, cortactin binding site, and SAM. Deletion of the C-terminal SAM causes the protein to be unable to interact with zinc ions, which may cause structural and functional changes in the protein. PMS has an autosomal dominant inheritance pattern, which is speculated to be related to haploid insufficiency in heterozygous individuals.

The mutation site identified in this study was previously reported in 3 children with ASD or language delay, including 1 patient harboring a de novo mutation^[16]. The other two patients are brothers; both have severe mental retardation, and their mother has germline mosaicism^[30]. This variation has not been included in the Human Gene Mutation Database (HGMD) and is reported as a pathogenic variant in ClinVar. The allele frequency of this mutation was less than 0.01% (16/160994) in the total population among the normal population in the Genome Aggregation Database (gnomAD) and 0% among the East Asian population. According to the variant classification guidelines of the American organization ACMG, this variant is classified as a pathogenic variant.

SHANK acts as the main scaffolding protein at the PSD, interacting with various synaptic molecules^[31]. For example, Shank proteins are associated with the N-methyl-D-aspartate receptor (NMDA-R) via the postsynaptic density-95/guanylate kinase-associated protein (GKAP) complex, with the metabotropic glutamate receptor (mGluR) via Homer, and the GluR1 α -amino-3-hydroxy-5-methyl-1-4-isoxazole propionic acid receptor (AMPA-R)^[32]. Some reports have indicated that Shank1 targeting of synapses is dependent on the PDZ, but the targeting of Shank2 and Shank3 depends on their C-terminal domains, including the SAM^[33, 34], and the PDZ of Shank3 also plays a role^[35]. Shank2 and Shank3 multimerize and form a platform or framework in the PSD that depends on Zn^{2+} binding to the SAM^[36, 37]. In contrast, Shank1 does not bind Zn^{2+} but forms a large framework complex with Homer in the PSD^[38]. In the PSD complex, the Shank3 protein binds to neuroligin^[39] and forms a complex with axon proteins in glutamate synapses. The current data show that the Shank protein can bind to glutamate receptors via cytoskeletal proteins, thereby regulating the size and volume of excitatory synapses and dendritic spines^[40].

The *SHANK3* gene is rich in GC motifs (with a GC content of up to 69%), and the encoded protein is highly expressed in the human striatum, especially in neuronal synapses. In synapses, neuroligin in the presynaptic membrane binds to neuroligin in the postsynaptic membrane and then binds to the Shank3 protein ^[4], forming a complex in the synapses of glutamatergic neurons. This complex plays a key role in maintaining normal synaptic function and dendritic spine morphology, as well as in regulating the balance between neuronal excitability and inhibition ^[41].

Phenotypic variability may be related to the type of *SHANK3* mutation. Studies have confirmed that large-scale deletions are generally associated with obvious structural abnormalities and social communication disorders, while small-scale deletions or point mutations are often associated with haploinsufficiency of *SHANK3* and with abnormalities including ASD, convulsions, abnormal electroencephalogram findings, hypotonia, sleep disorders, abnormal brain MRI findings and gastroesophageal reflux ^[7]. *SHANK3* gene deletion was found in ASD patients, suggesting that haploinsufficiency is a main cause of ASD ^[6].

Chiara Verpelli et al found that reduced metabotropic glutamate receptor subtype 5 (mGluR5) activity in Shank3-knockdown neurons can impair mGluR5-dependent signaling pathways and plasticity ^[42]. An allosteric agonist of mGluR5, 3-cyano-N-(1,3-diphenyl-1H-pyrazole-5-yl) benzamide (CDPPB), was proven to be brain penetrant, to reverse mGluR5 activity ^[43, 44] and to rescue ERK1/2 phosphorylation and miniature excitatory postsynaptic current (mEPSC) frequency. These results demonstrate that positive allosteric modulation of mGluR5 produces behavioral effects and suggest that mGluR5 activity could be a therapeutic target.

In addition, based on the locations of pathogenic and likely pathogenic mutations in the *SHANK3* gene (Fig. 4), we consider that the NMD mechanism may be involved in PMS. Further study is needed to determine whether the truncated protein product of the *SHANK3* gene has partial functionality. Then, we could study whether inhibition of the NMD process has a therapeutic effect.

Conclusion

The *SHANK3* gene is a common pathogenic gene in children with ASD and developmental retardation. Genetic testing is essential for children with similar problems. Researchers have found that CDPPB is a potent and selective positive allosteric modulator of mGluR5. In addition, it is important to further study whether NMD is involved in the expression of *SHANK3* and to further investigate PTCs. The results of such studies may indicate a new therapeutic direction for PMS.

Declarations

Ethical Approval and Consent to participate

This study involving human participants was reviewed and approved by the Ethics Committee of Shanghai Children's Hospital. Informed consent was obtained from the parents of the patient for the use

of their blood samples for genetic testing.

Consent for publication

Written informed consent for participation in this study and for publication was provided by the parents of the patient.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on a reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Quanmei Xu and Simei Wang: acquisition of data, analysis and interpretation of data, drafting and revision of the manuscript;

Yilin Wang: study conceptualization and conduction of experiments;

Chunmei Wang, Yuanfeng Zhang, Juefei Wang, and Jinju Luo: analysis and interpretation of data, critical revision of the manuscript for important intellectual content;

Yucai Chen: design, guidance and supervision of experiments.

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Figures

a

b

Figure 1

a Pedigree of the patient's nuclear family b Sanger sequencing results at the position of the c.3679dup mutation in the SHANK3 gene. Electropherograms showing the DNA sequence at the position of the c.3679dup (p.A1227Gfs*69) mutation in SHANK3 in this patient (top). His parents and older sister did not harbor this duplication (bottom). The duplication caused a frameshift mutation contributing to a premature termination codon.

Figure 2

The relative expression levels of SHANK3 in members of the patient's nuclear family were determined by RT-PCR. The patient exhibited significantly lower expression of the SHANK3 gene (harboring the c.3679dup mutation) than his parents and older sister. Each dataset for the transcription level was generated from triplicate experiments, and the values are presented as the means $\pm$ SEMs. Statistical analyses of the RT-PCR data were performed to compare the means of the samples with the 2-AAct method. Asterisks denote statistically significant differences (Student's t-test, ** $P < 0.05$, *** $P < 0.01$).

mutations

Figure 3

Proportions of various mutation types among the known pathogenic and likely pathogenic mutations in the SHANK3 gene.

The possible process by which mRNA degradation is mediated by a nonsense mutation (i.e., nonsense-mediated mRNA decay, NMD) in heterozygotes harboring premature termination codons (PTCs). NMD prevents the transcription of genes with PTCs. Some mutations not recognized by NMD can result in the translation of truncated proteins. The different functions of these truncated proteins affect the genetic pattern of diseases.