A 30 Mainland Chinese cohort of patients with Phelan-McDermid syndrome: genotype-phenotype correlation and the role of SHANK3 haploinsufficiency in the important phenotypes

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Abstract

This is the first study describing a cohort of Mainland China patients broaden the clinical and molecular spectrum of Phelan-McDermid syndrome (PMS) or 22q13 deletion syndrome. A total of 30 Mainland China patients were clinically and genetically evaluated. We discover that nineteen patients with 22q13.3 deletions, one patient with terminal deletions and duplications, one patient with duplications, and nine patients with SHANK3 mutations were included. Six novel heterozygous variants, c.3838_3839insGG, c.3088delC, c.3526G>T, c.3372dupC, c.3120delC and c.3942delC, were firstly reported. Besides, we demonstrated speech delay, DD/ID, ASD, hypotonia and hyperactivity were prominent clinical features. Since most reported cases used to genotype-phenotype analyses are caused by 22q13 deletions usually encompassing many genes including SHANK3, we performed genotype-phenotype analysis, and found hypotonia was 100% of cases with loss of SHANK3 alone and there was no significant difference between loss of SHANK3 alone and deletions encompass more than SHANK3 gene regarding hypotonia, DD/ID, ASD, increased pain tolerance, gait abnormalities, impulsiveness, repetitive behaviors, regression and nonstop crying which are high frequency in loss of SHANK3 alone group. This analysis improves the understanding that SHANK3 haploinsufficiency is a major contributor to the neurological phenotypes of PMS and also responsible for other important phenotypes such as hypotonia.

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This is the first study describing a cohort of Mainland China patients broaden the clinical and molecular spectrum of Phelan-McDermid syndrome (PMS) or 22q13 deletion syndrome. A total of 30 Mainland China

patients were clinically and genetically evaluated. We discover that nineteen patients with 22q13.3 deletions, one patient with terminal deletions and duplications, one patient with duplications, and nine patients with*SHANK3* mutations were included. Six novel heterozygous variants, c.3838_3839insGG, c.3088delC, c.3526G>T, c.3372dupC, c.3120delC and c.3942delC, were firstly reported. Besides, we demonstrated speech delay, DD/ID, ASD, hypotonia and hyperactivity were prominent clinical features. Since most reported cases used to genotype-phenotype analyses are caused by 22q13 deletions usually encompassing many genes including *SHANK3* , we performed genotype-phenotype analysis, and found hypotonia was 100% of cases with loss of *SHANK3* alone and there was no significant difference between loss of *SHANK3* alone and deletions encompass more than*SHANK3* gene regarding hypotonia, DD/ID, ASD, increased pain tolerance, gait abnormalities, impulsiveness, repetitive behaviors, regression and nonstop crying which are high frequency in loss of*SHANK3* alone group. This analysis improves the understanding that*SHANK3* haploinsufficiency is a major contributor to the neurological phenotypes of PMS and also responsible for other important phenotypes such as hypotonia.

Key words: Phelan-McDermid syndrome (PMS), Mainland China,*SHANK3* haploinsufficiency, genotype-phenotype correlation

Introduction

Phelan-McDermid syndrome (PMS, OMIM 606232), also called 22q13 deletion syndrome is a rare developmental disorder with considerable clinical heterogeneity (Durand et al., 2007; Ponson et al., 2018; Prasad et al., 2000; Precht et al., 1998). Common clinical features include loss or severely delayed speech, developmental delay (DD), intellectual disability (ID), autism spectrum disorder (ASD), hypotonia, seizures, gait disturbance, increased pain tolerance, recurring upper respiratory tract infections, gastroesophageal reflux, and minor dysmorphic features including dysplastic ears, bulbous nose; had long eyelashes; full lips, high arched palate, hyperextensibility, full cheeks and periorbital fullness(Phelan et al., 2001; Soorya et al., 2013).

PMS is caused by deletions, and duplications, ranging from hundreds of kilobases (kb) to over nine megabases (Mb) in size in the 22q13 region (Bonaglia et al., 2011). Chromosomal abnormalities include simple terminal deletions, interstitial deletions, translocations, ring chromosomes, and duplications. Almost all individuals identified to date involve *SHANK3* , mapping to the distal end of 22q13.33 (Delahaye et al., 2009; Dhar et al., 2010; Misceo et al., 2011; Nesslinger et al., 1994; Ponson et al., 2018). *SHANK3* encodes a scaffolding protein enriched in the postsynaptic density of glutamatergic synapses and play a critical role in synaptic function and dendrite formation (Monteiro & Feng, 2017; Yi et al., 2016). Deletions or point mutations in*SHANK3* have been identified in patients ascertained for ASD at a rate of about 2% (Boccuto et al., 2013), intellectual disability (ID) at a rate of about 2% (Leblond et al., 2014), and schizophrenia at a rate of 0.6–2.16% (de Sena Cortabitarte et al., 2017; Gauthier et al., 2010).

Currently, the hypothesis is that *SHANK3*haploinsufficiency is responsible for major neurological features of PMS (Bonaglia et al., 2001; Delahaye et al., 2009; Wilson et al., 2003). Wilson and colleagues detected few correlations between the deletion size and the most neurological features. They have also reported correlations between deletion size and other important phenotypes including hypotonia, head circumference, recurrent ear infections, pointed chin, and dental anomalies (Wilson et al., 2003). In addition, genotype-phenotype analyses suggest that the size of deletion is predictive of phenotypic severity. Specifically, developmental delay (Luciani et al., 2003; Sarasua et al., 2011; Wilson et al., 2003), hypotonia (Luciani et al., 2003; Sarasua et al., 2011; Wilson et al., 2003), dysmorphic features (Samogy-Costa et al., 2019; Sarasua et al., 2011; Soorya et al., 2013), language status (Samogy-Costa et al., 2019; Sarasua et al., 2014), social communication deficits related to ASD (Samogy-Costa et al., 2019; Soorya et al., 2013), renal abnormalities (Samogy-Costa et al., 2019; Soorya et al., 2013), lymphedema (Samogy-Costa et al., 2019; Soorya et al., 2013), seizures (Soorya et al., 2013) show a higher incidence or increased severity with the larger deletions. However, genotype-phenotype correlations analyses have largely focused on patients with 22q13 deletions, only two reports on PMS have contained a few patients carrying *SHANK3* mutations or deletions only disrupt *SHANK3*(Samogy-Costa et al., 2019; Soorya et al., 2013). The lack of phenotype comparisons between

patients with *SHANK3* mutations (or deletions only disrupt *SHANK3*) and patients with deletions encompass more than *SHANK3* gene have hindered exploring the role of*SHANK3* deficiency in the important phenotypes in PMS.

Over the past few years, more than 1400 cases were identified worldwide. However, thus far, only four 22q13 deletions and a *SHANK3*mutation in patients with ID have been reported in Mainland China (Gong et al., 2012). Here, we report 30 previously undescribed Mainland Chinese patients with PMS. Our main goal was to explore the contribution of *SHANK3*haploinsufficiency as contributing to the important phenotypes besides neurological features. We also present clinical profile and genetic spectrum of the patients, aiming at expanding the molecular and phenotypic spectrum of PMS.

1.

Methods

Editorial Policies and Ethical Considerations

The study was approved by Ethics Committee of Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University (XHEC-D-2020-072) and parents provided informed parental consent prior to their inclusion in the study.

Cohort

Twenty-nine previously unreported patients with clinical and genetic diagnosis of PMS in Mainland China from the China League of PMS Rare Disease were recruited in this study from 2018 to 2020. This cohort included 20 males (66%) and 10 females (34%), with age ranging from 1.9 to 9 years old (mean = 4.46, SD = 2.18). Parents or guardians provided genetic exams, medical record review and completed a standardized medical history questionnaire. The questionnaire included queries about the individuals' developmental/neurological features, behavioral abnormalities, and additional clinical features. Level of developmental delay, autism diagnosis, stature, head circumference, MRI information, perinatal events, growth parameters at birth were abstracted from medical record review. In addition to that, 22 families also provided pictures of their affected children. Two clinicians from Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University assesed pictures of the patients in order to analyze their morphological features.

Genetic testing

All patients have a deletion or duplication at 22q13.3 encompassing*SHANK3* . Different exams and platforms were applied: array-CGH (n = 2), SNP array (n = 16) or WES (n = 2) were performed in 20 individuals. Two deletions only disrupt *SHANK3* were validated by MLPA or QPCR (see details in Table 1). *SHANK3* mutations were identified by whole exome sequencing (WES) and then validated by Sanger sequencing in nine individuals. Nine genetic tests were conducted by our research team and the others by outsourced laboratories.

Statistical analysis

Children were divided into two groups to investigate the role of*SHANK3* haploinsufficiency in the important phenotypes. We excluded from analysis a child with duplication other than deletions on 22q13. Group 1 (n=17) included individuals with deletions encompass more than *SHANK3* gene. Group 2 (n=12) consists of individuals with*SHANK3* mutations or deletions only encompass *SHANK3*.Fisher's exact two-sided test was used to assess statistical significance in important phenotypes between two groups. Results were judged to be statistically significant at $P < 0.05$ and of borderline significance at $0.05 < P < 0.10$.

Results

3.1 Characterization of 22q13.3 deletions and *SHANK3*mutations in PMS patients

We reported 30 children identified by a custom 22q13 microarray or WES, nineteen children were 22q13 terminal deletions, one child was terminal deletions accompanied by proximal duplications and one was duplication (Table 1 and Fig. 1). Deletions or duplications ranged from 2.05 kb to 8.7 Mb with a mean size of 3944 kb. Three children had a small deletion of 2.1 to 45.7 kb that only consists of *SHANK3* gene. Nine children with *SHANK3* mutations tested through WES were also observed (Table 2 and Fig. 2). Human Genome Variation Society guidelines were used to describe variants. As reported previously (Kolevzon et al., 2011), the human genome reference assembly (GRCh37/hg19) misses the beginning of exon 11, nucleotide and amino acid positions were corrected according to the *SHANK3* mRNA (NM_033517.1) and protein (NP_277052.1) in RefSeq. The variants included eight frameshift and one nonsense (Table 2, Fig. 2). Remarkably, we found an identical frameshift mutation, c.3679dupG (p. Ala1227Glyfs*69) in two unrelated patients. Mutations were de novo in nine patients. Six variants caused the loss of the Homer-binding, the Cortactin-binding and the Sterile Alpha Motif (SAM) domains, one variant disrupted Homer-binding domains and caused the loss of the Cortactin-binding and SAM domains, and one variant caused the loss of the Cortactin-binding and the SAM domains (Fig. 2). In addition, we assessed pathogenicity of *SHANK3* mutations in our cohort. Variants listed in table 2 meet the following criteria: (1) loss-of-function variants (frameshift and nonsense, splice site), (2) De novo (both maternity and paternity confirmed) in a patient with the disease and no family history, and (3) absent from control databases (EVS and gnomAD). One variant (c.3942delC, p.Gly1041Alafs*37) was likely pathogenic and the other variants were pathogenic according to the ACMG guidelines (Richards et al., 2015). Six novel heterozygous variants, c.3838_3839insGG, c.3088delC, c.3526G>T, c.3372dupC, c.3120delC and c.3942delC, were firstly reported.

3.2 Overall prevalence of clinical phenotype

Some of the most common phenotype seen in our cases (Tables 3 and 4 and S1 Table) were speech delay (100%), DD/ID (88%), ASD (81%), hypotonia (73%), distinctive behavioral abnormalities such as hyperactivity (83%), impulsiveness (77%) and repetitive behaviors (70%), developmental/neurological abnormalities like increased pain tolerance (60%) and gait abnormalities (53%). Other common problems included seizures (23%), chewing difficulties (30%), biting self or others (30%), hair pulling (30%), aggressive behavior (33%), nonstop crying (47%), sleep disturbance (23%), frequent diarrhea/constipation (23 %) and eczema (27%), and minor dysmorphic traits included descending palpebral fissure (32 %), ear anomalies (27 %), periorbital fullness (27 %), strabismus (27 %) and high forehead (23 %).

3.3 Development, language skills and neurological features

DD/ID was evaluated by analyzing the patients' medical records or questionnaire (n=27), with four patients ranging from severe to extremely severe and nine patients ranging from mild to moderate. Twelve cases were diagnosed DD/ID in which we were not able to assess the degree of intellectual disability, and two were not ID (Tables 3 and 4 and S1 Table).

All individuals (21/21, 100 %) could walk independently (Table 3 and S1 Table) by 3 years of age, although prominent fine and gross motor delays in all individuals at the time of test. The mean age when individuals acquired this skill was 20 months, ranging from 12 to 33 months of age. The majority of individuals showed hypotonia (24/30, 80%) and gait abnormalities (16/30, 53%).

Language delay was notable (20/20, 100%) over the age of 3 years; results are summarized in table 3. In total, 52 % of the cases (11/21) had no speech, 10 had "sentences" (spoke single word or spoke in phrases or sentences). The degree of language delay in ten patients ranged from mild to moderate and seven ranged from severe to extremely severe.

Seven participants (7/30, 23%) had any type of seizures (Table 2). Abnormal electroencephalography (EEG) were described in six participants (6/19, 32%), including three without clinical seizures. MRI showed abnormal findings in fourteen participants (13/21, 62%), including delayed myelination (n = 2), abnormal corpus callosum (n = 5), enlargement of ventricles (n = 2), white matter abnormalities (n = 1) , generous extracerebral spaces(n = 1) and large cisterna magna (n = 2).

3.4 Behavioral features

By the age of 3 years, ASD were prominent, with 81% (17/21) receiving a diagnosis of ASD. As seen in table 3, the majority of patients were reported as hyperactivity (25/30, 83%), impulsiveness (23/30, 77%) and repetitive behaviors (21/30, 70%) such as hand flapping, teeth grinding. Patients were also prone to nonstop crying (14/30, 47%) and regression (13/30, 43%) affecting language, cognitive and motor.

3.5 Other Clinical Features

According to parent report, the majority of participants had increased pain tolerance (18/30, 60%). In addition, chewing difficulties (9/30, 30%), eczema (8/30, 27%) diarrhea/constipation (7/30, 23%), any kidney anomalies (5/24, 21%), and recurring upper respiratory tract infections (7/30, 23%) are common. Renal problems found in 17–38% of patients with PMS, were not present in our cohort (Kolevzon et al., 2014; Samogy-Costa et al., 2019). Similarly, hearing loss reported in 3% of cases with PMS, were uncommon (Kolevzon et al., 2014). Lymphedema, hypothyroid, diabetes, vitiligo and enzyme deficiency have been reported in patients with PMS (Kolevzon et al., 2014; Samogy-Costa et al., 2019; Sarasua et al., 2014; Soorya et al., 2013), but were absent in individuals in our cases (Table 3).

3.6 Dysmorphic features

A total of 22 patients were performed dysmorphology examinations by two clinicians and all had at least one abnormal feature (Table 4 and S2 Table). Dysmorphic features in our cohort were not as so common as reported in previous studies (Samogy-Costa et al., 2019; Sarasua et al., 2014; Soorya et al., 2013). The most frequent features in this study were ear anomalies (6/22, 27%), descending palpebral fissure (7/22, 32%), periorbital fullness (6/22, 27%), strabismus (6/22, 27%), high forehead (5/22, 23%).

3.7 *SHANK3* mutations (or deletions only disrupt*SHANK3*) effects

To investigate the role of *SHANK3* haploinsufficiency in the important phenotypes, we divided patients into two groups. Group 1 (n=17) included individuals with deletions encompass more than*SHANK3* gene. Group 2 (n=12) consists of individuals with*SHANK3* mutations or deletions only encompass *SHANK3.* Aa shown in table 5, the frequency of the hypotonia was higher in patients in the group 2 compared to patients in the group 1 (100% vs. 71%), with statistical significance near the borderline (Fisher's exact two-sided test P =0.059), suggesting the haploinsufficiency for *SHANK3* may contribute to the hypotonia. There was no significant difference between group1 and group 2 with respect to other clinical features including a high frequency of the DD/ID (10/11, 91%), ASD(7/8, 88%), increased pain tolerance (7/12, 58%), gait abnormalities (6/12, 50%), impulsiveness (11/12, 92%), repetitive behaviors (9/12, 75%), regression (7/11, 63%), nonstop crying (7/12, 58%) and minor dysmorphic traits including ear anomalies (4/10, 40%) and descending palpebral fissure (3/10, 30%) in loss of *SHANK3*alone group (Table 5). In addition, as shown in figure 3, children in group 1 did not show severe dysmorphic traits compared to children in group 2. Patient 3 had short philtrum and down-turned mouth, patient 26 had long eyelashes and down-turned mouth, patient 7 had descending palpebral fissure, strabismus and wide nasal bridge, patient 11 had bulbous nose and fleshy ears and patient 12 had low set ears.

Discussion

Up to now, only four cases with 22q13 deletions or a *SHANK3*mutation were reported in a cohort of patients with ID in Mainland China (Gong et al., 2012). To make up for the gap, the 30 participants with PMS in Mainland China were conducted by our team to broaden the clinical and genetic spectrum of PMS.

Findings in our cases indicate *SHANK3* mutations are fully penetrant in accordance to previous estimates (De Rubeis et al., 2018). Notably, seven out of eight variants lead to the loss of Homer-binding, the Cortactin-binding and the SAM domains of the protein, which are critical for *SHANK3* interactions with other PSD proteins. Homer-binding domain of *SHANK3* binds to the group 1 metabotropic glutamate receptors, such as mGluR1/5 (Tu et al., 1999). Functional studies have found impairments in hippocampal

synaptic transmission and plasticity in an exon 21 deletion (coding for the Homer binding domain) mouse model (Kouser et al., 2013). SAM domain of *SHANK3* have been shown be crucial for the postsynaptic localization (Boeckers et al., 2005). Two patients (S13 and S15) carry a c.3679dupG causing a premature stop codon at position 1227 causing the loss of the Homer-binding, the Cortactin-binding and the SAM domains of the protein. Functional studies on c.3679dupG have been shown to affect neuronal development and decreased growth cone motility (Durand et al., 2012).

The key symptoms of PMS are speech delay, DD/ID, ASD and hypotonia, which is in accordance with previous studies (Durand et al., 2012). We found a high frequency of hyperactivity (83%) and impulsiveness (77%), which have been previously reported in the literature. Only one child was able to speak full sentences in our cases, and about 100% of children were able to walk but with a delayed onset, which is compatible with a different PMS cohort (Durand et al., 2012).

We compared phenotypes between patients with loss of *SHANK3* alone and that with deletions encompass more than *SHANK3* gene and found that there were no significant difference between two groups in DD/ID, absence of speech and ASD in accordance with a previous study(Wilson et al., 2003). We also demonstrated hypotonia frequency was slightly higher in the loss of *SHANK3* alone group. However, hypotonia frequency showed a correlation with the larger deletions in their study. The smallest deletion in their study encompassed *SHANK3, RABL2B* and ACR genes which cannot implicate the role of *SHANK3* alone in hypotonia. In addition, we observed a high frequency of the increased pain tolerance, gait abnormalities, impulsiveness, repetitive behaviors, regression, nonstop crying, and minor dysmorphic traits including ear anomalies and descending palpebral fissure in loss of *SHANK3*alone group which are in line with previous estimates in individuals with PMS due to *SHANK3* point mutations (De Rubeis et al., 2018). There was no significant difference between two groups in these features. These results indicate that *SHANK3* haploinsufficiency affects these important phenotypes. Several studies have examined genotype-phenotype associations in *SHANK3* deficiency and results are inconsistent. Phenotypes correlated with deletion size in their studies included renal abnormalities, lymphedema, large or fleshy hands, dolichocephaly, facial asymmetry, feeding problems, genital anomalies, head circumference, recurrent ear infections, pointed chin, and dental anomalies. However, only two reports on PMS have consists of a few patients carrying *SHANK3* mutations or deletions only disrupt*SHANK3* (Samogy-Costa et al., 2019; Soorya et al., 2013). In addition, these medical comorbidities frequency were all low or absent in two groups in our study probably leading to the insignificance because of small sample size. Latha et.al found deletion size was statistical significance associated with ASD. Our evaluation of ASD depended on medical record review while their study included evaluations using standard diagnostic scales with ASD (e.g., ADOS-2 and ADI-R).

There were several limitations to this analysis. We obtained medical history by medical record review or questionnaires completed by parents and the collected data may be subject to recall or information bias. In addition, assessments using standard diagnostic scales with ASD and developmental delay were not available, and thus analyses depend on medical record review. The phenotype comparisons identified in our analysis are heavily influenced by sample size, future studies could be done in larger samples to provide a clear role of *SHANK3*haploinsufficiency in the important phenotypes in PMS.

In summary, this is the first detailed report of the comparisons of phenotypes between PMS patients with deletions encompass many genes including *SHANK3* and loss of *SHANK3* alone. Our findings show loss of *SHANK3* alone is sufficient to produce the characteristic phenotypes of PMS, including developmental/neurological abnormalities, behavioral features, gastrointestinal problems and dysmorphic features. We also observed a high frequency of the speech delay, DD/ID, ASD, hypotonia, increased pain tolerance, impulsiveness, repetitive behaviors, regression, nonstop crying, and minor dysmorphic traits including ear anomalies and descending palpebral fissure in loss of *SHANK3* alone group and there was no significant difference between two groups regarding these important features. These findings extend the role of *SHANK3* haploinsufficiency in PMS beyond its well-known role at the neurological features.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

All data are shown in this article.

Authors' contributions

NX, HL, TY, FL and YY: conception and design of the study; NX, FL and YY: drafting the manuscript or figures;YS, review and editing the manuscript; JX and BX: evaluation of the pictures of individuals for the morphological analysis; LW, YF, LX and YZ: acquisition and analysis of data. All authors read and approved the final manuscript.

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Figure legends

Figure 1 Distribution of deletions and duplications among 21 China mainland patients that varied from 2 kb to 8.7 Mb.

Figure 2 Distribution of *SHANK3* mutations in our cases. Recurrent mutations are indicated in bold. Protein domains are from UniProt; the homer and cortactin binding sites are indicated as previously reported (Durand et al., 2007).

Figure 3 Representative images of patients with PMS showing mild dysmorphism. There was no significant difference between patients with 22q13 deletions encompass more than *SHANK3* gene and patients with loss of *SHANK3* alone in dysmorphic features.

Table 1 Details of the 22q13.3 deletions in 21 individuals with PMS.

Table 2 Summarization of SHANK3 gene variants in 9 patients with PMS.

Table 3 Developmental and behavioral features of patients with PMS in our cohort as compared to the literature.

Table 4 Dysmorphic features in patients with Phelan–McDermid syndrome.

Table 5 Comparison of prevalence of phenotypes between patients with 22q13 deletions encompass more than SHANK3 gene and loss of SHANK3 alone.

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