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SHANK3 gene: Genotype-Phenotype relationship in a sample with Autism spectrum disorder and/or Intellectual Developmental Disability

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Abstract

Background. Autism spectrum disorder (ASD) is a complex and life-long neurodevelopmental disorder that has a strong genetic influence. *SHANK3* gene is a rare genetic variant that has been described in the literature as a strong candidate for the molecular modelling of ASD. *SHANK3* mutations leads to a synaptic and circuitry defects that may result in neurodevelopmental disorders, such as ASD and Phelan-McDermid Syndrome (PMS). PMS is caused by 22q13.3 deletions and it is characterized by global developmental disorder (GDD) or intellectual disability disorder (IDD), absent or severely delayed speech, hypotonia, mild dysmorphisms and ASD or autistic-like behaviour. In the present study we aim to add genotype-phenotype description in a subset of three individuals with a *SHANK3* germline mutation.

Patients and Methods. We provide a detail clinical and genetic data of three patients with *SHANK3* gene mutations: two identified with deletion and one with duplication. All underwent an extensive clinical evaluation, including information collected from parents, Autism Diagnostic Interview Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS) tests to diagnose or rule out ASD. Neurological and dysmorphology examinations and clinical genetics evaluations were performed too. Additionally, an intellectual and functional evaluations with Griffiths Mental Development Scales and Vineland-II, respectively was done.

Results. The patient 1 have a terminal deletion of chromosome 22 (q13.31q13.33), encompassing 51 genes (include *SHANK3* gene) and the patient 3 have an interstitial deletion, involving partially of the *SHANK3* gene. Both patients were diagnosed with PMS associated to GDD or IDD. The patient 2 was diagnosed with ASD and he has a duplication in the end of the chromosome 22 (q13.33), involving three genes: *SHANK3, ACR, RABL2B*.

Discussion and Conclusion. Our findings are broadly in line with those reported in previous studies. Moreover, our study showed that the *SHANK3* haploinsufficiency due to interstitial deletion, only disrupting the *SHANK3* gene, is enough to cause a set of features associated with PMS, namely neurobehavioral manifestations. Besides, this case highlights the hypothesis that perhaps not only the haploinsufficiency of *SHANK3* is associated to ASD, but also the *SHANK3* overexpression.

Keywords

SHANK3; Phelan-McDermid Syndrome; 22q13.3 deletion syndrome; 22q13.3 duplication; Autism Spectrum Disorder; Intellectual Developmental Disorder;

Abbreviations

ABC – Adaptive Behaviour Composite

ADI-R – Autism Diagnostic Interview-Revised

ADOS – Autism Diagnostic Observation Schedule

ASD – Autism Spectrum Disorder

array-CGH – Array-comparative genomic hybridization

CNV – Copy number variant

DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

FISH – Fluorescence *in situ* hybridization

GDD – Global developmental delay

GDQ – Global developmental quotient

HP-CHUC - Hospital Pediátrico of Centro Hospitalar e Universitário de Coimbra

IDD – Intellectual developmental disorder

MLPA – Multiplex Ligation-dependent Probe Amplification

MRI – Magnetic Resonance Imaging

OMIM - Online Mendelian Inheritance in Man

PMS – Phelan-McDermid Syndrome

SHANK3 – SH3 and multiple ankyrin repeat domains 3

VABS – Vineland Adaptive Behaviour Scale

BACKGROUND

Autism spectrum disorder (ASD) is a complex and life-long neurodevelopmental disorder, which affect approximately 1 in 160 children all over the world [1]. ASD is characterized by impairments in social communication and interaction and repetitive, restricted and stereotyped patterns of behaviour and interests. These symptoms must be present from early childhood, limit or impair everyday functioning and are not better explained by intellectual developmental disorder (IDD) or global developmental delay (GDD). However, IDD, GDD and ASD frequently co-occur [2].

ASD has a strong genetic influence and abundant evidence pointing to hundreds of genetic variants involved [3]. Nowadays, rare genetic variants are increasingly being described as etiological factors in ASD and IDD. Here, we focus our attention on high-impact rare variant – *SH3 and multiple ankyrin repeat domains 3* (*SHANK3*) gene, which has been described as a strong candidate for the molecular modelling of the ASD. However, *SHANK3* mutations are not a common cause of autism, being responsible for only 0.5% or less of the ASD cases [4].

SHANK3 gene is located at the minimal telomeric region of chromosome 22 and it expresses a protein found in many tissues, but most abundant in the brain [5,6]. SH3 and multiple ankyrin repeat domains 3 (SHANK3) is a structural protein of the postsynaptic density of excitatory glutamatergic synapses and it acts as a multidomain scaffolding protein, interacting with many postsynaptic proteins and orchestrating the assembly of the macromolecular postsynaptic signalling complex at the synapse (Figure 1). This protein has other functions, such as actin-based cytoskeletal remodelling, synapse formation, alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptor endocytosis, regulation of synaptic transmission and plasticity. It also plays a role in dendritic spine formation and maturation [7-10].

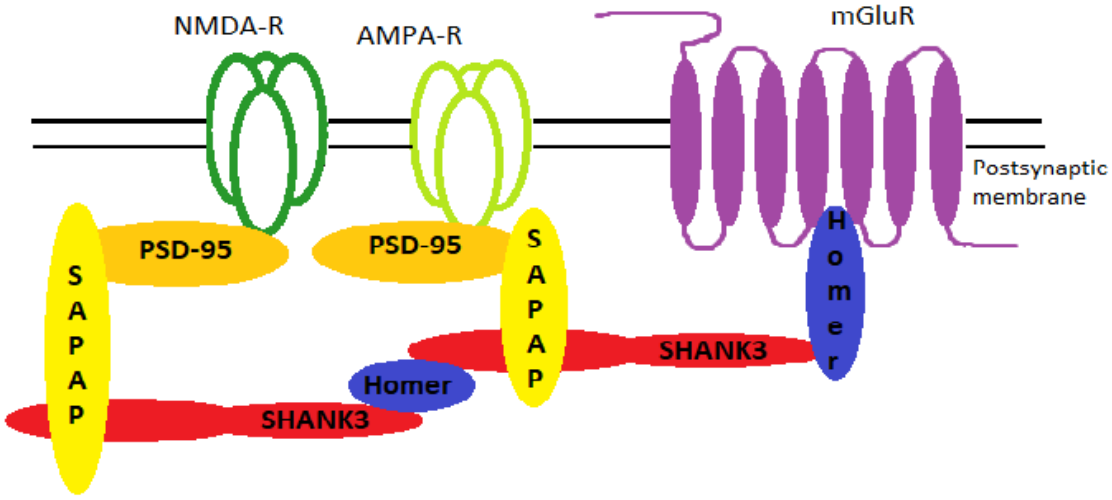

Figure 1. A simplified molecular organization of the postsynaptic *SHANK3* are responsible to organize and stabilize the postsynaptic density. PSD-95 and SAPAP serves as a functional bridge interconnecting *SHANK3* and ionotropic receptors (AMPA-R and NMDA-R). *SHANK3* binds Homer, which mediates mGluR anchoring [4, 7].

Mutations or deletions can occur providing loss of one functional copy of *SHANK3* and, as a consequence, its haploinsufficiency, leads to synaptic and circuitry defects that may have a neurodevelopmental and neuropsychiatric impact, causing disorders as GDD/IDD, monogenic form of ASD, Phelan-McDermid Syndrome (PMS), schizophrenia type 15 and atypical bipolar disorder [10,11]. Remarkably, a study reported an individual, who was diagnosed with Asperger Syndrome, that had three copies of the *SHANK3* gene [6]. In this way, not only the haploinsufficiency, but also overexpression of *SHANK3* can be associated with ASD, suggesting that abnormal *SHANK3* gene dosage or expression might, by itself, represent a risk factor to ASD [12].

PMS is caused by a deletion near to the end of the long arm of chromosome 22 (22q13 region). The size of these deletions varies from less than 100 Kb to more than 9 Mb. Although more than 90 genes can be deleted, most of the neurobehavioral manifestations are thought to be caused by haploinsufficiency of *SHANK3* [6]. Generally, it is characterized by GDD or IDD, absent or severely delayed speech, hypotonia, minor facial dysmorphic features [13, 14] and, in more than 50% of patients, ASD or autistic-like behaviour [5]. The main facial dysmorphism are fleshy hands, dysplastic toenails, long eyelashes, dolichocephaly, poorly formed or large ears, wide brow, deep-set eyes, flat midface, wide nasal bridge, pointed chin and bulbous nose. Additionally, medical

conditions have been reported, including renal abnormalities, cardiac defects, seizures, gastrointestinal problems, lymphedema and arachnoid cyst [14, 15]. Regression, that is, the loss of already acquired skills, is another characteristic of PMS. However, the frequency, the age of onset and the specification of the skills lost in regression are incompletely defined [16].

Several studies [15,17, 18] evidence that the deletion size and the phenotypic severity are positively correlated. Specially, a relationship between deletion size and dysmorphic features, developmental delay, and speech ability have been reported in the last years. Therefore, individuals with large deletions may have less favourable development trajectories than those with small deletions. On the other hand, some studies [6, 19] proof that the individuals with a point mutation and intragenic deletions in *SHANK3* share the main neurobehavioral features of the PMS, increasing the support that the haploinsufficiency of *SHANK3* leads to these features.

Several studies [14, 15, 20] of genotype-phenotype correlation have been conducted in the last years, but there is no consensus among the obtained results, strengthening the importance of the subject. In our study we aim to add genotype-phenotype description in subset of three individuals with a *SHANK3* germline mutation.

METHODS

Participants

The study includes three probands with *SHANK3* gene mutations: two identified with deletion, and one with duplication. The three subjects are Caucasian, two males and one female, currently with 5, 8 and 13 years old. All children are being followed in the Neurodevelopmental and Autism Unit of *Hospital Pediátrico* of *Centro Hospitalar e Universitário de Coimbra* (HP-CHUC), a Universitary Portuguese Paediatric Hospital that is a reference for autism in Portugal, serving children from birth up to the age of 18.

This study was conducted in accordance with declaration of Helsinki. Parents or caregiver provided written informed consent for participation.

Clinical evaluation

Detailed clinical data about the patients enrolled in this study was collected from parents, especially the pre and perinatal history, neuromotor and language development with particular attention to the milestones of neurodevelopment, behaviour and family history. Additionally, clinical genetics evaluations and dysmorphology examinations were performed by clinical geneticists.

Besides extensive clinical evaluation the *gold-standard* ASD diagnostic tools includes *Autism Diagnostic Interview-Revised* (ADI-R) [21], *Autism Diagnostic Observation Schedule* (ADOS) [22] and according to the *Diagnostic and Statistical Manual of Mental Disorders*, 5th edition (DSM-5) criteria.

Autism Diagnostic Interview – Revised (ADI-R) [21] is an investigator-based parent interview about the individual's early childhood and current social and communication development and stereotyped, repetitive behaviours and interests. The ADI-R has good reliability and validity [21]. ASD score between 10 and 30 (maximum rate). A positive score for autism on the ADI-R is results above seven for nonverbal subjects and eight for subjects with verbal communication simultaneously with ten in social interaction area and three in the repetitive behaviours and stereotyped patterns. Higher scores represent greater clinical severity.

Autism Diagnostic Observation Schedule (ADOS) [22] is a semi-structured interactive observation session that involves play and activities for young children and non-verbal individuals, and activities and an interview for older and verbal subjects. Individuals are tested with one of four different modules appropriate for their age and verbal ability. ADOS results corresponding to the minimum cut-off for ASD in all areas of communication and social interaction of the four existing modules: Module 1: total cut-off for communication and social interaction = 7; Module 2: total cut-off for communication and social interaction = 8; Module 3: total cut-off for communication and social interaction = 7; Module 4: total cut-off for communication and social interaction = 7. As a semi-structured ASD diagnostic tool, ADOS has shown strong predictive validity against best estimate diagnoses, making it a common choice among phenotyping measures. Higher scores represent greater clinical severity.

The Portuguese version of the ADI-R and ADOS are administered by two experienced clinical neurodevelopmental paediatricians and psychologists who met standard requirements for research reliability.

Furthermore, developmental quotient (DQ) was evaluated with *Griffiths Mental Development Scale* (GMDS) [23] and functional level assessed with *Vineland Adaptive Behaviour Scale, Second Edition* (Vineland-II) [24].

Additionally, a cerebral magnetic resonance imaging (MRI) was performed in one patient (Patient 1).

Genetic testing

The genetic tests of patients 1 and 2 were conducted by our laboratory (*Laboratório de Citogenética e Genómica* of the Faculty of Medicine of the University of Coimbra) and the genetic tests of patient 3 were performed by an out-sourced laboratory (*CGC genetics* in Oporto).

For the clinical genetic diagnostic evaluation of ASD, DNA samples of all patients were analysed using, firstly, array-comparative genomic hybridization (array-CGH) for Patient 1 and 3, and Multiplex Ligation-dependent Probe Amplification (MLPA) for Patient 2; secondly, to validate the genetic diagnostic, a karyotype with conventional cytogenetics and fluorescence in situ hybridization (FISH) analysis for 15 (q11.2q13) for Patient 1 and a MLPA for patient 3. Fragile X PCR-based test (only to be performed routinely for male patients, Patients 1 and 2) and metabolic screening were also conducted [25].

RESULTS

The main phenotypical characteristics of the three subjects are summarized on Table 1.

Concerning the genetic investigation, as shown in Table 2, two patients had confirmed *SHANK3* deficiency secondary to 22q13.3 deletion and one patient presented duplication of 22q13.33.

Table 1. Main clinical features with the *SHANK3* deletions or duplication

Major findings	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Female
Age current (years)	5	13	8
Age of referral	2 years and 9 months	3 years and 7 months	3 years and 7 months
Reason for referral	GDD	ASD	GDD
Psychomotor development			
▪ **Sit unaided (months)**	NA	6	17
▪ **Began walking (months)**	23	12	24
▪ **First words (months)**	36	12	36
▪ **First sentences (months)**	48	36	36
▪ **GDQ**	68	99	65
▪ **ABC**	NA	70	63
ADOS	Not Autism	Autism	Not Autism
ADI-R	Negative	Positive	Negative
DSM-5	Did not meet criteria for ASD	Met criteria for ASD	Did not meet criteria for ASD
Clinical features			
▪ **Craniofacial dysmorphisms**	+ (retrognatism and low-set ears)	−	−
▪ **Speech delay**	+	−	+
▪ **Autistic-like behaviour**	+	−	+
▪ **Neurological examination**	Clumsy	Clumsy	Clumsy
Cerebral MRI	Mild delayed myelination, moderate thinning of corpus callosum	Not performed	Not performed

Legend: +, present; −, absent; ABC, Adaptive behaviour Composite; ASD, Autism Spectrum Disorder; GDD, Global developmental delay; GDQ, Global developmental quotient; MRI, magnetic resonance imaging; NA, not available.

Table 2. Description of the genetic changes in 3 patients with 22q13 deletions and duplication

Patient	Diagnosis	Ascertainment method	Validation	Pathological rearrangement	Coordinates of CNV (hg19) or MLPA	Del 22q13 size (Kb)	Inheritance pattern	Additional genomic findings
1	PMS	Array-CGH	Karyotype; FISH	Terminal deletion (q13.31q13.33)	47730190 – 51219009	3400	Father negative (mother NA)	22q11.21 deletion (chr22: 18661724 – 19010508)
2	ASD	MLPA		Terminal duplication (q13.33)	22q13.33 *SHANK3; ACR; RABL2B*	NA	Maternal	Not found
3	PMS	Array-CGH	MLPA	Interstitial deletion (q13.33)	*SHANK3* (exons 9-22)	40	NA	Not found

Legend: array-CGH - array-comparative genomic hybridization; ASD - Autism Spectrum Disorder; CNV- copy number variation; FISH - Fluorescence in situ hybridization; hg, human genoma version; MLPA, Multiplex Ligation-dependent Probe Amplification; NA, Not available; PMS, Phelan-McDermid Syndrome.

Patient 1

Patient 1 is a 5 years old boy, the third child of healthy, unrelated, parents. The pregnancy was complicated by gestational diabetes, controlled with a diet, and by hypertension in the peripartum period, without previous diagnosis. He was born at 38 gestational weeks, by eutocic delivery, with 2520g (3rd percentile), 46,5cm (3rd percentile) and a head circumference of 33,3cm (15th percentile). Family history was unremarkable.

At birth, he presented microretrognathia and mild malnutrition signs. He had neonatal jaundice, but he did not need phototherapy session. Hypertonia was detected at 3 months of age and once again at 2 years old. However, this signal was not confirmed at the time of admission into our Autism Unit, thus we cannot reject the hypothesis that it was a reporting error. At 9 months, he did not both sit unsupported and able to perform a pincer grasp. He began walking at 23 months of age and his first words was at 36 months.

At 2 years and 9 months old, he was presented to Autism Unit of HP-CHUC with special impairment in cognitive, speech, language and autonomy areas. The observation of clinical details showed deficits in social communication and interaction, mainly manifested by the absence of verbal communication and the incapacity to point to any pictures or specific body parts. His general growth was within the normal range. The patient exhibited facial dysmorphisms: micrognatia and low-set ears. He had difficulty chewing and had drooling problems. He was a clumsy child, with uncoordinated gait, but functional, without ataxia. Based on clinic evaluations and genetic tests, he was diagnosed with PMS.

He did not fulfil criteria for ASD diagnosis. Specifically, he did not meet DSM-5 criteria, he also did not meet ASD cut-offs, on the ADOS (total communication and social interaction >12), neither ASD cut-offs, on at least two of three domains of the ADI-R (social interaction >10; communication >8; repetitive behaviours >3). In the Griffiths Scales, he had a GDQ = 68 (lower than the mean for his age – normal range 100 ±15).

At that point, he made a cerebral MRI, which revealed several no diagnostic alterations, namely mild delayed myelination, especially at frontal and temporal lobes, moderate thinning of corpus callosum and mild microcephaly.

At 4 years old, the patient had mild IDD and autistic-like behaviour. He was already able to use sentences. He had repetitive behaviours: open-close doors and drawers and order objects.

Although autistic-like behaviour remains, the patient had significant improvements in social communication and interaction areas: he makes eye contact, point to pictures or body parts, ask for help and show interest in peers. He frequented speech therapy, occupational therapy and physiotherapy.

The 180k array-CGH analysis showed a terminal deletion of chromosome 22q13.31q13.33, between the positions 47730190 and 51219009. Deletion size was approximately 3400 Kb, involving 51 genes, 30 of it described in the Online Mendelian Inheritance in Man (OMIM) database and 10 reported in the OMIM Morbid Map (OMIM ID: 607144-*ALG12*, 605908-*MLC1*, 610053-*TUBGCP6*, 603560-*SBF1*, 604272-*SCO2*, 131222-*TYMP*, 612395-*CHKB*, 607574-*ARSA*, 606230-*SHANK3*, 102480-*ACR*). The 22q13 deletion was later confirmed by conventional cytogenetic and FISH. This alteration was compatible with the PMS.

Furthermore, array-CGH analyse revealed a deletion of 22q11.21 between the positions 18661724 and 19010508. Deletion size was 348 Kb, involving 11 genes: 2 described in OMIM database (OMIM ID: 601279-*DGCR6*, 606810-*PRODH*) and the *PRODH* gene reported in the OMIM Morbid Map, associated with susceptibility to schizophrenia.

Father's analysis, using the same array platform, did not report any *SHANK3* deletions*.* The patient's mother was not available for further testing.

Patient 2

This 13-year-old boy was born as the first child from healthy, nonconsanguineous, parents. He was delivered by caesarean section at 41 weeks gestation, after an uneventful pregnancy. He had a birth weight of 4610g (97^{th} percentile), height of 51cm (80^{th} percentile) and head circumference of 37cm (97^{th} percentile). Early postnatal history was normal. Early motor skills were acquired at the normal age: he sat unaided at 6 months of age and walked at 12 months; he had the first words at 12 months and the first sentence at 36 months. Family history was negative to neurodevelopmental or neuropsychiatric disorders.

He was first seen at the Autism Unit of HP-CHUC at 3 years and 7 months old when he was referred by his paediatrician for suspect of ASD. ADI-R (social interaction >10; communication >8; repetitive behaviours >3), ADOS (total communication and social interaction >12), and DSM-5 criteria supported this diagnosis of ASD. In the Griffiths Scales, he had a GDQ = 99

(normal for his age – normal range 100 ±15). Vineland-II adaptive behaviour composite (ABC) score was of 70 (normal range 100 ±15), suggesting a functional age lower than the expected for his age.

At 5 years old, he frequented pre-primary school, with special education and speech therapy. In terms of behaviour, he exhibited oppositional and defiant behaviour, did not show interest in general tasks and failed follow orders, but he was controllable – he ended up performing all the tasks that were requested. Additionally, clinical evaluation no major or minor dysmorphic features were found, and he showed a clumsy but non-pathologic neurological examination.

The patient presents a normal karyotype (46, XY). MLPA exposed a duplication in the end of the long arm of the chromosome 22 (q13.33), involving three genes: *SHANK3, ACR, RABL2B*. Both parents was analysed by means of MLPA, but just the patient´s healthy mother showed the occurrence of the duplication. Father's analysis did not report any *SHANK3* duplication.

Patient 3

This patient is an 8 years old girl, the second child of healthy, unrelated parents. The pregnancy was well monitored and held without complications. She was born at 37 weeks gestation with 2760g (15^{th} percentile), 47cm (15^{th} percentile) and a head circumference 35cm (85^{th} percentile). In terms of her neurodevelopmental milestones, she had motor delay with sit unaided at 17 months of age and walked at 24 months, and she had the first words at 24 months of age and the first sentence at 36 months. Her parents and her brother are healthy and without learning disorders. Family history was negative to congenital anomalies or developmental delay/IDD.

At 3 years and 7 months old, she was referred to the Autism Unit of HP-CHUC by her paediatrician for suspect of ASD. She was diagnosed previously, in other hospital, with PMS associated to GDD. A full clinical evaluation showed that she collaborated with all the tasks that were requested, she manifested a good/normal eye contact and an adjusted behaviour for her stage of developmental (she point to pictures or body parts, answer a simple questions, show interest in peers and have symbolic games). On medical genetics evaluation, the patient was described as non-dysmorphic and the physical developmental was within the normal limits. There were no neurological signs evident on examination and her muscle tone were noted to be normal. At that time, she frequented speech therapy and occupational therapy.

ADOS and ADI-R tests revealed that she had some characteristics of ASD, but not in sufficient number or intensity to make a formal diagnosis, so this diagnosis was not confirmed. In the Griffiths Scales, she had a GDQ of 65 (lower than the mean for her age – normal range 100 ±15) and according to the Vineland-II scale, she had an ABC of 63 (functional age lower than the expected for her age – normal range 100 ±15).

The 750k array-CGH analysis showed an interstitial deletion with approximately 40 Kb of size in 22q13.33, involving partial of the *SHANK3* gene. After, a specific molecular study of this gene was done, by MLPA, which confirmed a deletion spanning exons 9 to 22 of *SHANK3*. This mutation is not described in the literature. However, once included many exons of *SHANK3* gene, there was a huge probability in being pathogenic. In this way, the results stablished the diagnosis of PMS.

We do not know the results of parental genetic testing, but both parents are healthy.

DISCUSSION

PMS was first reported in 1985 and, during the following years, hundreds of diagnoses have been done around the world [26]. However, the first clear association between ASD and *SHANK3* deficiency was only published in 2000 [27]. Since then, several patients with ASD and IDD have been described in the literature as having *SHANK3* variants. Similarly, the present study reports three cases with mutations of *SHANK3* that were diagnosed with ASD or IDD.

Patients 1 and 3 have a terminal deletion of 22q13 and a 22q13.33 interstitial deletion, respectively, and both were diagnosed with PMS. They did not fulfil criteria for an ASD diagnosis. However, autistic-like behaviour had been reported in both cases.

Patient 2, who met criteria for ASD, show a 22q13.33 duplication, inherited from his healthy mother. Thus, given that Patients 1 and 3 have the same diagnostic, we will first discuss these two cases. Then, we will argue Patient 2.

Most individuals with PMS are described as having GDD, IDD and language and speech impairment. Most of the patients was between the severe to profound range of ID [14, 15, 20]. In our research, both Patients 1 and 3 have mild ID and mild to moderate delayed speech

(Table 4). As we have noted, those patients have been delayed in achieving language milestones. In Patient 1, the moderate findings may be due to medium-sized deletions. In fact, according to previous studies, higher severity of phenotype is associated to larger deletions sizes [15, 18]. In the case of Patient 3, due to lack of studies, the specific phenotype associated with interstitial deletions is unknown.

Table 4. Clinical characteristics in patients with PMS in our study as compared to the literature

Medical features	Patient 1	Patient 3	Soorya et al. [15] n= 32	Sarasua et al. [20] n=201	Rubeis et al.[a] [14] n=17
Developmental delay	+	+	100%	100%	100%
Intellectual disability	+ (mild)	+ (mild)	96.7% (severe to profound=77%)	NA	100% (severe to profound=65%)
Delayed speech	+	+	100%	100%	100%
Hypotonia	−	−	75%	75%	94%
ASD	−	−	84.4%	31%	69%
Dysmorphic features	+	−	100%	NA	100% (n=11)

Legend: +, present; −, absent; ASD, Autism Spectrum Disorder; NA, not available.

[a] Analysis of PMS individuals carrying *SHANK3* point mutations.

Individuals with PMS may be diagnosed with ASD or, alternatively, may be described as having autistic-like behaviour. In our case, both children (Patients 1 and 3) did not receive an ASD diagnosis (Table 4), but ADOS and ADI-R tests revealed that they had some characteristics of ASD. Sarasua et al. [20] found that, in a total of 201 individuals with PMS diagnosis, 31% of individuals had also ASD diagnosis. Poson et al. [28] showed that in a total of 18 individual with PMS, when both ADI-R and ADOS criteria were required, just 39% of subjects met all criteria for ASD diagnosis. Despite that, when was only used the ADI-R criteria, 60% of individuals met the criteria for ASD diagnosis. By contrast, Soorya et al. [15] and Phelan et al. [29] reported that 84% and 94% of individuals, respectively, met the criteria for ASD. There seems to be a discrepancy in the diverse studies about rates of ASD diagnosis in children with PMS. Two plausible explanations can be that unconnected cases utilize different standardized

assessments tools or most of the individuals with PMS have moderate to severe IDD and GDD, and autism-specific diagnostic evaluation tools have been reported to possess limited specificity in these cases [30].

Patient 1 has repetitive behaviour, which is commonly descript in the literature associated to PMS. Aggressive behaviour, restricted interest, hyperactivity, self-injury and sleep disturbance had also been observed in previous researches, however our Patients 1 and 3 did not show these behaviours [14, 15].

Patient 1 exhibited a facial dysmorphism (micrognatia and abnormalities of ears) and Patient 3 did not evidence facial dysmorphic features (Table 4). In a prospective clinical evaluation of 32 patients with a 22q13 deletion, Soorya et al. [15] reported that all patients presented at least one dysmorphic feature. The most common dysmorphic features, observed in more than 40% of cases, were: large fleshy hands, bulbous nose, long eyelashes and ear anomalies; micrognatia appeared in 13% of the patients. Patient 1 had feeding problems (chewing difficulties), which are a very common comorbidity in PMS. Others common clinical features have been reported in other studies, as renal abnormalities, cardiac defects, seizures, gastrointestinal problems, lymphedema [5, 14,15], however, we do not have clinical information about the presence of this clinical features in our patients.

In our study, none of the children had a history of regression, a clinical feature increasingly described in children with PMS. Reierson et al. [16], for example, found that 43% of the 42 patients in their study had a history of regression. Moreover, they reported that the average age of onset was around 6 years and motor skills was the most common skills lost.

Almost all papers reported hypotonia as one of the main clinical features of PMS (Table 4). Soorya et al. [15] observed that the hypotonia was present in 100% of the 16 children who underwent neurological examinations, despite just 75% of the 32 patients were described by their parents as having hypotonia. In the study carried out by Rubeis and colleagues [14], the hypotonia was also present in 94% of subjects. However, our results are not consistent with those of previous studies. Patient 3 had a normal muscle tone and the neurological examination of Patient 1 at 2 years old showed hypertonia (we should note that, as we mentioned above, the signal in Patient 1 was not confirmed by our Autism Unit at 2 years and 9 months old).

Patient 1 has MRI performed, which revealed mild delayed myelination and moderate thinning of corpus callosum, alterations commonly found in individuals with PMS. The main structural

brain abnormalities reported in children with PMS are: thinning or hypoplasia of the corpus callosum, white matter changes (such as delayed myelination, generalized white matter atrophy and nonspecific white matter hyperintensities), ventricular dilatation and arachnoid cysts [31].

Previous genotype-phenotype analyses have reported that the increase in deletion size is positively correlated with severity of phenotypes [20, 32]. Sarasua el al. [20] found that individuals with larger deletions tended to be more developmentally delayed (severity of developmental delay, speech ability and walking ability). We can also assume that probably one or more deleted genes have an additional negative effect on development. We are not sure which additional genes contribute to the phenotype, although recent data suggest that *RABL2B* and *IB2* encompassed by the deletion also contribute to phenotype expression [32]. In our case, the patient with terminal deletion, who has a deletion of *SHANK3* gene and other genes presents in the 22q13 region, has a worst phenotype severity that the patient with interstitial deletion, who has a deletion only of *SHANK3* gene, which seems to support our hypothesis.

Analysing now the case of the second patient, who carries 22q13.33 duplication, the presence of the same duplication in the healthy mother is consistent with being benign. So, we can consider as our first working hypotheses that this duplication is a false-positive finding for an etiologic variant, which means that maybe this mutation is not responsible for the child's phenotype.

Theoretically, duplications of 22q13 should occur at the same frequency as the deletions resulted from the interchromosomal nonallelic homologous recombination [33]. However, until now, for unknown reasons, this duplication has rarely been described. Two hypothetical reasons for this fact are that the effect of duplication has no observable consequence or it is lethal [33] Patient 2 was diagnosed with ASD — a disorder presents many times in 22q13 deletion syndrome. In contrast with PMS, this patient did not have delayed speech and her GDQ is normal for her age.

Moessner et al. [19] and Durand et al. [6] reported two different cases of 22q13 duplication, both associated to neurodevelopmental disorder. Durand et al. [6] identified two affected brothers as a result of a paternal translocation t(14,22)(p11.2;q13.33). The daughter had an 800 Kb terminal 22q deletion and she was diagnosed with PMS. Additionally, her brother had a 22qter partial trisomy, resulted of three copies of *SHANK3* and another 24 genes, and he was diagnosed with Asperger syndrome (mild ASD). He demonstrated precocious language

development, fluent speech and impaired social communication. Notably, in the boy, the presence of an additional copy of 22q13 improved his language ability but caused a severe impairment in social communication. These results highlight the importance of a fine gene dosage for the development of speech, language and social communication.

Moessner et al. [19] studied a female patient with a 3.2 Mb deletion of chromosome 22q13.31-33 due to an unbalanced 22q13.31-33 monosomy — der(22) t(14;22) (q32.33;q13.31). Her derivative chromosome was inherited from her father, who was phenotypically normal and had a de novo balanced translocation — t(14;22) (q32.33;q13.31). Her sister had a partial 22q13.3 trisomy, which was due to inheritance of another derivative chromosome — der(14) t(14;22)(q32.33;q13.31). The two children developed different pathologies. The girl with 22q13 deletion had the autism and her sister was diagnosed with an attention-deficit/hyperactivity disorder and mild cognitive impairment.

These two cases have a similar duplication to our patient 2, but the three cases have different phenotypes. The different outcomes observed may be explained by the size of duplication: in our case the duplication only involved three genes (*SHANK3, ACR* and *RABL2B*), in the case of Durand and colleagues [6], the duplication had 800 kb and, in the case of Moessner and colleagues [19], the duplication had 3.2 Mb. Although, this does not explain the reason why patient's mother has the same duplication as her son and does not present the same phenotype. Thus, we cannot rule out the proposed first hypothesis that the duplication found in our patient can be coincident with ASD diagnosis. On the other hand, the patient's mother may have a mild neurodevelopmental impairment that has never been diagnosed.

Both loss and gain of one copy of *SHANK3* may be associated with neurodevelopmental impairment, suggesting that a proper *SHANK3* dosage is critical for normal development and functionality of the brain. In this way, the neurological impairment that occur in patients with deletions or duplications of 22q13 reinforces the idea that the gene dosage effects, can be on the basis of some neurodevelopmental and neuropsychiatric disorders [34]. The effects of *SHANK3* dosage and its consequences are not yet well understood.

CONCLUSION

This study described the molecular genetic data, the clinical features and the outcomes of 3 cases with *SHANK3* deletion or duplication. The findings help us to better understand the phenotype associated with the *SHANK3* deficiency and overexpression.

Patients with PMS present a range of phenotype characteristics, namely in the speech, behaviour and neurological and neurodevelopmental features. Our study showed that the *SHANK3* haploinsufficiency due to interstitial deletion, only disrupting the *SHANK3* gene, is enough to cause a set of features associated with PMS. Until nowadays, few studies established the relationship between PMS phenotype and interstitial deletion. Thereby, in the future, further research should be done.

Finally, our case agrees with the hypothesis that perhaps not only the *SHANK3* deficiency is associated to ASD, but also the *SHANK3* overexpression. In this way, more studies should be also done for a better understanding of the gene dosage effect in individual's phenotype.

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