Phelan–McDermid Syndrome: Expanding the Phenotype

Original Article

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ABSTRACT

Background: Phelan-McDermid syndrome (PMS) is a rare neurodevelopmental disorder recognized by developmental delay, mild dysmorphisms, autism spectrum disorder (ASD), and intellectual disability (ID).
Objectives: The aim of this work was to describe an Egyptian cohort of PMS patients and investigate the phenotype-genotype correlation.
Patients and Methods: Four patients with dysmorphic features, delayed milestones and autistic behavior were subjected to clinical examination, karyotyping, Fluorescence In Situ Hybridization (FISH), Multiplex ligation dependent probe amplification (MLPA) Chromosomal microarray (CMA) analysis.
Results: One patient presented with microcephaly. Echocardiography showed patent foramen ovale (PFO) and bilateral abnormal superior vena cava (SVC) in one patient. MRI revealed vermal hypoplasia and kinked corpus callosum in one patient and cerebral atrophy in another patient. All patients disclosed ID, while autistic behavior was noted in 3 patients. Karyotype detected no abnormality in 3 patients, while the fourth revealed ring chromosome 22. MLPA identified heterozygous deletion at 22q13 in 2 patients. FISH and CMA detected heterozygous deletion at 22q13 in one patient. Relation between patient's results and types of graft used showed no statistically significant differences between them.
Conclusion: Our study highlighted the occurrence of ASD in individuals with PMS due to *SHANK3* deletion, though, some individuals could compensate for such deletions. This report expanded the PMS phenotype and described some anomalies that to the best of our knowledge have not been previously described.

Key Words: CMA, 22q13 Deletion, FISH, MLPA, Phelan-McDermid syndrome, *SHANK3* gene.

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INTRODUCTION

Phelan-McDermid syndrome (PMS; OMIM: 606232) is caused by microdeletion on the terminal end of chromosome 22q (22q13.3) (**Ziats *et al*., 2019**). Patients are consistently indicated by global developmental delay, severely delayed language development, autism spectrum disorder (ASD) behaviors, intellectual disability, hypotonia and non-specific dysmorphic features (**Denayer *et al*., 2012; Phelan and McDermid, 2012**). The most common characteristics are dolichocephaly, long eyelashes, prominent or dysplastic ears, bulbous nose, full lips, pointed chin, large fleshy hands, and hypoplastic / dysplastic nails (**Deibert *et al*., 2019**). Macrocephaly and overgrowth are described in a higher incidence among these patients. Autistic disorders, seizures, congenital heart disease and renal abnormalities are usually accompanying the disease (**Dhar *et al*., 2010; Soorya *et al*., 2013**). Vision problems comprise myopia, strabismus, and retinitis pigmentosa (**Koolen *et al*., 2005**). Dental abnormalities are common including malocclusion, periodontal disease, and tooth decay (**Soorya *et al*., 2013**). The terminal deletion of 22q13 can be unveiled by karyotype study and FISH, that has the advantage of high resolution and accuracy, which is valuable for the diagnosis of microdeletion or microduplication syndromes. However, several terminal and interstitial deletions are very small. Therefore, the CMA has been the diagnostic tool of choice for elucidating multiple genetic problems (**Zhao and Wan, 2019**).

Our goal was to describe an Egyptian cohort of PMS patients and investigate the phenotype- genotype correlation.

Clinical reports

Case (1)

A female patient, 3.6 years old, she presented to the Clinical Genetics Department, National Research Centre,

Egypt, with autistic behavior and congenital heart disease. She was an offspring of first cousin consanguineous marriage with an older apparently normal sister. Her birth weight was average at first month of age, she developed aspiration pneumonia and cyanosis. The patient had delayed physical and mental milestones and walking has not been developed yet. There were minimal dysmorphic features including thick eyebrows, long eye lashes, short neck and low set ears (the parents do not have thick eyebrows or long eye lashes) (Fig. 1a). Anthropometric measurements at time of referral were within normal. Patient developed frequent epileptic seizures with coma. She suffered continuous constipation. Echocardiography was done showing patent foramen ovale (PFO) and bilateral abnormal superior vena cava (SVC). Brain MRI, abdominal-pelvic ultrasonography revealed normal results. Electroencephalogram (EEG) showed focal epileptogenic discharge. She had severe autistic behavior when evaluated using Autism Rating Scale (CARS).

Case (2)

A female patient 6.6 years old, presented with delayed mental milestone. She was an offspring of distant consanguineous mating. She has apparently normal two elder sisters and brother. Mother had a history of four successive abortions before her birth. Pregnancy and delivery histories were unremarkable. Patient had a history of sleep disorder, constipation, and repeated infections. No history of convulsion. She had some dysmorphic features in the form of bilateral epicanthal folds, large simple asymmetric ears. Anthropometric measurements were normal for age. Brain MRI showed vermal hypoplasia, kinked corpus callosum. EEG was normal. Autistic behavior was severe when evaluated by CARS.

Case (3)

A female patient 3.8 years was referred with delayed developmental milestone. She was the offspring of non-consanguineous marriage, pregnancy and delivery history were uneventful apart from low birth weight. Dysmorphic features were mid face hypoplasia and facial asymmetry, blepharophimosis, epicanthal folds, upwards slanting of palpebral fissure, broad nasal bridge, long philtrum, retromicrognathia, low set ears, hirsutism, multiple café au lait patches on back and abdomen, absent labia minora. Anthropometric measures revealed microcephaly (-5.8 SD), height and weight were at mean for her age. The IQ was 38, no autistic behavior was noted.

Case (4)

A male patient,15 years old, the offspring of non-consanguineous marriage. He showed severe delayed language and dysmorphic features including prominent nose, short philtrum, retrognathia, sparse scalp hair, bilateral soft tissue syndactyly between 2nd and 3rd toes, hirsutism, and bilateral lymphedema of lower limbs (Fig. 1b and c). Anthropometric measures were within normal. EEG revealed epileptogenic discharge, the MRI brain showed cortical atrophy and the IQ was 40. Autistic behavior was severe when evaluated by CARS.

PATIENTS AND METHODS:

All patients were subjected to routine G-banding chromosomal analysis. FISH, MLPA or CMA were also performed. This study was approved by the ethical committee of the National Research Centre. An informed consent was attained from the patients and their parents. Conventional cytogenetics analysis for the four patients was implemented conforming to the standard protocols (**Verma and Babu, 1995**). A total of 20 metaphases were analyzed for the patients and their parents and karyotyped according to the ISCN (**ISCN, 2016**).

MLPA assay was performed for the first two patients using the SALSA MLPA Probemix P245-B1 Microdeletion Syndromes-1A, according to the manufacturer's instruction (MRC-Holland, Netherlands). DNA denaturation and overnight hybridization of the MLPA probemix were done, followed by probe ligation and amplification on the next day. Separation of amplified products were done using Genetic Analyzer ABI 3500 (USA). Interpretation of the results was done utilizing Coffalyser.Net software (MRC-Holland). Ratios less than 0.75 were considered as deletion, between 0.75 and 1.30 as normal and more than 1.30 as duplication. While CMA was carried out for the third patient. CMA was performed by a CytoScan TM HD array (Affymetrix, Cambridge, UK) and Chromosome Analysis Suite (ChAS 3.1) software (Affymetrix, Santa Clara USA) according to the manufacturer's instructions. FISH was carried out for the fourth patient using Cytocell DiGeorge/VCFS TUPLE1 and 22q13.3 Deletion Syndrome FISH Probe Combination according to the manufacturer's instructions. The TUPLE1 probe is 113kb, labelled in red, and identifies most of the TUPLE1 (HIRA) gene. The N85A3 (44kb) probe, recognized in green, is situated within 22q13.3 and delineates the telomeric end of the *SHANK3* gene. The two unique sequences provide control probes for each other and allow the clarification of chromosome 22.

RESULTS:

A total of 4 patients, their age ranged from 3-15 years, 3 females and one male. Two of them were the offsprings of consanguineous marriage, 2 were low birth weight and all had delayed milestones and showed dysmorphic features (Figure 1). Detailed clinical and cytogenetic data were illustrated in (Table 1).

Cytogenetic Results

G-banding karyotype analysis in blood for the first three patients showed normal female karyotype. While the fourth patient showed a de novo 46,XY,r(22) in all analyzed metaphases. FISH was carried out for him which showed 22q13.3 deletion in the ring in all analyzed metaphases and interphases (Figure 2). MLPA assay for the first two patients showed heterozygous deletion at 22q13, PMS region. The deletion includes *SHANK3* and *RABL2B* genes (Figure 3). While CMA for the third patient showed around 2.127 Mb and 264.4 Kb heterozygous deletion at 22q13.32q13.33 and 22q13.33 respectively. The deletions were defined as chr22:48,737,989-50,864,696bp and chr22:50,916,642-51,181,078 (hg19) (Figure 4). The deleted region was accommodating several RefSeq/ OMIM genes comprising *TUBGCP6, MAPK8IP2, ARSA, SHANK3, ACR* and *RABL2B* genes.

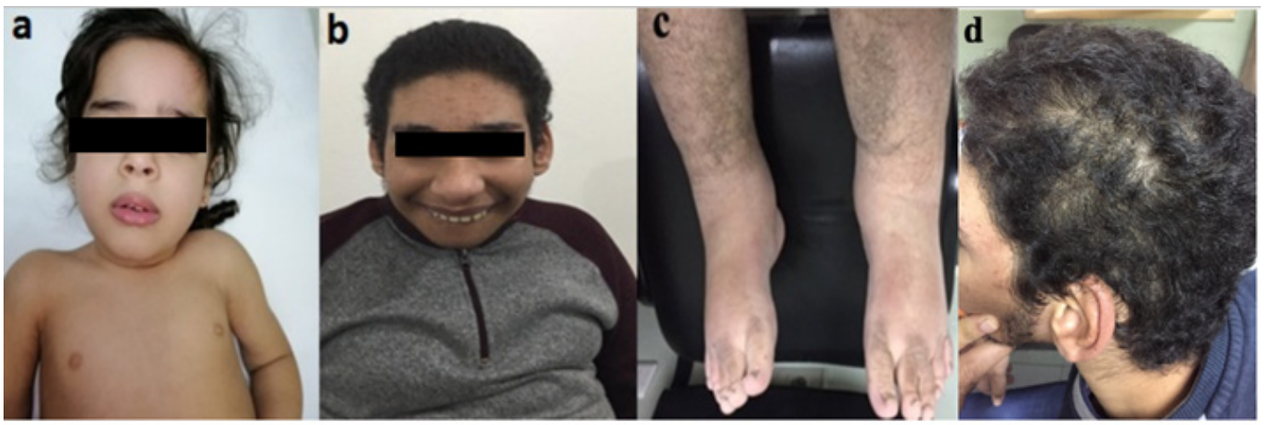

Fig. 1: a. 1st patient is showing broad forehead, thick eyebrows, upturned nose, short neck and low set ears, widely spaced nipples. b, c and d. of the 4th patient showing pear shaped bulbus nose, squint, hypertelorism, retrognathia, sparse scalp hair, short neck, bilateral syndactly between 2nd and 3rd toes, hirsutism, bilateral lymphedema of lower limbs. An informed consent and a permission for the publication of patients' faces was attained from the patients and patients' guardians. Patient 2 and 3 refused to be photographed.

Table 1: The clinical and cytogenetic findings of the Phelan–McDermid syndrome patients

	Case 1	Case 2	Case 3	Case 4
Age/gender	3/F	6.6/F	3.8/F	15/M
Consanguinity	+	+	-	-
Birth weight	average	average	low	low
Delayed milestones	+	+	+	+
Dysmorphic features	Long eye lashes, broad forehead, thick eyebrows, short neck and low set ears	Epicanthal folds, large simple asymmetric ears	Mid face hypoplasia and facial asymmetry, almond shaped eyes, blepharophimosis, upwards slanting of eyes, broad nasal bridge, epicanthal folds, long philtrum, retromicrognathia, low set ears, hirsutism, café au lait patches on back and abdomen, absent labia minora.	Prominent nose, retrognathia, bilateral syndactyly between 2nd and 3rd toes, bilateral lymphedema of lower limbs
Anthropometric measurements	Within normal	Within normal	Microcephaly (-5.8SD)	Within normal
Abdominal ultrasonography	Normal	Normal	Normal	Normal

Echocardiography	Patent foramen ovale and bilateral abnormal superior vena cava	Normal	Normal	Normal
EEG	Focal Epileptogenic discharge	Normal	Normal	Diffuse cerebral dysfunction
MRI brain	Normal	Vermal hypoplasia, kinked Corpus callosum	Normal	Cerebral atrophy
IQ	<34	<34	68	50
CARS	Severe autistic behavior	Severe autistic behavior	Normal	Severe autistic behavior
Karyotype	Normal	Normal	Normal	46,XY,r(22)
FISH				22q13.3 deletion
MLPA	Heterozygous deletion at 22q13.33	Heterozygous deletion at 22q13.33	-	-
CMA	-	-	Heterozygous deletion at 22q13.32q13.33 and at 22q13.33	-

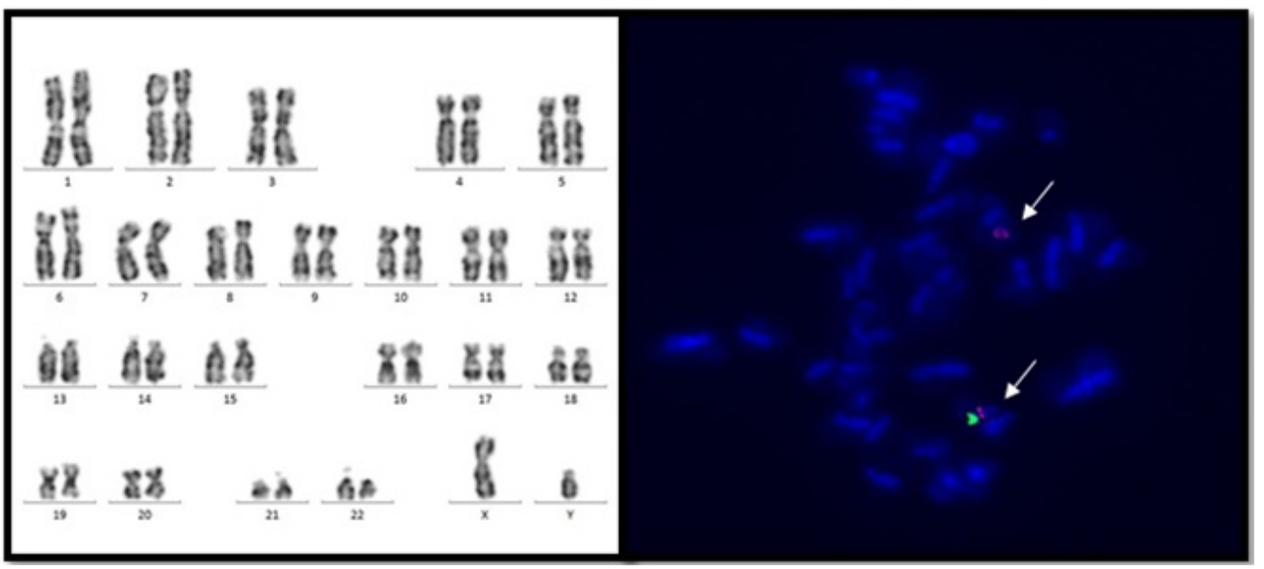

Fig. 2: a. Karyotype of the 4th patient showing ring chromosome 22; 46,XY,r (22). b. Metaphase FISH analysis showing 22q13.3 deletion in the ring (arrows show the normal chromosome and the ring chromosome 22 with the deletion).

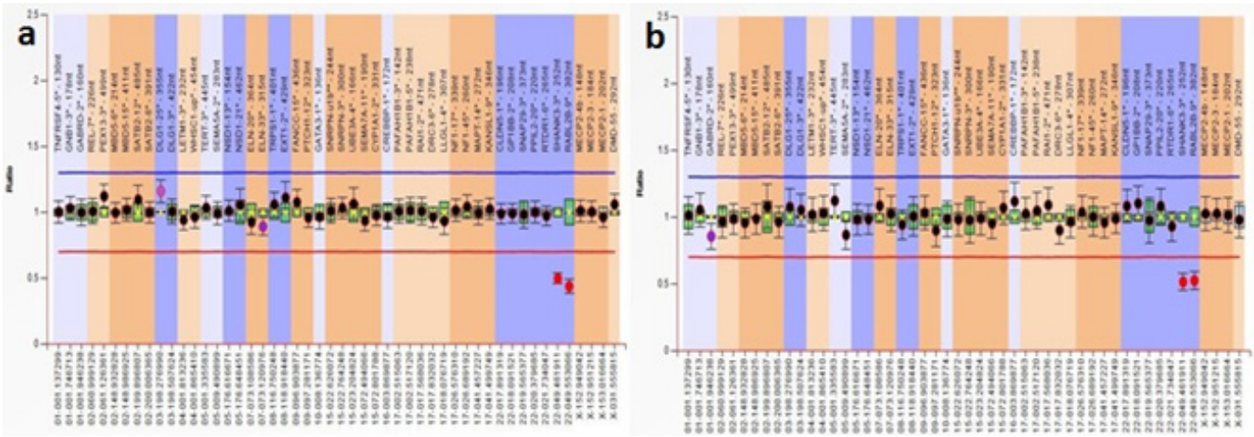

Fig. 3: Ratio charts of MLPA results for the 1st two patients using SALSA MLPA Probemix P245-B1 Microdeletion Syndromes-1A. The spots exemplify the MLPA probes, the lower red line indicates a peak ratio of 0.75, the probes below this line represents a deletion, the probes between the two lines are considered as normal two copies. The upper blue line denotes a peak ratio of 1.3 and any probes above this line characterizes a duplication. The chart is displaying deletion at 22q13, Phelan-McDermid syndrome region. The deletion is represented by the red spots below the deletion cut-offline (red) in the ratio chart.

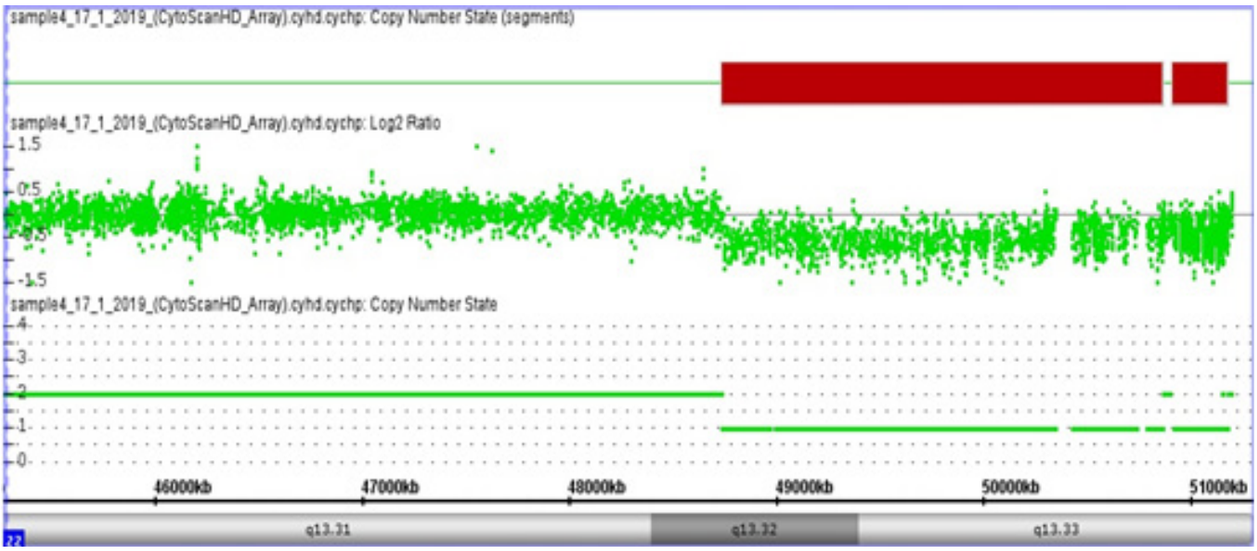

Fig. 4: The specified assessment of the CytosScan TM HD array (Affymetrix) of the 3rd patient displaying 2.127 Mb and 264.4 Kb heterozygous deletion at 22q13.32q13.33 and 22q13.33 respectively.

DISCUSSION

PMS is a neurodevelopmental disorder characterized by severely delayed language development, non-specific facial features, and intellectual disability. Genotype-phenotype correlations were investigated by different authors, showing conflicting results (**Luciani *et al*., 2003; Wilson *et al*., 2003; Jeffries *et al*., 2005; Sarasua *et al*., 2013**). Here in, we report on four new Egyptian patients with de novo 22q13.3 deletions. Patients of the current study demonstrated a variable range and severity of the phenotype. They shared intellectual disability, delayed or absent speech, autistic behaviors and non–constant dysmorphic features, ranging from minimal features to severe dysmorphism. Interestingly, our patients exhibited new findings which have not been previously reported including broad forehead, thick eyebrows, short neck and wide spaced nipples, facial asymmetry, blepharophimosis, arachnodactyly, retromicrognathia, hirsutism, café au lait patches and absent labia minora. These findings may extend the phenotypic spectrum of 22q13.3 deletion syndrome. However, bilateral toes syndactyly of the 4th patient and bilateral lymphedema have been denoted by previous investigators (**Nesslinger *et al*., 1994; Dhar *et al*., 2010; Mahajan *et al*., 2012; Sarasua *et al*., 2013; Soorya *et al*., 2013**). Furthermore, two patients complained of constipation which was reported by **Hussong *et al*., (2020)** in 19.4 % of their patients and they stated that

constipation is not considered a major problem in PMS. The anthropometric measurements were within normal in all patients, but the 3rd patient was microcephalic (-5.8 SD). Some patients may have microcephaly or macrocephaly; therefore, the measurement of the head circumference ought to be assessed regularly in PMS up to age 3 years (**Zhu *et al*., 2018**). The deleted region in that patient included *TUBGCP6, MAPK8IP2, ARSA, SHANK3, ACR* and *RABL2B* genes. Several authors recognized that *TUBGCP6* gene is related to microcephaly (**Hull *et al*., 2019; Martin *et al*., 2014**).

None of our patients presented with renal problems, although, renal abnormalities are deliberated to be common in PMS. Previous studies suggested a frequency of 38% (**Samogy-Costa *et al*., 2019**), but few studies did not describe renal problems (**De Rubeis *et al*., 2018)**.

The incidence of congenital heart defects in PMS is invariable, however, it is described in more than 25% of patients (**Phelan and McDermid., 2012**). The most common stated defects are atrial septal defect, patent ductus arteriosus, tricuspid valve regurgitation, and total anomalous pulmonary return (**Kolevzon *et al*., 2014**). Echocardiography of the first patient showed PFO and bilateral abnormal SVC which is one of the rarest variations of the systemic venous return anomalies, deliberated by cyanosis and right-to-left shunt physiology. To our knowledge this anomaly has not been previously reported in PMS.

The EEG displayed epileptogenic discharge in two patients. Prevalence estimates of seizure disorders range from 0% to 31% (**Philippe *et al*., 2008**). MRI brain revealed vermal hypoplasia and kinked corpus callosum in one patient and cerebral atrophy in another patient, this corroborates with the results previously mentioned in literature (**De Rubeis *et al*., 2018; Ismail *et al*., 2018**).

The IQ disclosed moderate/ severe ID in our patients. Moreover, autistic behavior was evident in three patients. Whereas **Ismail *et al*., (2018)** stated profound ID and autistic behavior in all their patients. *SHANK3* gene is the demanding gene in the deletion syndrome, which may instigate the neurodevelopmental delay of PMS (**Upadia *et al*., 2018; Phelan *et al*., 2022**), it is contiguous to a gene constellation (*ARSA* and *MAPK8IP2*) that possibly cause ASD (**Costales and Kolevzon *et al*., 2015; Mitz *et al*., 2018**). **Nevertheless, Tabet *et al*., (2017)** demonstrated the genomic and clinical heterogeneity of individuals with 22q13 relocations in a family with five affected siblings who inbred a *SHANK3* deletion from a normal mother, without ID nor autistic behavior, emphasizing that some persons can compensate for these deletions.

Karyotyping is necessary to confirm the presence of deletions; it must be implemented for children with dysmorphic features accompanied by ASD or developmental delays (**Phelan *et al*., 2022**). G-banding karyotype analysis detected no abnormality in 3 patients while the fourth patient revealed ring chromosome 22. Nearly, 20% of the Phelan–McDermid syndrome cases result from ring chromosome 22 or unbalanced translocation disrupting the 22q13 region (**Ismail *et al*., 2018**). The terminal deletion of 22q13 can be disclosed by karyotype analysis, but many interstitial and terminal deletions are too small. Consequently, the molecular cytogenetics technology has been the diagnostic tool of choice for unraveling the multiple genetic disorders. FISH analysis is valuable for detecting cases of chromosomal rearrangements such as the ring chromosome and mosaicism. Additional, testing by MLPA or other dosage-sensitive procedures for the detection of duplications and smaller intragenic deletions such as CMA, may be considered particularly after a negative result. In our study, MLPA showed heterozygous deletion at 22q13 in 2 patients. While FISH detected 22q13.3 deletion in one patient. However, CMA detected 2.127 Mb and 264.4 Kb heterozygous deletion at 22q13.32q13.33 and at 22q13.33 in one patient. The deleted region includes *TUBGCP6, MAPK8IP2, ARSA, SHANK3, ACR* and *RABL2B* genes. In consequence, the deletion size varies from 100 Kb to 95 Mb. The smallest deletion includes three genes *SHANK3*, *ACR* and *RABL2B* while the largest deletions include more than 90 genes, such deleted genes may be implicated in various manifestations of PMS (**Anderlid *et al*., 2002; Bonaglia *et al*., 2006**). Deletions smaller than 1 Mb are infrequent comprising (around 3%). Deletions with large sizes are accompanied with severe social-communication impairments associated to ASD (**Soorya *et al*., 2013**). However, other studies could not identify a relationship between the phenotypic severity and the deletion size (**Rozas *et al*., 2019**). Chromosomal microarray identifies most of the PMS patients that frequently result from structural rearrangements or deletions producing copy number loss of variable size on 22q13 but does not identify patients with *SHANK3* gene mutations or smaller intragenic duplications or deletions (**Dhar *et al*., 2010; Deibert *et al*., 2019**). *SHANK3* gene, the critical gene in the deletion syndrome, is responsible for the core phenotypic features of PMS (**Phelan *et al*., 2022**). Our results displayed the predominance of ASD in patients with PMS due to *SHANK3* deletion, similar to our previous results in patients with 22q13 deletions (**Ismail *et al*., 2018**).

Several reports have observed genotype-phenotype correlations with contradictory outcomes (**Sarasua *et al*., 2013**). Associations have been detected between larger deletion sizes and some dysmorphic features, developmental delay, hypotonia, and the absence of ASD diagnosis (**Wilson *et al*., 2003; Sarasua *et al*., 2013**). Despite small sample sizes, our study confirmed the clinical and genomic heterogeneity of PMS individuals. Moreover, our findings revealed the prevalence of ASD in patients with PMS resulting from *SHANK3* deletion,

this suggests that some cases could compensate for such deletions. This study further expanded the phenotype of PMS and described some anomalies that to the best of our knowledge have not been formerly described.

CONFLICT OF INTEREST

There are no conflicts of interest.

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