Chapter

First-Tier Array CGH in Clinically Variable Entity Diagnosis: 22q13.3 Deletion Syndrome

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Abstract

Phelan-McDermid (PMS) or 22q13 deletion syndrome (OMIM 606232) is a rare genetic disorder with highly variable clinical presentation. The phenotype includes generalized neonatal hypotonia, developmental delay with intellectual disability and delayed speech, mild dysmorphic features, and autistic behavior. The genetic defects of PMS consist of 22q13.3 deletions or chromosomal structural rearrangements involving *SHANK3* gene; the loss of function mutations of *SHANK3* gene was reported in a minority of cases. The 22q13.3 deletions vary in size, from 0.2 to over 9 Mb, and, although larger deletions are generally associated with more severe phenotypes, the genotype-phenotype correlations are not clear-cut for all patients. *SHANK3* is considered the main candidate gene for the neurologic features of PMS. PMS is a rare disorder, often underdiagnosed. There are no established clinical diagnostic criteria for PMS. The genetic tests typically used are chromosomal microarray and multiplex ligation-dependent probe amplification (MLPA) or fluorescent in situ hybridization (FISH) for copy number analysis of *SHANK3* gene; next-generation sequencing (NGS) or Sanger sequencing is used for pathogenic mutation screening of *SHANK3*. In this chapter, we report three cases with PMS and summarize the clinical and genetic diagnostic approaches of this condition, highlighting the role of chromosomal microarray technology in the identification of rare, but significantly impacting patient's life, DNA copy number abnormalities.

Keywords: chromosomal microarray, deletions, intellectual disability, speech delay, autistic features

1. Introduction

Phelan-McDermid or 22q13 deletion syndrome (OMIM 606232, PMS) is a genetic disorder characterized by a wide phenotypic spectrum that includes neonatal hypotonia, global developmental delay with cognitive deficits, absent to severely delayed speech (usually, more advanced perceptive language than expressive language), dysmorphic features (dolichocephaly, large or malformed ears, full brows, full cheeks, bulbous nose, pointed chin), behavioral abnormalities with autistic features (poor eye contact, stereotypic movements, impaired social interactions, aggressive behavior), and various neurological problems (reduced perception of pain, seizures, abnormal patterns of movements, habitual chewing or mouthing,

inability to regulate sweating) [1–3]. Patients with PMS are highly unlikely to ever function independently. Less often, congenital kidney anomalies [3], arachnoid cysts [1], and higher frequency of other pathological conditions (gastrointestinal disease, upper respiratory tract infections, ventriculomegaly, dysmyelination, morphological changes of the corpus callosum [4]) are described. No life-threatening malformations are associated with this disorder; however, as the number of patients reaching older ages is small, there may be yet a not identified potential for developing life-threatening conditions. Its incidence is not known, but the PMS Foundation estimates approximately 1949 patients worldwide, as of September 2018 on http://www.22q13.org; the prevalence of subjects with PMS in patients with neurodevelopmental delay was estimated at 0.27% [5]; it is present with equal frequency in both sexes.

The genetic anomalies associated with PMS involve 22q13.3 region, leading to *SHANK3* gene haploinsufficiency, in the vast majority of cases. Simple deletions were described in ~79% of patients; deletions generated by unbalanced translocations, ring chromosomes, or complex chromosomal rearrangements were reported in ~18% of patients. In addition, mutations involving *SHANK3* gene were detected in a minority of patients (~3%) with PMS phenotype [6].

Chromosome 22q13.3 deletions range from 0.2 to over 9 Mb [5, 7]. A critical region for PMS was refined in 2002 by Anderlid et al. [8], to 100 kb containing *SHANK3*, *ACR*, and *RABL2B*, with *SHANK3* being the candidate gene for the clinical features of PMS. In 2001, Bonaglia et al. [9] reported a patient with a phenotype consistent with PMS, bearing a translocation t(12;22) which disrupted *SHANK3* gene only. Subsequent studies, reporting small chromosomal deletions and mutations, brought further evidence supporting the role of *SHANK3* gene in PMS neuropsychiatric phenotype [10, 11]. Both contiguous gene deletion at 22q13.3, including SHANK3, and pathogenic variants of *SHANK3* were reported in PMS. Rarely, interstitial deletions with intact *SHANK3* and PMS-like phenotypes have been described, indicating that haploinsufficiency of other genes or a positional effect influencing *SHANK3* expression may cause the same phenotype in some patients [12–14].

SHANK3 encodes for a scaffold protein that connects ion channels and receptors in the postsynaptic density of glutamatergic synaptic membrane to the cytoskeleton, thus participating in a signal transduction pathway highly relevant to various forms of autism spectrum disorder (ASD) [1, 4, 15, 16]. Neurons differentiated from induced pluripotent stem cells from patients with PMS have reduced *SHANK3* expression and major defects in excitatory, but not inhibitory synaptic transmission [17]. Yi et al. [18] demonstrated, in 2016, that *SHANK3* haploinsufficiency decreases I_h channel function, leading to altered synaptic function.

The deletions in PMS patients span a large genomic region and encompass numerous protein coding genes; many of these genes are highly expressed in the brain [19]. Genotype-phenotype correlations connected larger deletion sizes with the severity of the developmental profile, hypotonia, increased number of medical comorbidities, dysmorphic features, and absence of an ASD diagnosis [12]; on the contrary, other studies suggested more severe autistic phenotype in patients with larger deletions, or even no association at all [4]. Additionally, it has been hypothesized that the increase in the deletion size may attenuate the effects of *SHANK3* deficiency, i.e., autistic features, due to additional genes concomitant deletions; also, the severe developmental and language impairments observed in patients with larger deletions make the evaluation of autistic features more difficult [12].

The genetic anomalies in PMS are de novo (80% of cases) or inherited from parents with balanced translocations, inversions, or mosaics (20% of cases); Sarasua

et al. [12] reported a 75% inheritance of the abnormal chromosome from the father, but no association with parental age at conception. PMS has been described both in mosaic and non-mosaic forms.

PMS is a rare disorder, with a broad phenotypic spectrum, that often goes underdiagnosed. As there are no specific clinical features suggestive for PMS, the diagnosis is genetic. Chromosomal microarray is the method most commonly used, as a first-tier approach; various techniques can be used for confirmation of copy number imbalance (fluorescent in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), or qPCR. We report three cases with PMS and summarize the clinical and genetic diagnostic approaches of this condition, highlighting the role of array-based comparative genomic hybridization (array CGH) technology in identification of rare, but significantly impacting patient's life, DNA copy number abnormalities.

2. Materials and methods

Our patients have been referred to clinical and genetic evaluation for developmental delay and behavior problems. The patients have been clinically evaluated by a multidisciplinary team including child psychiatry and child neurology specialists, as well as a psychologist. Array-based comparative genomic hybridization (array CGH) using two oligonucleotide platforms (180 and 60 K) was performed on genomic DNA (gDNA) isolated from peripheral blood, as recommended by manufacturer (Agilent Technologies, Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis Enzymatic Labeling for Blood, Cells, or Tissues Protocol, Version 7.3, March 2014). FISH using bacterial artificial chromosome (BAC) probes RP11-316I10 (22q11.21), CTA799F10, and cosmide n85a3 (22q13.33) was carried out according to standard protocol, in probands and parents.

All three patients were born to healthy, non-consanguineous parents, following uncomplicated pregnancies and with no perinatal events. Patients 1 and 2 are siblings; patient 3 is the second child, having a healthy sibling. **Table 1** summarizes the clinical characteristics of our patients.

The first patient, a 4-year-old boy, with a height of 91 cm (Pc 5), weight of 16 kg (Pc 25), and occipitofrontal circumference (OCP) of 49 cm (Pc 25), presented delayed psychomotor development (walked alone at 18 months, says no syllables); minor dysmorphic features: hypoplastic helix, bilateral epicanthus, long eyelashes, and thin upper lip; bilateral 2nd–3rd toes partial cutaneous syndactyly; and sacral dermal sinus. Neurological evaluation revealed gross and fine motor inabilities and absent speech. Psychiatric and psychologic evaluation showed severe intellectual disability, autistic features (absent psychical and visual contact, stereotypic play, no social interactions), and hyperkinesia with aggressive behavior. He had a history of repeated respiratory infections. Electroencephalography (EEG) and abdominal ultrasound investigations were normal. Brain MRI revealed minimal dysmyelination and corpus callosum hypotrophy. Metabolic screening tests for Pompe disease, neurolipidosis, mucopolysaccharidosis, glycoproteinosis, mucolipidosis type I, and serum amino acids were normal.

The second patient, a 2-year-old sister of patient 1, with a height of 81 cm (Pc 10), weight of 11.2 kg (Pc 25), and occipitofrontal circumference (OCP) of 47 cm (Pc 25–50), presented moderately delayed psychomotor development (walked alone at 1.5 year old; had first syllables at 6 months without any progression afterward), micrognathia, and bilateral 2nd–3rd toes partial cutaneous syndactyly. Neurological examination showed gait disorders (an instable gait, with left lower limb rotated

Features	Patient 1	Patient 2	Patient 3
Neonatal hypotonia	+	+	+
Normal to accelerated growth	+	+	+
Absent to severely delayed speech	+	+	+
Global developmental delay	+	+	+
Minor dysmorphic facial features	+	+	+
Behavior characteristics			
• Autistic-like affect and behavior	+	+	+
• Mouthing or chewing nonfood items	—	—	—
• Decreased perception of pain	NA	NA	NA
Relatively large and fleshy hands	+	+	—
Dysplastic toenails	—	—	—
sacral dimple	+	—	—
Decreased perspiration	NA	NA	NA
Feeding difficulties	—	—	—
Strabismus	—	—	—
Renal problems	—	—	—
Seizures	—	—	—

NA, not assessed; +, present; –, absent.

Table 1.
Clinical characteristics of our patients.

outside) and speech delay (she says only few syllables). Psychiatric and psychologic evaluation revealed moderate developmental delay and autistic features (did not respond to commands; was not sociable, playing alone, and imitating only few actions). Her history included prolonged neonatal icterus (Crigler-Najjar syndrome type II). Her EEG was normal.

The third patient, an 11-year-old girl, presented delayed psychomotor development; she walked alone at 2 years and said first syllables after 5 years. The neurological evaluation at 11 years and 7 months showed severe intellectual disability and severe speech delay (she said only few mono- and disyllabic words, understood few simple orders). Brain MRI showed cerebellar subarachnoid cyst.

All three patients were investigated with genome-wide array CGH platforms. Array CGH experiments were performed using SurePrint G3 Human CGH Microarray Kits 180 K containing 170,334 distinct oligonucleotide probes (patients 1 and 2) and 60 k containing 55,077 distinct oligonucleotide probes (patient 3). The median probe spacing in RefSeq genes was 11 kb for 180 K platform and 33 kb for 60 K platform, respectively. The genomic gDNA was extracted using PureLink Genomic DNA Mini Kit (Invitrogen, Thermo Fisher Scientific); the concentration and quality of gDNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Normal human gDNAs (*Agilent Human Reference DNA* Male or Female, according to the sex of the patient) were used as reference DNA. The experimental procedures were conducted using 1000 ng gDNA for 180 K microarray kit and 500 ng for 60 K microarray kit, for each sample (test and reference). After the enzymatic digestion of gDNA with *Alu*I and *Rsa*I, the reference DNA samples were labeled with cyanine 5-deoxyuridine triphosphate (Cy5-dUTP) and the patients DNA samples with Cy3-dUTP, respectively, using the

SureTag DNA Labeling Kit (Agilent Technologies). The labeled test and reference samples were pooled and purified together using Agilent Purification Columns. The DNA mixture was combined with Cot-1 DNA (Agilent Technologies), 10× aCGH blocking agent, and 2× HI-RPM hybridization buffer (Agilent Technologies); the mixture was dispensed to a microarray slide, assembled with an Agilent hybridization chamber, and incubated at 67°C for 24 h at 20 rpm. The hybridization was followed by a two-step washing procedure using Wash Buffer 1 and Wash Buffer 2 (Agilent Technologies). The microarray slides were scanned with Agilent SureScan Microarray Scanner system, and the data were extracted and analyzed using Agilent Cytogenomics software (Agilent Technologies). Copy number variations (CNVs) were called if they encompassed at least three consecutive probes with a log2 ratio value over 0.25 or below −0.25. For CNV clinical interpretation, public databases were used: UCSC (http://genome.ucsc.edu, accessed July 15, 2019); DGV (http://dgv.tcag.ca/dgv/app/), OMIM (http://www.omim.org/); DECIPHER (http://decipher.sanger.ac.uk/), and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/).

3. Results and discussion

PMS has been under continuous investigation since its description [20, 21] that benefited, in many cases, by the strong cooperation of the PMS Foundation. Most of the patients were recruited in the USA [7, 12, 20–23] and Western Europe [9, 24–29]; there are also reports from Korea [30] and China [31]. PMS seems to be underdiagnosed in many parts of the world. However, the wide use of chromosomal microarray as a first-tier test for patients with neurodevelopmental disorders, autism, and congenital malformations [32] has greatly improved both diagnostic sensitivity and yield.

The array CGH results of our patients revealed deletions of 22q13 including SHANK3 gene. The genomic profiling of the two siblings (patients 1 and 2) revealed an identical 1.38 Mb interstitial duplication and a 1.565 Mb distal deletion: arr[GRCh37] 22q13.31q13.33(48202588_49585589)x3 and 22q13.33(49628598_51193680)x1 (**Figure 1a**). The deletion was confirmed by FISH. The duplication contained 2 miRNAs and *FAM19A5*, a gene coding a protein expressed mainly in the brain, considered a putative immunomodulator in nervous cells [5], while the 1.56 Mb deleted region included 37 genes, among which are *ALG12*, *MLC1*, *SBF1*, *SCO2*, *TYMP*, *MAPK8IP2*, *ARSA*, *SHANK3*, *ACR*, *TUBGCP6*, and *CHKB* (**Figure 1b**). Karyotype analysis and FISH testing of both parents (CTA799F10 probe, 22q13.33) showed no visible anomalies.

Patient 3 had a 3.514 Mb deletion, arr[GRCh37] 22q13.31q13.33 (47664025_51178264)x1, containing 41 genes, among which are *ALG12*, *MLC1*, *SBF1*, *SCO2*, *TYMP*, *MAPK8IP2*, *SHANK3*, *ARSA*, *TUBGCP6*, and *CHKB* (**Figure 2a** and **b**). The result was confirmed by FISH, using RP11-316I10 probe (22q11.21) and cosmide n85a3 (22q13.33).

The cytogenetic findings in PMS may be variable. In a study gathering 201 PMS patients analyzed by array CGH, the vast majority had 22q13 terminal deletions. Duplications, varying in size from 0.02 to 6.84 Mb, localized centromeric to the deleted region were detected in 9% of patients [7]. Two of our patients (patients 1 and 2, siblings) have a similar pattern and a proximal duplication that accompanies a terminal deletion. This type of duplication-deletion cytogenetic changes have been reported for many chromosomes, and it is most likely mediated by a U-type exchange between sister chromatids [33]. Patient 3 had a simple terminal deletion.

22q13 region in the vicinity of SHANK3 gene is rich in genes with a high expression in the brain and also a high probability of *loss of function intolerance* [19, 34].

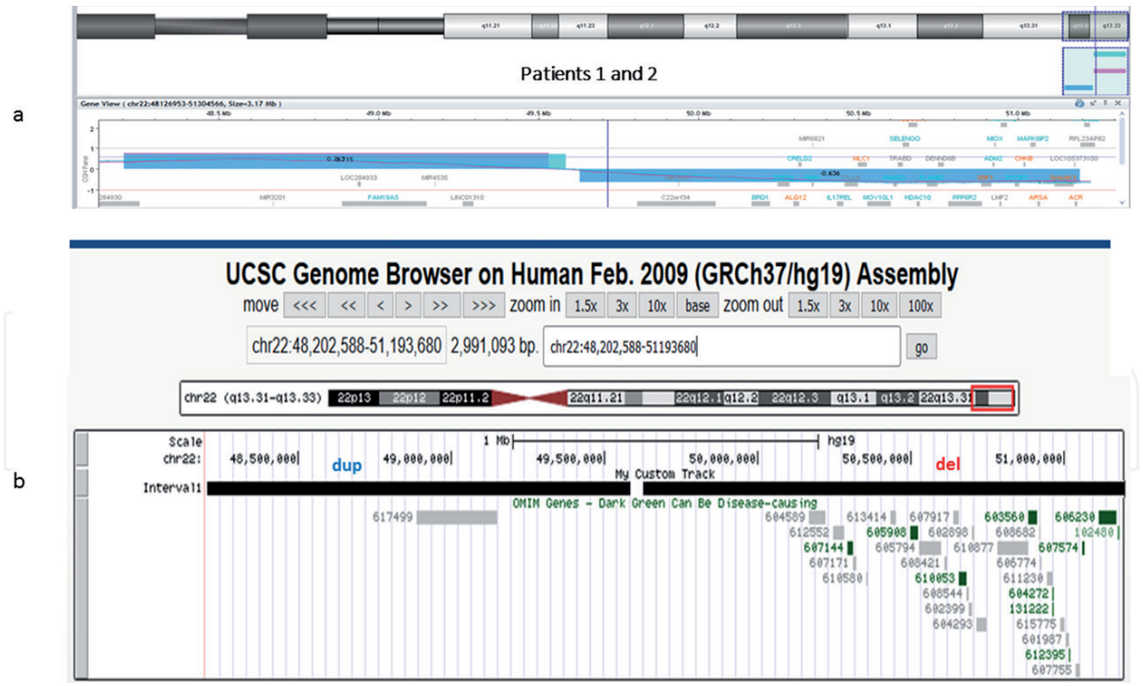

Figure 1.
(a) The genomic profiling of the two siblings (patients 1 and 2) revealing an identical 1.38 Mb interstitial duplication and a 1.565 Mb distal deletion and (b) UCSC Genome Browser window showing the genomic coordinates of the detected variants.

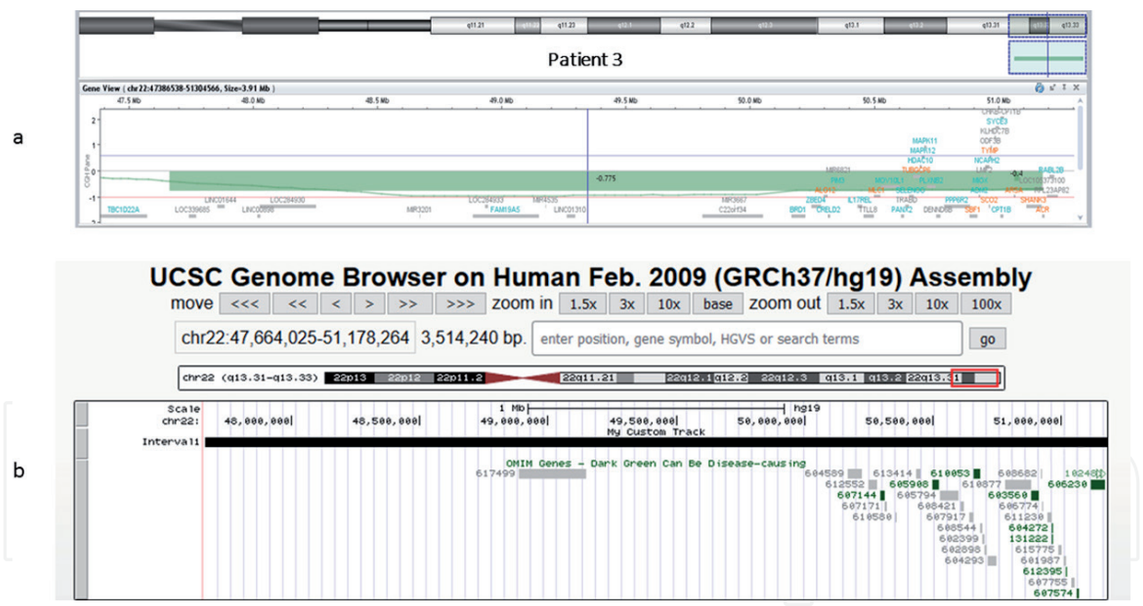

Figure 2.
(a) The genomic profiling of the patient 3 showing a 3.514 Mb deletion and (b) UCSC Genome Browser window showing the genomic coordinates of the detected variant.

The haploinsufficiency of these genes is considered to contribute not only to the variability of the phenotype but also to the neuropsychiatric features of PMS.

Among the genes located on 22q, *PARVB* and *SULT4A1* genes (telomeric to *SHANK3*) have been associated with increased severity of PMS [29]. Mitogen-activated protein kinase 8-interacting protein 2 (*MAPK8IP2*) localized centromeric to *SHANK3* codes a protein with high expressivity in the brain (postsynaptic density); experimental animal studies showed that mice lacking *MAPK8IP2* had motor and cognitive deficits [35]. *MLC1* gene variants have been reported as causal for megalencephalic leukoencephalopathy characterized by regression of development. Taking

into account the prevalence of regression symptoms in PMS, the relation between deletion of *MLC1* gene and regression might bring new insights [35].

Other genes in this region have been involved in particular aspects of PMS (e.g., *NUP50*, *CERK*, *KIAA1644*, *PHF21B*, *c22orf9*, *FBLN1*, *CELSR1*, or miRNA molecules located at 22q) [7]. Frye et al. [36] brought evidence that haploinsufficiency of genes related to mitochondrial functioning, located at or close to 22q13.3 (e.g., *TRMU*, *SCO2*, *TYMP*, *CPT1B*), may explain a part of the phenotypic variation in PMS patients.

PARVB and *WNT7B* gene loss as well as larger or more proximal deletions of 22q have been associated with absent speech [5, 7]. In addition, Soorya et al. [10] reported in 2013 that expressive and receptive language skills were not significantly associated with deletion size. Our patients 1 and 3 had absent speech; patient 2, evaluated at 2 years of age, also presented speech delay. The regions deleted in our patients did not correlate with the abovementioned genetic regions nor include *PARVB* and *WNT7B* genes. However, both of our patients exhibited ASD, which has been associated with smaller deleted segments [5]. Aggressive behavior, present in patient 1, has been assumed to be inversely correlated with the deletion size [7]. Brain structural anomalies, among which is corpus callosum hypoplasia, are reported in the literature to be present in 25–66% of patients [5]; this anomaly was present also in the first patient. Patient 3 has a cerebellar subarachnoid cyst, also reported in PMS patients [4]. Patient 2 did not undergo brain MRI.

4. Conclusion

PMS is a rare disorder, with a broad phenotypic spectrum, which often goes underdiagnosed. Although the phenotype is clinically heterogeneous, PMS should be considered in any patient with global developmental delay, absent or severely delayed speech, and autistic behavior, in association with some minor dysmorphic features (dolichocephaly, ear anomalies, periorbital fullness, epicanthal folds, hypertelorism, long eyelashes, bulbous nose, pointed chin, large fleshy hands) and hypotonia. The most common medical complications include seizures, gastrointestinal problems, renal anomalies, and respiratory infections [4]. Brain MRI images showed a high prevalence of arachnoid cysts, ventriculomegaly, dysmyelination, and morphological changes of the corpus callosum [4]. Clinical practice parameters reviewed by Kolevzon et al. [4] aimed to provide guidelines for evaluation and monitoring. The clinical evaluation and regular monitoring of patients with PMS should include psychiatric, psychological, neurologic, endocrinological, cardiologic, gastroenterological, nephrological, and pediatric examinations. After a review of all case series reported previously, the authors concluded that there are no specific features for the syndrome and the diagnosis of PMS is genetic [4]. Recommended genetic tests are chromosomal microarray (as a first-tier test), MLPA, and FISH; if these tests are not informative in a patient, mutations should be searched by NGS or Sanger sequencing.

Regarding the therapeutic strategy, patients can benefit from early intervention targeted to improve their muscle strength and communication abilities, including physical, speech, occupational, and behavioral therapies [1]. There are very few studies on different medications in patients with PMS [37]. One study investigated the effect of intranasal insulin—six individuals with PMS received 0.5–1.5 IU/day three times daily for 12 months, with good effects both on motor development and cognitive functions [38]. In another study on 25 children with PMS, intranasal insulin improved intellectual and behavioral development, especially in children older than 3 years, but most results were not statistically significant (Netherlands

Trial Registry ID: NTR3758). Studies on mice and human neuronal models brought evidence that insulin-like growth factor-1 (IGF-1) reverts synaptic deficits in PMS [4, 27], as well as in Rett syndrome [4, 27], most probably due to its positive effects on synaptic development, neurogenesis, and brain vessel growth [27]. Clinical studies with IGF-1 and related compounds in autism spectrum disorders, including PMS, are ongoing.

PMS is a rare disorder with a wide phenotypic spectrum and a large genomic landscape. *SHANK3* gene is considered the main culprit for the neuropsychiatric phenotype; however, its role must be investigated in the broader genetic landscape. Insights into the contribution of other genes at 22q13 are gained from genomic profiling, leading to refined genotype-phenotype correlations. PMS clinical diagnosis is challenging, as no pathognomonic features are described. Intellectual disability/global developmental delay, severe speech impairment, autistic features, motor impairments, and mild dysmorphic traits stand as the main clinical characteristics.

Genetic diagnosis of PMS has greatly benefited from the introduction of chromosomal microarray. As no characteristic clinical features are described, genomic profiling by microarray as a first-tier approach drives the diagnosis. Furthermore, the detailed molecular characterization has advanced the understanding of different gene contributions to PMS pathogenesis, thus paving the way for identification of therapeutic targets.

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Ethics

Written consent forms, for research and publishing the data of the patients, were obtained from the parents.

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