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General Article

GENERAL ARTICLE

Strong evidence for genotype–phenotype correlations in Phelan-McDermid syndrome: results from the developmental synaptopathies consortium

Tess Levy[1,2], Jennifer H. Foss-Feig[1,2], Catalina Betancur[3], Paige M. Siper[1,2,4], Maria del Pilar Trelles-Thorne[1,2,4], Danielle Halpern[1,2], Yitzchak Frank[1,2], Reymundo Lozano[1,2,5,6], Christina Layton[1,2], Bari Britvan[1,2], Jonathan A. Bernstein[7], Joseph D. Buxbaum[1,2,4,5,8], Elizabeth Berry-Kravis[9], Craig M. Powell[10,11], Siddharth Srivastava[12], Mustafa Sahin[12], Latha Soorya[13], Audrey Thurm[14], Alexander Kolevzon[1,2,4,6,*] and the Developmental Synaptopathies Consortium

[1]Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA, [2]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA, [3]Sorbonne Université, INSERM, CNRS, Neuroscience Paris Seine, Institut de Biologie Paris Seine, Paris 75005, France, [4]The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA, [5]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA, [6]Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA, [7]Department of Pediatrics, Stanford University, Stanford, CA, 94304, USA, [8]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA, [9]Department of Pediatrics, Neurological Sciences, Biochemistry, Rush University Medical Center, Chicago, Illinois 60612, USA, [10]Department of Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA, [11]Department of Psychiatry and Neuroscience Graduate Program, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA, [12]Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts; Rosamund Stone Zander Translational Neuroscience Center, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA, [13]Department of Psychiatry, Rush University Medical Center, Chicago, Illinois 60612, USA and [14]Neurodevelopmental and Behavioral Phenotyping Service, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20814, USA

*To whom correspondence should be addressed at. Tel: 212-659-9134; Fax: 212-996-8931; Email: alexander.kolevzon@mssm.edu

Abstract

Individuals with Phelan-McDermid syndrome (PMS) present with a wide range of developmental, medical, cognitive and behavioral abnormalities. Previous literature has begun to elucidate genotype–phenotype associations that may contribute

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to the wide spectrum of features. Here, we report results of genotype–phenotype associations in a cohort of 170 individuals with PMS. Genotypes were defined as Class I deletions (including *SHANK3* only or *SHANK3* with *ARSA* and/or *ACR* and *RABL2B*), Class II deletions (all other deletions) or sequence variants. Phenotype data were derived prospectively from direct evaluation, caregiver interview and questionnaires, and medical history. Analyses revealed individuals with Class I deletions or sequence variants had fewer delayed developmental milestones and higher cognitive ability compared to those with Class II deletions but had more skill regressions. Individuals with Class II deletions were more likely to have a variety of medical features, including renal abnormalities, spine abnormalities, and ataxic gait. Those with Class I deletions or sequence variants were more likely to have psychiatric diagnoses including bipolar disorder, depression, and schizophrenia. Autism spectrum disorder diagnoses did not differ between groups. This study represents the largest and most rigorous genotype–phenotype analysis in PMS to date and provides important information for considering clinical functioning, trajectories and comorbidities as a function of specific genetic alteration.

Introduction

Phelan-McDermid syndrome (PMS) is caused by haploinsufficiency of the *SHANK3* gene, resulting from either 22q13.33 deletions encompassing *SHANK3* or pathogenic sequence variants in *SHANK3* (1–4). PMS is characterized by early developmental delays; intellectual disability (ID), which is often severe; language deficits; hypotonia; and autism spectrum disorder (ASD) (5). Other common features include motor skill deficits, seizures, structural brain abnormalities, gastrointestinal problems, renal malformations, and non-specific dysmorphic features.

Defects in *SHANK3* have been identified as a relatively common cause of ASD (6–8), and ASD is frequent in PMS. The most recent estimates based on prospective assessments in large samples using gold-standard diagnostic tools such as the Autism Diagnostic Interview-Revised (ADI-R; (9)), the Autism Diagnostic Observation Schedule, second edition (ADOS-2; (10)) and the Diagnostic and Statistical Manual for Mental Disorders, fifth edition (DSM-5) criteria (11) establish the prevalence of ASD to be 58% (12). Global developmental delays and/or ID are present in the vast majority of individuals diagnosed with PMS thus far.

Psychiatric symptoms are prominent in a portion of individuals with PMS and have gained increased attention with multiple case reports of atypical bipolar disorder and catatonia (13–16) and a recent case series documenting neuropsychiatric decompensation and loss of skills during adolescence or early adulthood (17). Psychiatric presentations are typically characterized by pronounced mood disturbance, often with loss of functional skills and high rates of catatonia (17,18). Individuals with sequence variants in *SHANK3* may be at a higher risk for major psychiatric illness, highlighting the critical importance of this gene (17).

Given the significant heterogeneity in breadth and severity of the clinical presentation in PMS, as well as the large chromosomal region affected by deletions of varying sizes, many studies have explored possible relationships between genotype and phenotype. While *SHANK3* haploinsufficiency is sufficient for the psychiatric and neurological features of PMS, there is evidence to suggest that other genes in the region may play important roles based on genotype–phenotype studies (reviewed in Table 1) and reports of phenotypes associated with 22q13 deletions without *SHANK3* involvement (19–25).

In general, larger deletion size has been correlated with greater severity and presence of numerous clinical features, including developmental delay (2,21,26–28), language impairment (21,27–30), social communication impairment related to ASD (29,31), impairments in activities of daily living (2,30), feeding problems (21,27), hypotonia (2,26,27), recurrent ear infections (2), dental anomalies (2), renal abnormalities (29,31), cardiac abnormalities (31,32), lymphedema (29,31), and dysmorphic features (21,27–29,31,32). In addition, some reports have suggested that smaller deletions are associated with ASD (27,28) and aggressive behaviors (21,27,28). However, other studies have found no association between deletion size and clinical features (33–36).

Most studies examining genotype–phenotype relationships to date have employed small samples and used highly variable assessment methods for clinical features. In addition, few adequately corrected for multiple comparisons in their analytic methods, raising the risk of spurious results. Most analyses were based on individuals with deletions; only five studies to date have included individuals with *SHANK3* sequence variants (30,31,36–38), but with too few cases to draw meaningful conclusions about phenotype correlations.

Efforts to better understand the relationship between genotype and phenotype are crucial to inform assessment and monitoring procedures and to counsel families. Elucidating a role for neighboring genes will also motivate additional research to clarify the potential impact on the phenotype and may lead to targeted therapeutics. This study aims to evaluate the relationship between genotype and clinical features of PMS using a large sample of individuals who were prospectively evaluated with highly rigorous and standardized assessment methods.

Results

Genetics

Among the 170 participants, 136 had deletions (80%). Of those, 18 had ring chromosome 22 and five had unbalanced translocations. Deletion sizes ranged from 17 kb to 8.81 Mb (2.28 ± 2.76 Mb). Thirty-four participants had pathogenic ($n = 29$) or likely pathogenic ($n = 5$) sequence variants in *SHANK3*. Of those, 28 had a frameshift (82%), four had a nonsense (12%), one had a splice site (3%) and one had a de novo missense variant (3%). When parental DNA was available, all variants were de novo (26/34). Participants who carried deletions were split into two groups: 1) Class I deletions: deletions including only *SHANK3 or SHANK3* in combination with *ARSA* and/or *ACR* and *RABL2B*. These latter three genes are not expected to contribute to the phenotype of PMS because they are not constrained for protein truncating variants (pLI = 0 in the gnomAD database); in addition, *ARSA* is involved in a recessive disorder, metachromatic leukodystrophy. 2) Class II deletions: all deletions that did not qualify as Class I deletions. For the majority of genotype–phenotype analyses, Class I deletions and sequence variants were combined into one group. The Class I deletion and sequence variant group had 80 participants (46 Class I deletions +34 sequence variants), and the Class II deletion group had 90 participants.

Table 1. Literature review of studies examining genotype–phenotype correlations in PMS

Author, year	Sample	Phenotyping methods	Results
Wilson et al., 2003	N = 51 Deletions (130 kb—>9 Mb); five individuals with a translocation and duplication of a non-acrocentric chromosome were excluded.	A variety of methods, including the Developmental Profile II (DPII) and the Scales of Independent Behaviour-Revised (SIB-R). Used linear regression to obtain a correlation coefficient.	Larger deletions associated with SIB-R Broad Independence, Personal Living Skills, Community Living Skills and DPII Self-Help Skills. Larger deletions also associated with hypotonia, recurrent ear infections, pointed chin and dental anomalies. No significant associations between deletion size and motor, communication or social skills on the SIB-R or DPII. The majority of medical and dysmorphic features were not associated with deletion size.
Jeffries et al., 2005	N = 30 r (22) (deletions 69 kb—10.2 Mb)	Parental clinical history questionnaires, including the Parental Involvement Project (PIP), Autism Screening Questionnaire (ASQ), Social Communication Questionnaire (SCQ), parent-rated strengths and difficulties questionnaire (PSDQ), ADHD scale; photographs; body measurements. Ranked the *P*-values of regression coefficients based on the expected direction of effect plotted against the actual *P*-value. Assigned a *P*-value of ≤0.32 as the threshold for significance.	Based on the assigned *P*-value threshold ≤0.32, the following clinical features were associated with deletion size: general developmental delays, language delays, autistic features, genitourinary abnormalities, cardiovascular abnormalities and seizures. Only cardiovascular abnormalities were associated with deletion size at a *P*-value <0.05. Several minor dysmorphic features were also associated with deletion size (e.g. ear abnormalities, short philtrum, dysplastic toenails).
Philippe et al., 2008	N = 8 Deletions (150 kb—>9 Mb)	Brain MRI	Structural brain abnormalities were only present in three patients with larger deletions (>270 kb), including thinning or morphologically atypical corpus callosum, ventricular dilatation, arachnoid cysts and white matter hyper-intensities.
Dhar et al., 2010	N = 13 Deletions (95 kb—8.5 Mb), including 2 r (22)	Clinical profiling using a variety of measures and record review.	No significant correlations between phenotype and deletion size.
Sarasua et al., 2011	N = 71 Terminal deletions (0.22–9.22 Mb, mean 5.08 Mb)	Physical exam for physical features, medical history questionnaire. Two-sided Wilcoxon rank-sum tests without correction for multiple comparisons.	Larger deletions were associated with 84 characteristics, including eight physical features (dysplastic toenails, full brow, large or fleshy hands, macrocephaly, atypical reflexes, dolichocephaly, large stature, facial asymmetry) and six medical history features (neonatal hypotonia and feeding problems, walking delay, expressive language delay, male genital anomalies, neonatal hyporeflexia). Smaller deletions were associated with aggressive behavior and ASD.
Soorya et al., 2013	N = 32 30 deletions (101 kb—8.45 Mb), including 6 r (22); 2 sequence variants	Psychiatric, neurological and clinical genetics evaluations; ADI-R, ADOS-G, cognitive testing, Vineland Adaptive Behavior Scales II, medical record review. Spearman rank order correlations for continuous variables and Mann–Whitney U tests for dichotomous variables.	Larger deletions were associated with increased number of medical comorbidities, social communication impairment and total number of dysmorphic features. Lymphedema, asthma, cardiac abnormalities and renal abnormalities were only observed in larger deletions. Seizures, GERD, hypotonia, sleep disturbance and abnormal brain MRI findings were common and present in participants with the smallest deletions and/or sequence variants as well as in those with larger deletions.
Sarasua, Dwivedi et al., 2014	N = 70 Terminal deletions	Same cohort and methods as Sarasua et al. 2011. Examined 22 clinical features identified in Sarasua et al. 2011 and used association and receiver operating characteristic statistical methods, incorporating protein interaction networks, to identify 22q13 genomic locations and genes associated with clinical features (27).	Severity of speech/language delay, neonatal hypotonia, delayed walking, hair-pulling behavior, male genital anomalies, dysplastic toenails, large/fleshy hands, macrocephaly, short and tall stature, facial asymmetry and atypical reflexes were associated with specific genomic regions and candidate regions within the 22q13 region. The genomic region 41.9–46.6 Mb was associated with reduced prevalence of ASD and aggressive behavior.

(*Continued*)

Table 1. Continued

Author, year	Sample	Phenotyping methods	Results
Sarasua, Boccuto et al., 2014	N = 98 Terminal deletions, including 14 r (22). (Individuals with adjacent duplications, unbalanced translocations and interstitial deletions not involving *SHANK3* were excluded from the analyses.)	Cohort overlaps with Sarasua et al. 2011 and 2014a. Standardized medical history questionnaire and physical examination. Linear and logistic regression models to assess the effect of deletion size on phenotype.	Larger deletion size was significantly associated with language and motor developmental delays, dysmorphic features including macrocephaly, abnormal reflexes, neonatal hypotonia, neonatal feeding problems, strabismus and skin rashes. Independent walking, aggressive behavior and impulsiveness were associated with smaller deletion sizes. Seizures, hypotonia, birth weight and gestational age at birth were not associated with deletion size.
Oberman et al., 2015	N = 14 13 terminal deletions (0.22–9.18 Mb, mean 5.01 Mb), 1 sequence variant	Parent interview using the ADI-R and Vineland-II Pearson Correlation Coefficients	Larger deletions were associated with lower Vineland-II Communication, Motor and Daily Living Skills domains scores, and with lower ADI-R Restricted and Repetitive Behavior subdomain scores. No association between deletion size and ADI-R Social Communication sub-domain or Vineland-II socialization domain scores.
Tabet et al., 2017	N = 71 (8 not determined to include *SHANK3*) Deletions (45.8 kb—9.1 Mb)	Medical record review, including results from DSM-5, ADI-R, ADOS, Raven's Progressive Matrices, and Peabody Picture Vocabulary Test. Cluster analysis into quartiles based on deletion size and then the prevalence of each feature was measured each 50 kb in sliding windows of 1.5 Mb in the 22q13 region. Odds ratio were then calculated. Examined the burden of other CNVs outside 22q13 on phenotype.	Absence of speech (42.6–46.3 Mb), ophthalmic features (42.25–44.6 Mb) and gastroesophageal reflux (48.9–49.9 Mb) were associated to specific genomic regions. No region-specific associations with ASD, heart abnormalities, seizures and corpus callosum abnormalities. No correlation was found between the burden of other CNVs and any clinical features of PMS.
De Rubeis et al., 2018	N = 17 Sequence variants	Prospective direct clinical and psychological evaluation using a battery of standardized assessments, psychiatric, neurological and clinical genetics examination ($n = 12$); parent interview, neurological examination and medical record review ($n = 3$); caregiver reports and phone interview ($n = 2$).	All individuals had ID; common features included ASD (73%), severe speech deficits, hypotonia, motor skill deficits, regression, seizures, structural brain abnormalities, mild dysmorphic features, sleep disturbance, increased pain tolerance and feeding and gastrointestinal problems. Regression in motor and language skills occurred in 65% of the sample.
Droogmans et al., 2019	N = 15 Terminal deletions (76 kb—6.6 Mb)	Standardized questionnaires, interviews and observation, medical record review. Mann–Whitney test, Kruskal–Wallis test, Wilcoxon signed rank tests.	No significant differences in cognitive development, adaptive behavior, verbal and non-verbal communication, emotional and behavioral problems, ASD features or sensory processing were observed in relation to deletion size.
Samogy-Costa et al., 2019	N = 29 Terminal deletions (49 kb—9.1 Mb, mean 4.1 Mb)	Parent questionnaire and photographs for dysmorphic features. Mann–Whitney U tests and Kruskal–Wallis without correction for multiple comparisons.	Larger deletions associated with renal abnormalities, lymphedema and language impairment. No association with ASD, cardiac abnormalities, increased pain tolerance, hypotonia, recurring infections, GERD, sleep disturbance, seizures, constipation/diarrhea, strabismus, chewing, swallowing or hypohidrosis.
Xu et al., 2020	N = 29 20 terminal deletions (34 kb—8.7 Mb, mean 3.6 Mb); 9 sequence variants	Medical record review, dysmorphology evaluation via photographs, standardized medical history questionnaire. Compared the frequency of features between deletions that encompass more than the *SHANK3* gene versus sequence variants or deletions that only encompass *SHANK3*.	No statistically significant differences in the frequency of clinical features between patients with loss of *SHANK3* alone and those with deletions encompassing other genes besides *SHANK3*.

Abbreviations: ADHD, attention deficit hyperactivity disorder; ADI-R, Autism Diagnostic interview-Revised; ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, fifth edition; GERD, gastroesophageal reflux disease; ID, intellectual disability; MRI, magnetic resonance imaging; r (22), ring chromosome 22.

Table 2. Class I deletions and sequence variants vs. Class II deletions: primary analysis results

Variable	Class I deletions, sequence variants	Class II deletions	*p*-value	Effect size
		Medical features		
Renal abnormalities	0% (0/77)	24% (20/85)	0.000005*	Φ = 0.357
Cardiac defects	6% (5/80)	13% (11/87)	0.161	Φ = 0.109
Number of dysmorphic features	8.14 ± 5.05 (*n* = 65)	10.23 ± 4.95 (*n* = 78)	0.012	*d* = 0.418
Comorbid severe mental illness[†,‡]	19% (12/64)	3% (2/68)	0.003*	Φ = 0.257
		Developmental milestones		
Age of walking (m)	16.41 ± 4.54 (*n* = 51)	27.60 ± 14.40 (*n* = 50)	0.0000001*	*d* = 1.05
Single word achievement[†]	82% (45/55)	50% (26/52)	0.0005*	Φ = −0.337
Phrase speech achievement[†]	72% (39/54)	21% (11/52)	0.0000001*	Φ = −0.511
		Intellectual and adaptive functioning		
Full scale IQ/DQ[†]	31.73 ± 19.35 (*n* = 58)	20.34 ± 15.27 (*n* = 63)	0.001*	*d* = 0.653
Verbal IQ/DQ[†]	31.57 ± 22.05 (*n* = 58)	17.69 ± 15.86 (*n* = 63)	0.0005*	*d* = 0.722
Nonverbal IQ/DQ[†]	36.52 ± 22.32 (*n* = 59)	24.00 ± 16.98 (*n* = 63)	0.002*	*d* = 0.631
Vineland Adaptive Behavior Composite[†]	47.79 ± 19.11 (*n* = 58)	46.12 ± 14.47 (*n* = 59)	0.416	*d* = 0.010
		Regression		
Language regression	43% (24/56)	11% (7/63)	0.00008*	Φ = −0.361
General regression[§]	0:27, 1:7, 2:20	0:40, 1:9, 2:13	0.155	Φ = 0.179
		ASD symptomatology		
ASD consensus diagnosis	60% (45/75)	65% (55/85)	0.539	Φ = 0.049

Class I deletions include only *SHANK3* or *SHANK3* in combination with *ARSA*, *ACR* or *RABL2B*. Class II deletions include all deletions that did not qualify as Class I deletions. Values represent mean ± SD, percentages or score distribution. Abbreviations/symbols: ASD: autism spectrum disorder, d: Cohen's d effect size, DQ: developmental quotient, IQ: intellectual quotient, m: months, Φ: phi effect size.
[†]Only participants 5 years and older included in analysis.
[‡]Comorbid severe mental illness includes schizophrenia, schizoaffective disorder and bipolar disorder.
[§]0: no regression, 1: probable regression, 2: definite regression.
*Significant after Bonferroni correction.

The mean age of the Class I deletion and sequence variant group was 14 years (14.19 ± 8.9), and the mean age for the Class II deletion group was 11 years (10.7 ± 9.0). The ages were significantly different between groups ($p = 0.0005$); thus, where variables were expected to be impacted by age (e.g. developmental milestones, intellectual and adaptive functioning), only participants over 5 years of age were included in the analyses. The Class I deletion and sequence variant group had 46% females, and the Class II deletion group had 50% females, which was not significantly different.

Primary analysis: two-group analysis with primary variables

Individuals with Class II deletions were significantly more likely to have renal abnormalities, including hydronephrosis, vesicoureteral reflux, renal agenesis and polycystic kidney (Table 2). Individuals with Class I deletions or sequence variants were more likely to have a diagnosis of severe mental illness (bipolar disorder, schizophrenia or schizoaffective disorder). Full-scale, verbal and nonverbal IQ/DQ were significantly lower in those with Class II deletions. Participants with Class II deletions were significantly less likely to develop a single word and phrase speech and they took significantly more time to learn to walk independently. The Class I deletion and sequence variant group was more likely to have a regression of language, likely related to the greater attainment of skills. Primary variables that did not show differences, or did not withstand correction for multiple comparisons, include cardiac defects, number of dysmorphic features, adaptive behavior composite score, regression of general skills and ASD consensus diagnosis.

Exploratory analysis: two-group analysis with secondary variables

Medical features. Individuals with Class II deletions were significantly more likely to have ocular abnormalities (e.g. strabismus, nystagmus), early infancy hypotonia, hypotonia persisting after infancy, spine abnormalities (e.g. lordosis, scoliosis, kyphosis), and both apraxic and ataxic gait (Table 3). Those with Class I deletions and sequence variants were more likely to have a variety of comorbid mental illnesses including bipolar disorder, depression, and schizophrenia/schizoaffective disorder.

Medical features that did not show differences between groups include visual acuity abnormalities (e.g. myopia, hyperopia), hearing abnormalities, recurrent infections, thyroid dysfunction, pica, disrupted sleep, anxiety, obsessive compulsive disorder, poor feeding in early infancy, lymphedema, epilepsy, gastrointestinal dysfunction (e.g. reflux, constipation), and genital abnormalities (e.g. cryptorchidism, hydrocele) (Table 3).

Developmental milestones. Of individuals who developed single words (45/55 with Class I deletions and sequence variants, 26/52 with Class II deletions), those with Class II deletions developed single words significantly later than those with Class I deletions and sequence variants (Table 3). The age of phrase speech achievement was not significant; however, the number of participants with Class II deletions who achieved this skill at all was very low (11/52), and the difference likely would be significantly different with a larger sample. Walking independently was almost universally achieved and did not differ between the groups. The Class II deletion group was significantly less likely to achieve both daytime bladder and bowel control. Lastly, parents of individuals with Class II deletions reported the onset

Table 3. Exploratory analysis: two-group analysis with secondary variables

Variable	Class I deletions, sequence variants	Class II deletions	P-value	Effect size
		Medical features		
Visual acuity abnormalities	25% (19/75)	31% (22/71)	0.447	Φ = 0.063
Ocular abnormalities	19% (12/62)	39% (26/67)	0.015*	Φ = 0.213
Hearing abnormalities	14% (10/72)	19% (13/69)	0.426	Φ = 0.067
Recurrent infections	38% (29/76)	51% (44/87)	0.112	Φ = 0.125
Thyroid dysfunction	7% (5/75)	5% (4/87)	0.566	Φ = −0.045
Pica	36% (21/59)	44% (28/63)	0.319	Φ = 0.09
Disrupted sleep	63% (48/76)	49% (43/87)	0.078	Φ = −0.138
Bipolar disorder†	16% (10/64)	3% (2/68)	0.011*	Φ = −0.221
Depression†	13% (8/64)	2% (1/66)	0.014*	Φ = −0.216
Anxiety†	27% (17/64)	15% (10/66)	0.109	Φ = −0.141
Schizophrenia/Schizoaffective disorder†	9% (6/64)	0% (0/68)	0.010*	Φ = −0.225
Obsessive compulsive disorder†	11% (7/63)	6% (4/66)	0.305	Φ = −0.09
Early infancy hypotonia	58% (32/55)	85% (56/66)	0.001*	Φ = 0.298
Early infancy poor feeding	37% (20/54)	58% (36/62)	0.024	Φ = 0.21
Hypotonia persisting after infancy	78% (62/79)	93% (81/87)	0.006*	Φ = 0.211
Lymphedema	1% (1/72)	7% (6/86)	0.089	Φ = 0.135
Epilepsy	26% (19/73)	27% (22/83)	0.946	Φ = 0.005
Spine abnormalities	21% (15/73)	51% (42/83)	0.0001*	Φ = 0.311
Gastrointestinal dysfunction	83% (63/76)	78% (69/88)	0.47	Φ = −0.056
Apraxic gait	67% (28/42)	87% (39/45)	0.027	Φ = 0.238
Ataxic gait	20% (6/30)	83% (19/23)	0.000006*	Φ = 0.622
Genital abnormalities	4% (2/46)	5% (3/64)	0.933	Φ = 0.008
		Developmental milestones		
Achieved walking unaided	100% (52/52)	98% (50/51)	0.310	Φ = −0.1
First single word (m)	31.77 ± 21.77 (*n* = 43)	45.25 ± 17.79 (*n* = 24)	0.001	*d* = 0.678
First phrase (m)	49.97 ± 25.98 (*n* = 36)	67.70 ± 49.28 (*n* = 10)	0.240	*d* = 0.450
Achieved bladder control (daytime)†	38% (19/50)	14% (7/51)	0.005	Φ = −0.278
Achieved bowel control†	39% (20/51)	14% (7/51)	0.004	Φ = −0.289
Onset of developmental abnormality (m)	15.12 ± 10.00 (*n* = 59)	8.36 ± 5.86 (*n* = 64)	0.000016	*d* = 0.825
		Adaptive functioning		
Communication†	47.53 ± 20.07 (*n* = 58)	44.81 ± 15.40 (*n* = 59)	0.484	*d* = 0.152
Daily living skills†	47.59 ± 18.66 (*n* = 58)	46.56 ± 14.84 (n = 59)	0.688	*d* = 0.061
Socialization†	52.71 ± 20.63 (*n* = 58)	51.15 ± 15.68 (*n* = 59)	0.756	*d* = 0.085
		Language and communication		
EVT-2 score	38.11 ± 22.22 (*n* = 55)	23.54 ± 11.48 (*n* = 59)	0.000006	*d* = 0.824
PPVT-4 score	34.64 ± 21.51 (*n* = 55)	24.36 ± 11.73 (*n* = 59)	0.007	*d* = 0.593
Comprehension of simple language‡	0:16, 1:11, 2:11, 3:17, 4:1	0:3, 1:13, 2:20, 3:21, 4:5	0.006	V = 0.350 (df = 4)
Overall language level§	0:30, 1:6, 2:24	0:7, 1:12, 2:45	0.000012	V = 0.427 (df = 2)
MCDI words understood	226.88 ± 157.16 (*n* = 33)	127.62 ± 123.45 (*n* = 45)	0.006	*d* = 0.702
MCDI words produced	164.82 ± 173.83 (*n* = 33)	39.40 ± 103.57 (*n* = 45)	0.002	*d* = 0.877
		ASD symptomatology and behavioral comorbidities		
ADOS Comparison Score	6.29 (2.62) *n* = 59	5.88 (2.54) *n* = 58	0.329	*d* = 0.159
ADI-R met for ASD	72% (43/60)	68% (42/62)	0.509	Φ = −0.049
Sensory Profile Threshold Score¶	0:16, 1:10, 2:24	0:8, 1:10, 2:32	0.170	V = 0.189 (df = 2)
ABC irritability	7.54 (7.75) *n* = 54	8.04 (8.61) *n* = 53	0.968	*d* = 0.061
ABC lethargy	10.21 (8.85) *n* = 53	9.13 (8.88) *n* = 53	0.452	*d* = 0.122
ABC stereotypy	5.35 (5.16) *n* = 54	4.34 (4.98) *n* = 53	0.206	*d* = 0.199
ABC hyperactivity	18.64 (13.15) *n* = 53	15.79 (12.23) *n* = 52	0.27	*d* = 0.224
ABC inappropriate speech	2.93 (3.24) *n* = 54	1.25 (1.89) *n* = 53	0.006	*d* = 0.633
RBS-R total score	17.08 (16.68) *n* = 50	17.34 (13.11) *n* = 53	0.537	*d* = 0.017

Class I deletions include only *SHANK3* or *SHANK3* in combination with *ARSA*, *ACR* and/or *RABL2B*. Class II deletions include all deletions that did not qualify as Class I deletions. Values represent mean ± SD, percentages or score distribution. Abbreviations/symbols: ABC: Aberrant Behavior Checklist, ADI-R: Autism Diagnostic Interview-Revised, ADOS: Autism Diagnostic Observation Schedule, ASD: autism spectrum disorder, *d*: Cohen's d effect size, df: degrees of freedom, EVT-2: Expressive Vocabulary Test, second edition, MCDI: MacArthur Bates Communicative Index, m: months, Φ: phi effect size, PPVT-4: Peabody Picture Vocabulary Test, fourth edition, RBS-R: Repetitive Behavior Scale-Revised, V: Cramer's V effect size.

†Only participants 5 years and older included in analysis.

‡Comprehension of simple language (ADI-R): 0: in response to a request can usually perform an unexpected action with an unexpected object, 1: in response to a request can usually get an object from another room, but usually cannot carry out a new action on this object or put it in a new place, 2: understands more than 50 words but does not meet criteria for '0' or '1', 3: understands fewer than 50 words, but some comprehension of 'no' and names of a few favorite objects, foods or people or words within familiar routines, 4: little or no comprehension of words, even in context.

§Language level (ADI-R): 0: functional use of spontaneous, echoed or stereotyped language that, on a daily basis, involves phrases of three words or more that at least sometimes include a verb and are comprehensible to other people, 1: no functional use of three-word phrases in spontaneous, echoed or stereotyped speech, but uses speech on a daily basis with at least five different words in the last month, 2: fewer than five words total or speech not used on a daily basis.

¶Sensory Profile Threshold Score: 0:typical performance, 1: possible sensory differences, 2: definite sensory differences.

*Significant after Benjamini–Hochberg correction

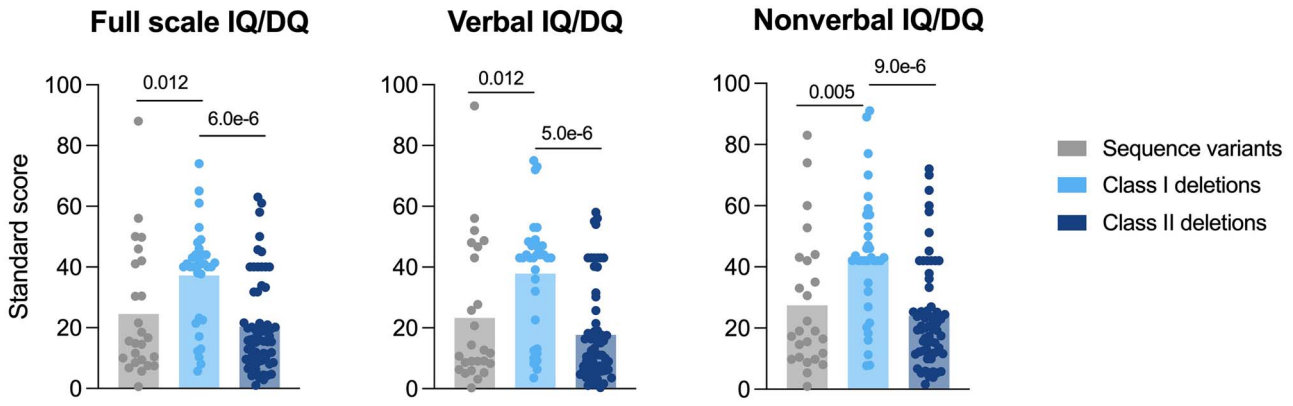

Figure 1. Exploratory analysis: three-group analysis of IQ/DQ. Full scale, verbal and nonverbal IQ/DQ in participants over 5 years with *SHANK3* sequence variants, Class I or Class II deletions. Abbreviations: DQ: Developmental quotient, IQ: intellectual quotient.

of developmental abnormality significantly earlier than parents of individuals with Class I deletions and sequence variants.

Adaptive functioning. There were no significant differences between groups on the Vineland-2 adaptive behavior domains (Table 3).

Language and communication. Participants with Class II deletions had significantly lower scores on both the Peabody Picture Vocabulary Test, fourth edition and the Expressive Vocabulary Test, second edition compared to those with Class I deletions and sequence variants, indicating lower receptive and expressive communication ability (Table 3). Class II deletion participants also showed significantly lower skills in overall language on the ADI-R. Additionally, individuals with Class II deletions could understand and produce significantly fewer words than individuals with Class I deletions and sequence variants, based on the MacArthur-Bates Communicative Developmental Inventories.

ASD symptomatology. There were no significant differences between groups in rates of ASD diagnoses, severity, sensory features on the Short Sensory Profile, or restricted and repetitive behaviors on the Repetitive Behavior Scale-Revised (Table 3). Participants with Class I deletions and sequence variants showed significantly more Inappropriate Speech on the Aberrant Behavior Checklist as compared to participants with Class II deletions ($p = 0.006$), a finding likely attributed to significant differences in language acquisition between groups.

Exploratory analysis: three-group analysis with primary variables

As expected, the Class I and Class II deletion groups differed in all the same variables as in the primary analyses: renal abnormalities, comorbid severe mental illness, IQ/DQs, milestone achievement and age, and language regression (Table 4, Fig. 1). However, the sequence variant group showed significantly lower scores than the Class I deletions in all IQ/DQ measures (verbal, nonverbal and full scale) and did not differ from the Class II deletions. There were no other differences between Class I deletions and sequence variants.

Discussion

This study represents the most rigorous genotype–phenotype analysis in PMS to date. Almost all (159/170) participants were prospectively evaluated by a team of psychiatrists, clinical psychologists, neurologists and geneticists/genetic counselors. Evaluations consisted of gold-standard direct assessments, structured and semi-structured caregiver interviews, and caregiver questionnaires. Clinical variables were chosen and delineated by primary and secondary analyses based on core features of PMS (e.g. ID, developmental milestones) and previous literature (e.g. renal abnormalities, ASD, severe mental illness, regression). Genetic groupings were chosen to differentiate features of *SHANK3* haploinsufficiency from potential haploinsufficiency of other disrupted genes. Given the large sample, this study is one of few to employ strict corrections for multiple comparisons.

Comparing the current findings to previous genotype–phenotype studies can be complex due to the various definitions of genotype groupings, phenotyping methods, and sample sizes (Table 1). Despite this, there are some findings that are fairly consistent throughout the literature, such as renal abnormalities being associated with larger deletions (29,31). Renal abnormalities were one of the most striking differences in our cohort (0% in Class I and sequence variants vs. 24% in Class II deletions), indicating that the renal abnormalities seen in PMS are not due to haploinsufficiency of *SHANK3*. Recent literature has suggested *CELSR1* as a candidate for the renal abnormalities seen in PMS (24,39). Among the individuals with renal abnormalities in our cohort ($n = 20$), 16 had *CELSR1* deleted. There were no obvious differences in type of renal abnormalities between the groups, though the sample size is limited. Further studies evaluating renal abnormalities in individuals with these genes deleted, including in a non-PMS cohort, are warranted to better understand their role.

Another frequent finding in the literature is the extent of developmental delays and ID being more severe in individuals with larger deletions (21,27–29,34). We aimed to further describe these differences by isolating motor milestones (walking), language milestones (single words, phrases), and adaptive behavior (bladder, bowel control), as well as assessing differences in achievement of skills and timing of skill attainment. Generally, participants with Class II deletions were significantly less likely to achieve developmental milestones, and if achieved, they developed them significantly later. This finding is in line with previous reports that individuals with larger deletions had more severe delays. Individuals with Class II deletions had more severe impairments in motor (longer to walk), language (less likely to achieve words and phrases, longer to achieve words if achieved), and daily living skills (less likely to achieve bladder and bowel control) development. However, it is important to note

Table 4. Exploratory analysis: three-group analysis with primary variables

Variable	Genetic class	Proportion/mean ± SD	Class I deletions vs. Class II deletions	Class I deletions vs. sequence variants	Class II deletions vs. sequence variants
		Medical features			
Renal abnormalities	Class I	0% (0/45)	0.0004*	n/a	0.003*
	Class II	24% (20/85)			
	Sequence	0% (0/32)			
Cardiac abnormalities	Class I	9% (4/46)	0.494	0.293	0.108
	Class II	13% 11/87			
	Sequence	3% (1/34)			
Number of dysmorphic features	Class I	8.26 ± 4.83 (*n* = 39)	0.036	0.707	0.052
	Class II	10.23 ± 4.95 (n = 78)			
	Sequence	8.0 ± 5.5 (*n* = 26)			
Comorbid severe mental illness[†],[‡]	Class I	18% (7/38)	0.006*	0.935	0.007*
	Class II	3% (2/68)			
	Sequence	19% (5/26)			
		Developmental milestones			
Age of walking	Class I	16.69 ± 5.08 (*n* = 32)	0.000008*	0.930	0.00003*
	Class II	27.60 ± 14.39 (*n* = 50)			
	Sequence	15.8 ± 3.4 (*n* = 21)			
Single word achievement[†]	Class I	88% (30/34)	0.0003*	0.116	0.095
	Class II	48% (26/52)			
	Sequence	71% (15/21)			
Phrase speech achievement[†]	Class I	79% (26/33)	0.0000002*	0.177	0.001*
	Class II	21% (11/52)			
	Sequence	62% (13/21)			
		Intellectual and adaptive functioning			
Full scale IQ/DQ[†]	Class I	37.17 ± 16.22 (*n* = 33)	0.000006*	0.012*	0.664
	Class II	20.34 ± 15.27 (*n* = 63)			
	Sequence	24.4 ± 21.1 (*n* = 25)			
Verbal IQ/DQ[†]	Class I	37.87 ± 19.35 (*n* = 33)	0.000005*	0.012*	0.442
	Class II	17.69 ± 15.85 (*n* = 63)			
	Sequence	23.3 ± 23 (*n* = 25)			
Nonverbal IQ/DQ[†]	Class I	43.16 ± 20.43 (*n* = 34)	0.000009*	0.005*	0.879
	Class II	24.00 ± 16.98 (*n* = 63)			
	Sequence	27.5 ± 21.9 (*n* = 25)			
Vineland adaptive behavior Composite[†]	Class I	49.44 ± 19.10 (*n* = 36)	0.170	0.418	0.754
	Class II	46.12 ± 14.47 (*n* = 59)			
	Sequence	45.1 ± 19.3 (*n* = 22)			
		Regression			
Language regression	Class I	34% (12/35)	0.005*	0.094	0.00001*
	Class II	11% (7/63)			
	Sequence	57% (12/21)			
General regression[§]	Class I	0:19, 1:5, 2:9	0.762	0.178	0.023
	Class II	0:40, 1:9, 2:13			
	Sequence	0: 8, 1: 2, 2: 11 (*n* = 21)			
		ASD symptomatology			
ASD consensus diagnosis	Class I	58% (25/43)	0.469	0.703	0.825
	Class II	65% (55/85)			
	Sequence	63% (20/32)			

Class I deletions include only SHANK3 or SHANK3 in combination with ARSA, ACR and/or RABL2B. Class II deletions include all deletions that did not qualify as Class I deletions. Values represent mean ± SD, percentages or score distribution. Abbreviations/Symbols: ASD: autism spectrum disorder, *d*: Cohen's d effect size, DQ: developmental quotient, IQ: intellectual quotient, n/a: not applicable, Φ: phi effect size.
[†]Only participants 5 years and older included in analysis.
[‡]Comorbid severe mental illness includes schizophrenia, schizoaffective disorder and bipolar disorder.
[§]0: no regression, 1: probable regression, 2: definite regression.
*Significant after Benjamini–Hochberg correction.

that both groups were significantly delayed when compared to typically developing individuals. For example, for those that achieved this milestone, individuals with Class I deletions and sequence variants achieved their first single word at an average of 31 months and Class II deletions at an average of 43 months; this is more than 18 months delayed for both groups compared to typically developing children. Our data provide additional evidence that while all individuals with PMS have developmental delays, those with larger deletions tend to have more severe delays. Besides *SHANK3*, only one other

gene in the 22q13.2qter region has been involved thus far in an autosomal dominant neurodevelopmental disorder, *TCF20*. Located at 22q13.2, it contributes to the phenotype of individuals with terminal deletions larger than 8.74 Mb. Loss of *TCF20* leads to a syndrome characterized by DD, ID, hypotonia, variable dysmorphic features, and behavioral disorders including ASD (40). *TCF20* is deleted in only one individual in our cohort; thus, other haploinsufficient genes contributing to the phenotype severity of larger deletions remain to be identified.

There are inconsistent findings in the literature regarding ASD diagnosis. Some studies have found differences in rates of ASD according to genotype (21,27,28) while others have not (29,34,36). Conflicting results may be due to differences in phenotyping methods or the absence of direct observation, genotype definitions, or other factors including difficulty diagnosing ASD in those with ID (41). Our study represents the largest cohort to date with prospectively assessed ASD diagnoses using gold standard assessments, and we found comparable rates of ASD between groups. Furthermore, we found similar profiles of ASD symptomatology including ASD severity, restricted and repetitive behaviors, and sensory symptoms between groups.

In addition, some features do not show consistent genotype–phenotype associations in prior studies, including seizures and/or epilepsy (2,27,29,34), cardiac defects (2,27,29,34), and gastrointestinal abnormalities (27,29,31). Lack of clear genotype–phenotype associations for these features were replicated in our cohort. The absence of significant associations with genotype indicates that *SHANK3* haploinsufficiency may be the primary mechanism of disease for some of these features, including seizures and possibly gastrointestinal problems, both shown to be common in individuals with *SHANK3* variants (37). For other features, such as congenital heart defects, larger cohorts and more precise definition of the relevant phenotypes may be necessary to show a genotype–phenotype correlation.

There are previously reported findings in the literature that were not replicated in our study. These include lymphedema, where previous studies have found genotype–phenotype differences (29,31); however, we did not. This may be due to the low prevalence of lymphedema in our cohort (4%) and the relatively young age of those with Class II deletions. Indeed, if we include only individuals 15 years or older in analysis, those with Class II deletions were more likely to have a diagnosis of lymphedema, and the *p*-value becomes nominally significant (0.047). Inclusion of additional adolescents and adults may help clarify this finding. Heterozygous loss-of-function variants in *CELSR1* have been reported in individuals with hereditary lymphedema (42,43). *CELSR1* is located at 22q13.31 and is included in 22q13 terminal deletions larger than 4.3 Mb. Of the six individuals with Class II deletions and lymphedema in our cohort, all had a deletion of *CELSR1*.

Among the new findings we report is a significant difference in rates of diagnosis of severe mental illness, wherein individuals with Class I deletions and sequence variants are more likely to be diagnosed with bipolar disorder, depression, schizophrenia and/or, schizoaffective disorder. Comorbid mental illness, and schizophrenia in particular, is challenging to accurately diagnose in PMS given the extent of cognitive and language delays. However, severe illness such as bipolar disorder, even if presenting atypically in PMS, has recently been highlighted in the literature (17,18) and has a major impact on functioning level and quality of life (17). For many of the individuals in our cohort, regression and neuropsychiatric decompensation likely triggered genetic testing, or additional testing with higher resolution techniques, and led to the diagnosis of PMS. It is therefore possible that the subset of individuals with small deletions and sequence variants is enriched with a higher prevalence of severe mental illness. Furthermore, the diagnosis of mental illness in general is complex in PMS, and individuals who are higher functioning at baseline may be more easily identified, reflecting an ascertainment bias. Broader access to genetic testing and ongoing prospective studies tracking the natural history of PMS may shed more light on this important issue.

In exploratory analyses looking at sequence variants compared to Class I and Class II deletions independently, we found unexpected differences in cognitive ability. Because sequence variants in *SHANK3* are sufficient to impact cognitive functioning (30,31,36–38) and Class I deletions only included *SHANK3* and other genes thought not to play a role in the phenotype, these two groups were predicted to not show significant differences. And yet, all cognitive functioning variables (full scale, verbal and nonverbal IQ/DQ) were significantly lower for individuals with sequence variants than those with Class I deletions. Furthermore, individuals with sequence variants did not differ from the more severely impaired individuals with Class II deletions in terms of IQ/DQ. Before concluding this finding represents a true difference between groups, several alternative explanations are worth considering given the biological plausibility that sequence variants and Class I deletions are functionally similar. First, ascertainment bias may be relevant since most sequence variants in our cohort were identified by whole exome sequencing while most deletions were identified by chromosomal microarray. This is relevant because until recently, exome sequencing was ordered mainly for severe or complex neurodevelopmental disorders, while chromosomal microarray analysis has been standard of care in the assessment of neurodevelopmental disorders for more than a decade. However, if only more severely affected individuals with sequence variants were diagnosed, we would expect to see differences in other measures such as milestone achievement or language measures, which we did not. Because unexplained regression is also often an indication for sequencing, we further explored regression in this group. For individuals with sequence variants who had data on regression, 62% (21/34) had regression in language, general skills or both. All of these individuals had a reported regression before they were diagnosed. Therefore, our cohort of individuals with sequence variants may be overrepresented by individuals with regression, potentially explaining the difference we see in current cognitive ability but not in historical achievement of milestones. As sequencing panels and exome sequencing become more commonly ordered as part of clinical assessments in ASD or ID, further basic functional and clinical analyses should be done to compare sequence variants to deletions in PMS.

This study is limited by potential ascertainment bias in terms of who receives genetic diagnoses of PMS and the wide age range of participants. Additionally, standardized tests developed for typically developing individuals at times limited our ability to derive precise scores reflecting variability among participants, as scores were often subject to floor effects and/or some participants were unable to engage at all with a subset of standardized tests. Furthermore, because we included participants from a diverse set of studies which employed various assessment batteries, most variables did not have full sample sizes.

In sum, in our cohort, we found divergent phenotypic profiles of individuals with PMS who have Class I deletions, sequence variants and Class II deletions. Individuals with Class I deletions and sequence variants tended to attain more complex developmental milestones and reached them at a younger age. These individuals were more likely to exhibit greater language and

communication skills. In our cohort, they were also more likely to lose these skills and to be affected by a variety of mental illnesses. Individuals with Class II deletions tended to have more medical complexity and were more likely to present with renal, ocular, spine, and gait abnormalities. Individuals with Class II deletions were less likely to achieve developmental milestones and therefore less likely to regress, being unable to lose skills they never achieved. Overall, developmental and intellectual disability tended to be more severe in the group with Class II deletions. Findings present at consistent rates across all genotypic groups include ASD diagnosis and symptoms, behavioral abnormalities, and various medical comorbidities, including gastrointestinal abnormalities, recurrent infections, and seizures.

This is the largest prospective study to date and replicates several important findings from the literature in addition to highlighting new associations for further study. Taken together, results from our study have important implications for assessment, monitoring, and for counseling families.

Materials and Methods

Informed consent was obtained from participants' caregivers for study participation and publication. The cohort included 170 individuals (82 female, 88 male) with PMS between the ages of one and 45 years (12 ± 9.1, mean $\pm$ SD). Ninety-three participants were enrolled in studies at the Seaver Autism Center for Research and Treatment at the Icahn School of Medicine at Mount Sinai. An additional 66 participants were enrolled by partner sites through the Rare Disease Clinical Research Network Developmental Synaptopathies Consortium (DSC), as part of a PMS phenotyping and natural history study (Boston's Children's Hospital $n = 15$, National Institute of Mental Health $n = 16$, Rush University Medical Center $n = 25$, Stanford University $n = 5$, University of Texas Southwestern $n = 5$). Eleven participants were enrolled by Seaver Autism Center staff at a PMS Foundation conference. Studies were approved by the Institutional Review Board (IRB) for the protection of human subjects at Mount Sinai (Study IDs: 98–0436, 10-0527, 12-1718) and Boston Children's Hospital (Study ID: P00013300), which serves as the central IRB for the DSC.

For 159 participants, a comprehensive battery that included standardized assessments, semi-structured interviews, and caregiver report questionnaires was used to examine medical comorbidities, intellectual, and adaptive functioning, expressive and receptive language, ASD symptomatology, and behavioral comorbidities. Medical evaluation included neurological, psychiatric, and clinical genetics examinations and a review of each participant's medical history. Evaluations were conducted by child and adolescent psychiatrists, psychologists, neurologists, clinical geneticists, and genetic counselors. For the 11 participants enrolled at the PMS Foundation conference, a medical history form was collected.

Genetics

Genetic reports were reviewed by a genetic counselor and the diagnosis of PMS was confirmed for each participant, defined as having either a deletion encompassing *SHANK3* (MIM: 606230) or a pathogenic sequence variant in *SHANK3* according to standards established by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Microarray results were aligned to the hg19 reference genome and sequence variants to reference transcript NM_033517.1.

Medical comorbidities

Medical histories for participants were collected by a psychiatrist or neurologist ($n = 159$) or by parent form ($n = 11$). A psychiatric evaluation assessed development, current mental status, psychiatric comorbidity, and ASD features ($n = 159$). A neurological examination assessed reflexes, motor skills, sensory response, balance, and coordination ($n = 143$). A clinical geneticist or genetic counselor assessed dysmorphisms ($n = 143$). Dysmorphic features evaluated included head abnormalities (including microcephaly and macrocephaly), ear abnormalities, dental and palate abnormalities, facial dysmorphisms, hand and feet abnormalities, and other body dysmorphisms.

Intellectual and adaptive functioning

Cognitive functioning was assessed by clinical psychologists using the Stanford-Binet Intelligence Scales, fifth edition ($n = 28$) (44), the Differential Abilities Scales, second edition ($n = 9$) (45), or the Mullen Scales of Early Learning ($n = 107$) (46). The Stanford-Binet or Differential Abilities Scales is typically attempted first; if participants are not able to comply with the receptive or expressive language demands of these tests, the Mullen Scales (which are out-of-age range for children over 5 years, 8 months) are completed instead. Intellectual quotients (IQ) were calculated for individuals who completed the Stanford-Binet or Differential Abilities Scales, while developmental quotient (DQ) scores, using age equivalents, were calculated for individuals who completed the Mullen scales. Adaptive functioning was measured using the Vineland Adaptive Behavior Scales, second edition ($n = 136$) (47). A consensus diagnosis of ID was made based upon DSM-5 criteria (11).

Milestones

Developmental milestones were recorded using the ADI-R ($n = 126$) (9).

Language and communication. Expressive and receptive language abilities were assessed using the clinician-administered Expressive Vocabulary Test, second edition (EVT-2; $n = 114$) (48) and the Peabody Picture Vocabulary Test, fourth edition (PPVT-4; $n = 114$) (49). Individuals who were administered, but could not complete, the EVT-2 ($n = 72$) or PPVT-4 ($n = 74$) were assigned a basal score of 20. Items on the ADI-R regarding current comprehension of language and overall language were also used to assess language and communication. Parent report from the MacArthur-Bates Communicative Development Inventories ($n = 78$) (50,51) was used to evaluate vocabulary and comprehension.

Regression

Regression was assessed using the ADI-R, defined as a loss of developmental skills for at least three months.

ASD symptomatology. Consensus ASD diagnoses were determined for each participant using gold-standard diagnostic testing and clinical evaluation. The ADOS-2 ($n = 117$) (10) and the ADI-R (9) were administered by research-reliable clinical psychologists—research reliability is achieved after advanced research training and a high level of inter-rater agreement (>80% for ADOS-2, >90% on ADI-R) with scoring of a skilled examiner. A psychiatric evaluation, completed by a physician, assessed DSM-5 (11) criteria for ASD. To further evaluate features of the

ASD phenotype in this cohort, several caregiver report forms were administered, including the Short Sensory Profile ($n = 104$) (52), the Aberrant Behavior Checklist ($n = 107$) (53,54), and the Repetitive Behavior Scale, Revised ($n = 103$) (55).

Statistical analysis

Genotype. Class I deletions and sequence variants were combined into one group for comparison to Class II deletions in two-group analyses. To explore the sequence variant group, three-group analyses were run (Class I deletions vs. Class II deletions vs. sequence variants).

Phenotype. Fourteen primary variables were selected to represent core features of PMS and the best replicated findings from previous literature. Core features were selected based upon the most consistent findings in the literature (e.g. developmental delay, intellectual disability). An additional 46 variables were included as secondary variables. Variables represented six key domains: medical features, intellectual and adaptive functioning, developmental milestones, language and communication, regression, and ASD symptomatology/behavioral comorbidities.

Tests. Associations between genotype class and continuous variables were assessed using Mann–Whitney U tests. Chi square analyses were employed for analyses between genotype class and discrete and nominal variables. *p*-values are listed to three decimal points or until the first nonzero value. Bonferroni corrections for multiple comparisons were made for the primary analyses, with a significant *p*-value set at 0.00357. Benjamini–Hochberg corrections, with a false discovery rate of 0.05, were made for each set of exploratory analyses. *P*-values below 0.015 were significant for the two-group analysis on secondary variables, and values below 0.012 were significant for the three-group analysis on primary variables.

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