The Journal of Rare DISORDERS

A PROSPECTIVE STUDY OF NEUROLOGICAL ABNORMALITIES IN PHELAN-MCDERMID SYNDROME

Yitzchak Frank MD,[1,2] Jesslyn M. Jamison BA,[1,2] M. Pilar Trelles MD,[1,2] Paige M. Siper PhD,[1,2] Teresa Tavassoli PhD,[1,2] Latha V. A Soorya PhD,[3] Stacey Lurie BA,[1,4] Hillary T. Rieger BA,[1,2] Elyana Feldman BA,[1,2] Thomas Naidich MD,[5,6,7] Ting Wang PhD,[1,2,8,9] Danielle Halpern PsyD,[1,2] Joseph D. Buxbaum PhD,[1,2,8,9,10,11] Alexander Kolevzon MD[1,2,9,11,12]

[1] Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY, USA, [2] Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA [3]Department of Psychiatry, Rush University Medical Center, Chicago, IL, USA,[4] Ferkauf Graduate School of Psychology, Yeshiva University, Bronx, NY, USA, [5]Department of Radiology Icahn School of Medicine at Mount Sinai, New York, NY, USA,[6]Department of Neurosurgery, Icahn School of Medicine at Mount Sinai, New York, NY, USA, [7]Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA,[8] Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA,[9] Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA,[10] Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA,[11]Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA [12]Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

ABSTRACT

Background: Phelan-McDermid syndrome (PMS) occurs as a result of a chromosomal abnormality, most frequently deletion, in the long arm of chromosome 22, involving the *SHANK3* gene. Our goal was to prospectively assess the neurological phenotype in this syndrome.

METHODS: Twenty-nine participants were recruited from ongoing studies in PMS at the Seaver Autism Center. They had a structured, uniform neurological examination performed by a pediatric neurologist (Y.F.). Abnormal findings were graded as mild, moderate or severe. In addition, reports of seizures, magnetic resonance imaging (MRI) and electroencephalograms (EEG) were reviewed. We calculated the frequency and severity of neurological abnormalities, as well as correlations between items on the neurological examination and items on the Mullen Scales of Early Learning and on the Vineland Adaptive Behavioral Scales (VABS).

RESULTS: Hypotonia, abnormal gait, fine motor coordination deficits, and expressive and receptive language delays were present in all participants. Attention deficits were present in 96% (severe in 62%), and abnormal visual tracking was present in 86%. A history of seizures was obtained in 44.8% of participants but 46.1% of these were febrile only. Of the 13 EEG reports available for review – 69.2% were abnormal- with epileptic features present in 53.8%. Abnormalities were present in 62.5% of MRI reports. Correlations were found between neurological abnormalities and scores on the Mullen and Vineland Scales.

CONCLUSIONS: Neurological abnormalities are very common in PMS and are correlated with measures of cognitive and adaptive functioning. The neurological examination may be used for clinical diagnosis, identification of PMS phenotypes, and, in the future, for evaluation of therapy.

INTRODUCTION

Phelan-McDermid syndrome (PMS), also called 22q13 deletion syndrome, is characterized by global developmental delay, intellectual disability, speech and language abnormalities, autism spectrum disorder (ASD), hypotonia, and mild dysmorphic features.[1,2,3,4,5] It is likely underdiagnosed due to lack of a specific clinical phenotype and insufficient genetic testing. While its true prevalence remains unknown, PMS is among the more prevalent rare causes of ASD.[6] The syndrome is the result of a chromosomal abnormality including terminal or interstitial deletion involving the long arm of chromosome 22 in the 22q13.3 region, unbalanced chromosome translocation, other structural chromosomal rearrangement, or loss of function mutations in *SHANK3.*[7,8,9] There is mixed evidence for a relationship between deletion size and presence and severity of clinical features.[4,7,8,10,11] A core of neurological and behavioral symptoms and signs characterize the syndrome, including hypotonia, speech and language abnormalities and ASD, and are related to loss of the *SHANK3* gene.[8,12,13,14,15,16,17] This gene encodes a scaffolding protein in the post synaptic density (PSD) of excitatory synapses, and plays an important role in the

development and maintenance of these synapses. It is strongly expressed in the cerebral cortex and cerebellum. Genetic manipulation of *Shank3* in model systems leads to changes in the key components of glutamatergic synapses including glutamate receptors, dendritic spine density, and electrophysiological function.[18,19,20,21,22]

To date, there have been a few large prospective studies reporting the results of standardized developmental testing in PMS[4,23] but no prospective standardized neurological examinations. Our knowledge of the neurological phenotype in PMS is therefore mostly based on small studies or case reports [24,25,26,27] which do not include a full neurological examination, or on information obtained historically from caregivers, referring physicians, or questionnaires[1,9,14] The most frequently described neurological abnormality is hypotonia, reported in 75-100% of individuals PMS, while other neurological abnormalities have only infrequently been reported.[1,4,7,8,9,16,28,29] Seizures have been reported in 14-70% of cases[1,2,4,7,9,13,14,16,24-26, 28-30] and several studies have documented abnormal electroencephalograms (EEG).[4,24,26,30] There are only a few imaging studies which describe a variety of structural brain changes.[4,16,25,29,31,32]

The frequency and degree of reported neurological abnormalities are highly variable. Given that the brain is the most affected organ in this syndrome, and that a neurological examination is an important tool used to study brain abnormalities - there is clearly a need for a systematic effort to study the neurological phenotype in PMS. In addition, a structured neurological examination can be used to compare cohorts of PMS individuals and to assess response to therapy.

The purpose of our study is to characterize the neurological phenotype of PMS through a prospective, structured neurological examination that will record the type, frequency and severity of neurological abnormalities in a cohort of participants with PMS, and in addition, to review results of EEG and MRI testing in these participants.

METHODS

Participants

Twenty nine children and adults, 16 males (55.2%) and 13 females (44.8%), between the ages of 20 months and 45 years (M_{age}= 8.7 years, SD_{age}= 9.58 years) participated in this study. Twenty two (76%) were between the ages of 2 -18 years, and only 3 were adults. Participants were recruited as part of ongoing studies in PMS at the Seaver Autism Center for Research and Treatment at the Icahn School of Medicine at Mount Sinai. Caregivers provided informed consent and the study was approved by the Mount Sinai Program for the Protection of Human Subjects. A neurological interview and clinical examination were part of a comprehensive assessment protocol, which also included psychiatric, cognitive, behavioral, and autism-focused diagnostic testing. Nineteen of the 29 participants included in the study were previously described by Soorya et al. (2013).[4] All participants had *SHANK3* deletions or mutation confirmed by chromosomal microarray or sequencing respectively. A diagnosis of ASD was confirmed in 24 of the 29 participants (82.8%) according to clinical consensus based on psychiatric evaluation using the Diagnostic and Statistical Manual for Mental Disorders, Fifth Edition (DSM-5), the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS-2).[33,34,35] Data on cognitive functioning was obtained with the Mullen Scales of Early Learning.[36] Data on adaptive functioning was available using the Vineland Adaptive Behavior Scales.[37] All participants had moderate to severe intellectual disability.

Procedure

An interview with parents and a uniform pediatric neurological examination were done by a pediatric neurologist (Y.F.) The interview included history of seizures, and presence of other medical problems or medications which may have affected the nervous system or the neurological examination. Medical records provided by families were used to supplement parent history.

The neurological examination included the following domains: motor (muscle tone, strength, gait and fine motor coordination), visual motor coordination, speech and language, sensory, cranial nerves, and behavior (visual tracking, stereotypy, eye contact, hyperactivity, attention, impulsivity and aggressiveness). Abnormal features on most parts of the examination were graded as mildly, moderately and severely abnormal (see Table I). Other parts of the examination including sensory and cranial nerves examinations were graded as normal or abnormal.

Table I: Neurological Exam in Phelan-McDermid Syndrome

	Grading System				
Exam	**Assessment**	**Score**			
		Normal	Abnormal		
Motor			**Mild**	**Moderate**	**Severe**
Muscle Tone	Muscle resistance to passive movement of limbs against gravity	Age appropriate	Mildly increased/ decreased muscle passive resistance	Moderately increase/decreased muscle passive resistance	Severely increased/ decreased muscle passive resistance
Muscle Strength	Active resistance to pulling/pushing of limbs, and observation	Fully resistant	Partially resistant	Did not resist to push/pull	Cannot hold limbs against gravity.
Deep Tendon Reflexes	Reflex hammer	Present	Absent		
Gait	Observation of gait: hypotonic, hypertonic, ataxic	Age appropriate	Clinician assessment	Clinician assessment	Clinician assessment
Fine Motor Coordination	Manipulation of test blocks and raisins				
Interest in Objects	Interest in reaching towards blocks and raisins	Age appropriate	Takes and manipulates	Takes, but does not manipulate	Has no interest in blocks and raisins
Object Manipulation	Ability to manipulate blocks and raisins	Age appropriate	Bangs blocks/ builds a tower	Takes blocks with or without a pincer grasp	Does not take or does not regard blocks
Visual Motor Coordination	Copies geometrical shapes	Age appropriate	Copies circle	Scribbles	No use of pencil
Language					
Expressive	Verbalizing and vocalizing	Age appropriate	Speaks in sentences or words	Vocalizes	No vocalizations
Receptive	Ability to follow instructions	Age appropriate or follows 2-step instructions	Follows 1--step instructions	Sporadic responses to instructions	Does not follow instructions
Behavior					
Visual Tracking	Tracking of a large red ball	Persistent and full tracking 180°	Tracks, but non-persistent	Gaze fixed on ball but does not track.	Does not look at the ball
Stereotypy	Frequency during the exam	None	Infrequent (1-2 times)	Moderate (4-5 times)	Very frequent (> 5 times)
Eye Contact	Frequency during the exam	Sustained	Frequent but not sustained	Intermittent	Rare (1-2 times during the exam) or absent

1. Muscle tone, strength and deep tendon reflexes

Muscle tone was assessed by moving the participant's limbs against gravity. The grading of decreased muscle tone (hypotonia) or increased muscle tone (hypertonia) - mild, moderate, or severe - was made by the examiner based on clinical norms for decreased or increased passive resistance to movements, in consideration with the participant's age.

Muscle strength was assessed by evaluating a participant's active resistance to the examiner's pulling and pushing their arms and legs, and watching them get up from the floor and climbing on a chair. Muscle weakness was graded as mildly abnormal when a participant partially resisted arm pull or push, or needed to hold on to a person or an object to get up from the floor. It was graded as moderately abnormal when they could not resist the pull or push of a limb, and as severely abnormal when they could not hold their arms and legs against gravity.

Deep tendon reflexes were graded as normal (elicited), or abnormal (not elicited). A flexor plantar reflex (obtained by scratching the bottom of the foot) was scored as normal and an extensor response was abnormal.

2. Gait

Gait was observed while the participant walked on a flat surface and while going up and down stairs. The degree of gait abnormality was graded, based on clinical experience, as mild, moderate and severe. Gait abnormalities can be the result of a number of neurological dysfunctions including hypertonia (e.g., circumduction, spastic diplegic gait, toe walking, foot deviation, knee flexion, or upper extremity posturing), hypotonia (e.g., reduced leg raising, waddling gait, foot drop), and ataxia (e.g., reduced balance, tendency to fall to one side, inability to walk on a narrow base). We, therefore, classified the abnormalities as hypertonic, hypotonic and ataxic.

3. Fine motor coordination

Fine motor coordination was assessed by presenting blocks and raisins to participants and demonstrating banging the blocks together, building a tower of blocks, and putting the raisins into a container. We graded the interest in reaching towards, taking and manipulating the test objects (interest in objects), and the actual ability to take and manipulate objects in an age appropriate manner (object manipulation) separately. Grading for interest in objects was: within normal limits (WNL) for age (normal), takes and manipulates (mildly abnormal), takes but does not manipulate (moderately abnormal), and no interest in the object (severely abnormal). Grading for object manipulation was: WNL for age (normal), can build a tower or bang blocks (mildly abnormal), takes the blocks using pincer grasp or incomplete pincer grasp (moderately abnormal) and does not regard or take the blocks (markedly abnormal). The grading of the fine motor coordination domains (as well as for some other domains including visual motor coordination and language) are relative to our group of individuals with PMS, and does not compare to grading of subjects with typical development. For instance, infants normally reach towards and grab a test block by four months of age, transfer blocks from one hand to the other by six months, have a pincer grasp by nine months, bang two blocks together by 12 months, and build a tower of two blocks by 20 months.[38] Our participants, aged 20 months or older, should have all been able to at least reach and take the blocks using a pincer grasp, bang blocks together and build a tower of two blocks or more. Grading their performance in comparison to subjects with typical development would classify most of them as "severely abnormal" and would not have demonstrated the inter-subject difference in the degree of abnormality (See **Table I** for methods of assessment and grading of abnormalities).

4. Visual-motor coordination

Visual motor coordination was assessed by participants' ability to copy geometrical shapes including a line, circle, square and triangle[39] and was graded as: WNL for age (normal), draws a circle or a line (mildly abnormal), scribbles (moderately abnormal), and no use of pencil (severely abnormal).

5. Language

Language was evaluated by observing the participants' expressive language (verbalizing and vocalizing) and receptive language (ability to follow instructions). Expressive language was scored as WNL for age (normal), use of words or short sentences (mildly abnormal), having vocalizations only but no words (moderately abnormal), and having no vocalizations (markedly abnormal). Receptive language was scored as WNL for age or able to follow 2-step instructions (normal), able to follow 1-step instructions (mildly abnormal), only

sporadic response to instructions (moderately abnormal), and unable to follow instructions (markedly abnormal).

6. Visual tracking

Visual tracking was assessed by moving a large red ball across the participant's visual field (180 degrees), while holding their head fixed. A full, continuous, smooth, tracking was graded normal. An inability to persistently track full field was graded mildly abnormal. Looking at the ball but inability to track was graded moderately abnormal, while not being able to look at the ball was graded markedly abnormal.

7. Stereotypic motor activity:

Stereotypic activity was graded as normal when none was present during the examination, mildly abnormal- when observed only infrequently (1-2 times during an examination period of about 1 hour), moderately abnormal when observed more frequently (4-5 times during the examination), and severely abnormal when observed very frequently (more than 5 times during the examination).

8. Eye contact

Eye contact grading was based on the frequency of the participant's eye contact with the examiner, and was graded WNL when sustained, mildly abnormal when frequent but not sustained, moderately abnormal when occurring intermittently, and markedly abnormal when rare (occurring only 1-2 times or absent during the examination).

9. Hyperactivity, inattention, impulsivity and aggression

Participants' activity level, attention span, impulse control and aggressiveness were observed during the examination, and graded based on comparison with typical subjects as WNL, mildly abnormal, moderately abnormal, and markedly abnormal.

10. Sensory examination

The participant's response to touch and light pain (pin) applied to their extremities was graded as normal when response was obtained, and abnormal for no response. Other sensory modalities, including "cortical" sensory modalities (e.g., 2-point discrimination; graphesthesia; stereognosis), could not be examined due to the severity of cognitive and language impairment in our participants.

11. Cranial Nerves

Cranial nerves II-XII examination included vision (behavioral response to a visual stimulus, a fundoscopic exam, and visual fields gross integrity), extra-ocular muscle movements, pupillary responses to light, facial movements, facial sensation, tongue movements and gag reflex. The results were graded as normal or abnormal.

12. Head circumference

Head circumference was measured (in cm) using a standard measuring tape. Age and gender percentages were obtained from the Nellhaus head circumference chart.[40] Macrocephalus was determined when head circumference was greater than 2 standard deviations (SD) above the mean. Microcephalus was determined when head circumference was less than 2 SD below the mean.

13. History of seizures: EEG and MRI reports

History of past and present seizures, type of seizures (afebrile or febrile), and the state of seizure control, were obtained from parents and medical records. EEG reports were available for 13 of the 29 participants (44.8%). Seven were routine EEG tests and six were prolonged (24 hours or longer) video-EEG monitoring. EEG reports were reviewed for background activity (normal or abnormal for age), and for the presence of epileptiform activity (focal or generalized). Magnetic resonance images and reports were available for 24 of our 29 participants (82.7%). Eighteen were obtained from other medical facilities and six were performed at our institution as part of a separate research protocol.

DATA ANALYSIS

SPSS 23 was used to analyze the data. Descriptive statistics were calculated for all variables identified through the neurological exam. Pearson correlation coefficients were calculated to examine the relationship between items on the neurological examination and standardized scores on the Mullen Scales of Early Learning and the Vineland Adaptive Behavior Scales.

RESULTS

Parent interview:

Thirteen participants (44.8%) had a history of feeding problems in early infancy, and nine others (31.0%) were described as hypotonic. Those problems did not cause significant disability (e.g., hospitalization). Abnormal attention span was reported by the majority of parents (93.1%). Hyperactivity was reported by 44.8%, sleep problems were reported in 51.7% of the participants, and were frequent or occurred every night in about 38%. Only 6 participants (20.7%) were taking medications

targeting behavior. Stereotypic activity was reported in 81% of the participants. None of the participants had medical or neurological conditions that could impact the interpretation of the neurological exam. Results are summarized in **Table II**.

Table II: Neurologic Findings in Phelan-McDermid Syndrome

	Total Abnormal (%)	Severity of Abnormalities Mild (%)	Moderate (%)	Severe (%)
Motor				
Hypotonia	100.0	55.2	34.5	10.3
Strength	10.3	10.3	0.0	0.0
Gait *				
Hypertonic	72.4	37.9	34.5	0.0
Hypotonic	75.9	20.7	55.2	0.0
Ataxic	31.0	13.8	17.2	0.0
All*	100.0	24.1	58.6	0.0
Fine Motor Coordination				
Interest in Objects	100.0	41.3	34.5	24.2
Object Manipulation	100.0	31.0	44.9	24.1
Visual-Motor Coordination	100.0	6.9	20.7	72.4
Language				
Expressive	100.0	17.2	69.0	13.8
Receptive	100.0	13.8	27.6	58.6
Behavior				
Visual Tracking	86.2	75.9	6.9	3.4
Stereotypy	68.9	27.6	37.9	3.4
Eye Contact	89.6	10.3	48.3	31.0
Hyperactivity	44.8	10.3	24.1	10.3
Attention	96.4	10.3	24.1	62.0
Impulse Control	51.7	20.7	13.8	17.2
Aggression	17.2	6.9	3.4	6.9

* 5 participants (17.3%) did not walk at the time of the examination.

Neurological Exam (summarized in Table II):

1. Muscle tone, strength, and deep tendon reflexes

Hypotonia was present in all participants on neurological exam. It was mildly abnormal in 16 (55.2%) moderately abnormal in 10 (34.5%) and severely abnormal in 3 (10.3%). Although two of the participants had mildly increased ankle dorsiflexion tone and two others had intermittent "cortical thumbs" posturing, none had generalized hypertonia. Strength was grossly normal in 26 participants (89.7%) and mildly abnormal in three (10.3%). Deep tendon reflexes in the upper extremities were elicited in 79.3% of the participants, and in the lower extremities - in 86.2%. Non-sustained clonus was present in two participants (6.9%). An extensor plantar reflex was present in two participants (6.9%).

2. Gait

Five participants (17.3%) could not walk independently at the time of the examination. Of those, one was an adult with a history of gradually deteriorating gait after an accident, and the other four were young children, ages 20-24 months, with delayed development. All others (24/29) had an abnormal gait (mildly abnormal in seven (24.1%) and moderately abnormal in 17 (58.6%). Hypotonic gait abnormalities were found in 22 participants (75.9%), hypertonic gait abnormalities in 21 participants (72.4%), and ataxic - in nine participants (31.0%). Most participants had gait abnormalities reflecting more than one neurological difficulty (e.g. the same participant could have posturing of upper extremities (a hypertonic abnormality), and waddling gait (a hypotonic abnormality)).

3. Fine motor coordination

None of the participants had normal interest in objects. Twelve of 29 participants (41.3%) showed interest in taking and manipulating the test blocks (mildly abnormal), and 10 (34.5%) took the blocks without manipulating them (moderately abnormal). Four (13.8%) looked at but did not take the blocks, and three (10.4%) did not look at the blocks - together 24.2% - (severely abnormal). Similarly, none of the participants had a normal object manipulation. Three participants (10.3%) could build a tower of at least two blocks and six participants (20.7%) could bang two blocks together. These 9 participants (31.0%) were graded mildly abnormal. Ten (34.5%) took the blocks using a pincer grasp, but could not bang blocks and three (10.4%) took the blocks but did not have a pincer grasp. These 13 participants (44.8%) were scored as "moderately abnormal". The remaining 7 participants (24.1%) who either did not regard or did not take the blocks were graded as severely abnormal.

4. Visual-motor coordination

None of the participants demonstrated age appropriate visual-motor coordination. One participant, age 14, could draw a circle and one, age 4:10, was able to copy a line. These two participants (6.9%) were graded mildly abnormal. Six participants (20.7%) could scribble (moderately abnormal), and 21 (72.4%) did not have any use of a pencil (markedly abnormal).

5. Language

None of the participants had normal language for age, and the majority had markedly abnormal language. Two participants (6.9%) - a nine year old boy and a 4:10-girl spoke in short sentences, and three participants (10.3%) spoke in single words. Together these participants (17.2%) were graded mildly abnormal. The majority of the participants (20/29; 69.0%) could vocalize, but did not use any words (moderately abnormal). Four participants (13.8%) did not vocalize (severely abnormal). Similarly, receptive language was markedly impaired in our participants: none were normal for age and none could follow a two-step instruction. Four participants (13.8%) could follow a one-step instruction (scored mildly abnormal) and eight participants (27.6%) had sporadic responses (moderately abnormal). The majority (17/29; 58.6%) did not follow any instruction (severely abnormal).

6. Visual tracking

Twenty-five participants (86.2%) had abnormal visual tracking. Twenty two (75.9%) were graded mildly abnormal. Two participants (6.9%) were graded moderately abnormal, and one participant (3.4%) was graded severely abnormal.

7. Stereotypic motor activity

Stereotypic motor activity was observed in 20/29 participants (68.9%). It was graded mildly abnormal in eight participants (27.6%), moderately abnormal in 11 participants (37.9%), and severely abnormal in one participant (3.4%).

8. Eye contact:

Eye contact with the examiner was impaired in 26/29 participants (89.6%). It was graded mildly abnormal in three participants (10.3%) moderately abnormal in 14 (48.3%) and severely abnormal in nine (31.0%).

9. Hyperactivity, inattention, impulsivity and aggressiveness:

Most participants were not hyperactive during the examination. Activity level was normal (typical) in 16 participants (55.2%), mildly abnormal (increased) in three (10.3%) moderately abnormal (increased) in seven (24.1%), and severely abnormal (increased) in three (10.3%). Attention span was deficient in most participants (96.4%). It was graded mildly abnormal in three (10.3%), moderately abnormal in seven (24.1%) and severely abnormal in 18 (62.0%). Impulse control was graded normal in 14/29 participants (48.3%) mildly abnormal in six (20.7%), moderately abnormal in four (13.8%) and severely abnormal in five (17.2%). Aggression was infrequent in our participants (5/29, 17.2%). Most participants (24/29, 82.8%) did not demonstrate any aggression during the examination; two (6.9%) were graded mildly abnormal, one (3.4%) was graded moderately abnormal, and two (6.9%) were graded severely abnormal.

10. Sensory examination

All participants responded symmetrically to touch and pain stimuli.

11. Cranial nerves examination

Cranial nerves examination did not reveal abnormalities. There were no gross visual field defects.

12. Head circumference

Head circumference was normal for age in 22 participants (75.9%). Six participants (20.7%) had a head circumference measurement at or above the 98th percentile. One participant had a head circumference in the 2nd percentile, and none had head circumference measuring below the 2nd percentile (microcephaly).

13. History of seizures, review of EEG and MRI

A history of seizures was reported in 13 of the 29 participants (44.8%). Six of the 13 (46.1%) had seizures only with fever. Two participants (6.9%) had seizures in the past, but were free of seizures at the time of the examination and were not receiving anticonvulsant treatment. Five participants (17.2%) had an active seizure disorder at the time of the examination three (10.3%) were controlled with medications and two (6.9%) were not fully controlled. Nine of 13 EEGs (69.2%) were abnormal. Seven of the 9 (53.8%) demonstrated epileptiform abnormalities (focal or generalized) and 4/9 (30.8%) had slow background. There were no reports of electroencephalographic seizures.

Abnormalities were present in 15 of 24 MRIs (62.5%). Eleven of the MRIs had more than one abnormality, with a total of 32 reported structural changes. The most common were white matter abnormalities including periventricular leukomalacia, present in 8 scans and constituting 25% of the total number of abnormalities. Large ventricles were found in 6 cases, constituting 18.75% of abnormalities found, and arachnoid cysts in 5 cases, constituting 15.6% of the total number of abnormalities. Other less common changes were atrophy and abnormalities of cortical grey matter, cerebellum, and corpus callosum. Subdural hematomas were found in 2 cases one reportedly occurred after an endoscopic fenestration of an enlarging middle cranial fossa arachnoid cyst, and the other was incidentally found on an MRI taken at age two because of developmental delay.

Correlational Analyses with Measures of Cognitive and Adaptive Functioning

Items on the neurological examination were correlated with raw scores on the Vineland Adaptive Behavior Scales, Second Edition, and the Mullen Scales of Early Learning. [36, 41]

On the motor examination, *Hypotonia* was moderately correlated with Gross Motor (r=.59, *p*=.001) and Fine Motor (r=-.44, *p*=.018) scores on the Vineland-II and Mullen Fine Motor scores (r=.43, *p*=.033). *Gait* was strongly correlated with Vineland-II Gross Motor scores (r=-.811, *p*<.001) and moderately correlated with Vineland-II Fine Motor scores (r=-.56, *p*=.002) and Mullen Fine Motor scores (r=.43, p=.034). The neurological *expressive language* exam was strongly correlated with Vineland-II Expressive Language Scores (r=-.77, *p*<.001) and moderately correlated with both Mullen Expressive Language (r=-.58, *p*=.002) and Receptive Language scores (r=-.54, *p*=.005). The neurological *receptive language* exam was moderately correlated with Vineland-II Receptive (r=-.65, *p*<.001) and Expressive Language scores (r=-.58, *p*=.002) and the Mullen Receptive (r=.65, *p*<.001) and Expressive Language scores (r=-.58, *p*=.002). The Visual Reception scale on the Mullen, which measures visual discrimination and memory was also correlated with expressive (r=-.44, *p*=.028) and receptive language (r=-.67, *p*<.001) scores on the neurological exam.

DISCUSSION

Neurological abnormalities were very common in our

sample of participants with PMS. Almost all had abnormalities in the gross and fine motor, visual motor, and speech and language parts of the examination. There were no gross cranial nerve or sensory abnormalities. Neuroimaging and EEG abnormalities were common.

Although some of the neurological abnormalities found in our study are similar to those described in the literature, our study is conceptually and methodically different in that it provides a full neurological examination for each participant and describes specific neurological abnormalities rather than developmental delays. The motor system examination, including muscle tone, gait, and fine motor coordination, was abnormal in all our participants. Hypotonia was present in all participants. Although commonly a component of a "lower motor neuron" syndrome, where pathology is located in the spinal cord, peripheral nerves or muscle, hypotonia can also be a sign of central nervous system abnormalities, and has been described in genetic conditions affecting the brain, including ASD.[42] Based on our results and previous clinical reports of PMS, hypotonia can be considered a core neurological sign of this syndrome. Similarly, gait abnormality was also present in all our participants who could walk. We recognized different types of gait abnormalities, including hypotonic, hypertonic, and ataxic gait, suggesting that multiple brain systems are affected in PMS including the cerebellum, frontal cortex, and basal ganglia.[43]

Other significant motor abnormalities identified in our sample were difficulties in reaching, grabbing and manipulating objects - "fine motor" activities which involve motor planning, motor coordination, and attention. These impairments were the result of a combination of factors including lack of interest in the task and an inability to plan and perform motor movements necessary to carry out such tasks. Our participants also performed very poorly on a visual-motor coordination task, which included both fine and visual perceptual components and required them to copy simple geometrical shapes.[39] Over seventy percent of participants had no ability to use a pencil, a task all should have been able to accomplish, only two participants could copy a line or a circle. For reference, children can normally hold a pencil and scribble before age two, can copy a vertical line by age three, and copy a circle at age 3-4.[38] Together, these findings suggest that visual perception (a sensory function), visual-motor connections, attention factors, motor planning and fine motor performance, controlled by multiple brain regions and connections, are impaired in PMS.

Over 80 percent of our participants did not have any spoken language. Receptive language was similarly impaired in all participants, with the majority (86.2%) unable to consistently follow a one-step simple command. These findings confirm previous reports of severe speech and language impairment in PMS.[4,7,23] In contrast, participants with PMS in other studies[9,26] varied in their language ability, ranging from those with no language to some with functional language and only minor articulation difficulties. This variability in the severity of speech and language impairment, as well as variability in other cognitive and neurologic deficits in PMS, remains to be clarified.

The sensory examination (the response to touch and mild pain stimuli) did not detect abnormalities, suggesting that the peripheral nerves and spinal cord are not grossly abnormal. A more detailed sensory examination that includes the modalities of position, vibration, graphesthesia, stereognosis, 2-point discrimination, and subjective appreciation of various sensory stimuli, could not be done because of the cognitive and language limitations of the participants. PMS subjects are reported to have reduced response to temperature and other sensory sensitivities.[3,44] Those sensory differences cannot be identified by the neurological examination alone, and require other neurological and observational tests.

A large head circumference (OFC ≥ 98th percentile) was found in ~ 20% of our sample. None of our participants had a head circumference below the 2nd percentile (microcephaly). These findings are consistent with one study reporting macrocephaly in 18% of a group of PMS subjects. However, that study also reported microcephaly in 11% of their sample[10] – an abnormality not observed in our study. Large head circumference has been reported in idiopathic ASD and may be related to an increased rate of head growth during a period in infancy or early childhood,[45] although it is not known whether this is the same pathophysiology of large head circumference in some PMS subjects.

Our study confirms a high prevalence of seizures and EEG abnormalities in PMS. Approximately forty-five percent of our participants were reported to have seizures, and almost 70% of the EEG results were abnormal. Seizures were febrile or afebrile, generalized or focal, and were usually not intractable. It is difficult to assess the

prevalence of a seizure disorder in PMS because most of the case studies are small and the source of data is variable. To our knowledge, there have not been prospective studies which include seizure evaluation and EEG testing. Although Figura et al. (2014)[24] reported an "atypical EEG pattern, characterized by multifocal paroxysmal abnormalities, prevalent over the frontal–central or frontal–temporal regions, with sleep activation", most reports, including ours, have not observed a particular type of seizures or EEG pattern. Seizure disorders and EEG abnormalities are, in general, common in ASD,[46] and are also common in certain genetic syndromes with documented synaptic pathology, such as Rett syndrome and Tuberous Sclerosis. [47] *SHANK3* deficiency with its synaptic pathology and resultant dysregulation in glutamatergic circuits may therefore increase vulnerability to seizures.[21] Although, most *Shank3* heterozygous mice do not show seizures or EEG abnormalities,[20] the increased risk of seizures or EEG abnormalities in PMS is thought to be related to *SHANK3* deficiency.

MRI abnormalities were found in the majority of those participants for whom MRI data was available, and were diverse, including white matter (periventricular, corpus callosum), grey matter and cerebellar abnormalities, and arachnoid cysts. Philippe et al (2008)[26] reported brain MRI abnormalities in 5 of 8 children with *SHANK3* deletions, including a thin or atypical corpus callosum, white matter hyperintensities, arachnoid cysts, ventricular dilatation and bilateral periventricular nodular heterotopias. Figura et al. (2014)[24] reported, similarly, a variety of brain MRI abnormalities in 22q13.3 deletion subjects, including reduced myelination, agenesis or thinning of the corpus callosum, ventricular dilatation, and cortical atrophy. Neuroimaging abnormalities reported by Aldinger et al (2013)[31] in 10 subjects with PMS included corpus callosum thinning (9/10), abnormally thin white matter (7/10) and enlarged ventricles (8/10). Vermian hypoplasia and/or mega cisterna magna (MCM) were found in 8/10 participants. The authors raised the question of whether posterior fossa/cerebellar abnormalities are a structural brain signature of PMS. Hypoplasia of the cerebellar vermis with enlarged cisterna magna was also found in two adult brothers with PMS, intellectual disability and speech abnormalities.[32] Other MRI reports of PMS, including ours, do not show a predominance of posterior fossa/cerebellar abnormalities.[16,24,26,29]

We found that the neurological examination correlated with performance on measures of cognitive and adaptive functioning. The neurological exam motor scores were correlated with motor performance on the Mullen and Vineland-II and not with language scores. Language scores given during the neurological examination were correlated with expressive and receptive language scores on the Mullen and Vineland-II and not with motor scores. The fact that items on our neurological examination correlated with performance on standardized measures supports the use of the neurological examination as a tool for assessing brain function in patients with PMS. The Mullen is a valuable quantitative developmental assessment, administered by specially trained clinicians, while the neurological examination is a "bedside" tool that can be used by pediatricians and pediatric neurologists who may have earlier contact with PMS children.

There are a number of limitations to our study. Validated age appropriate quantitative measures for many items on the neurological examination are not readily available, especially for populations of severely affected individuals. The fact that the same structured examination of all participants was performed by the same clinically experienced pediatric neurologist increases the validity of our grading and partially mitigated this difficulty.

Another limitation was the fact that part of our EEG and MRI data was obtained through clinical reports from other institutions. For this reason, we restricted our investigation to general types of EEG and MRI abnormalities, reported in standard fashion in clinical reports.

Last, full understanding of the neurological phenotype of PMS requires knowledge of the natural history and evolvement of the neurological abnormalities. As most of our participants were children, this knowledge will accumulate through results from an ongoing longitudinal study.

In conclusion, our findings confirm that neurological abnormalities are very common and a core component of the PMS phenotype. A neurological examination is therefore an important tool which can be used to identify elements of the clinical phenotype of PMS and quantify the neurological deficits. Future studies should also examine potential genotype/phenotype correlations and whether the neurological examination may be used as an outcome measure of change in response to treatment.

Funding Support

This work was supported by grants from the Beatrice and

Samuel A. Seaver Foundation. AK received research support from NIMH (R34 MH100276-01), NINDS (U54 NS092090-01), and the Autism Science Foundation.

12Financial conflicts of interest: Dr. Kolevzon consults to Vencerx Therapeutics, Supernus Pharmaceuticals, Fulcrum Therapeutics, Genentech, and Ovid Therapeutics. Dr. Joseph Buxbaum and the Icahn School of Medicine hold a shared patent for insulin-like growth factor-1 (IGF-1) in Phelan-McDermid syndrome.

13 REFERENCES

1. Nesslinger NJ, Gorskit JL, Kurczynski TW, Shapira SK, Siegel-bartelt J, Dumanski JP, et al. Clinical , Cytogenetic , and Molecular Characterization of Seven Patients with Deletions of Chromosome 22q13.3. *Am J Hum Genet* 1994;54:464–72.
2. Phelan MC, Rogers RC, Saul RA, Stapleton GA, Sweet K, McDermid H, et al. 22q13 Deletion Syndrome. *Am J Med Genet* 2001;101:91–9.
3. Phelan MC. Deletion 22q13.3 syndrome. *Orphanet J Rare Dis* 2008;3:14.
4. Soorya L, Kolevzon A, Zweifach J, Lim T, Dobry Y, Schwartz L, et al. Prospective investigation of autism and genotype-phenotype correlations in 22q13 deletion syndrome and SHANK3 deficiency. *Mol Autism* 2013;4:18.
5. Kolevzon A, Angarita B, Bush L, Wang AT, Frank Y, Yang A, et al. Phelan-McDermid syndrome : a review of the literature and practice parameters for medical assessment and monitoring. *J Neurodev Disord* 2014;6:39.
6. Betancur C, and Buxbaum JD. SHANK3 haploinsufficiency: a “common” but underdiagnosed highly penetrant monogenetic cause of autism spectrum disorders. *Molecular Autism* 2013;4:17.
7. Luciani JJ, Mas P De, Depetris D, Bottani A, Prieur M, Jonveaux P, et al. Telomeric 22q13 deletions resulting from rings, simple deletions, and translocations: cytogenetic, molecular, and clinical analyses of 32 new observations. *J Med Genet* 2003;40:690–697.
8. Koolen DA, Reardon W, Rosser EM, Lacombe D, Hurst JA, Law CJ, et al. Molecular characterisation of patients with subtelomeric 22q abnormalities using chromosome specific array-based comparative genomic hybridisation. *Eur J Hum Genet* 2005;13:1019–24.
9. Sarasua SM, Boccuto L, Sharp JL, Dwivedi A, Chen CF, Rollins JD, et al. Clinical and genomic evaluation of 201 patients with Phelan-McDermid syndrome. *Hum Genet* 2014;133:847–59.
10. Sarasua SM, Dwivedi A, Boccuto L, Rollins JD, Chen C-F, Rogers RC, et al. Association between deletion size and important phenotypes expands the genomic region of interest in Phelan-McDermid syndrome (22q13 deletion syndrome). *J Med Genet* 2011;48:761–6.
11. Oberman LM, Boccuto L, Cascio L, Sarasua S, Kaufmann WE. Autism spectrum disorder in Phelan-McDermid syndrome : initial characterization and genotype-phenotype correlations. *Orphanet J Rare Dis* 2015;10:105.
12. Goizet C, Wxcoffier E, Taine L, Taupiac E, El Moneim AA, Arveiler B, et al. Case with autistic syndrome and chromosome 22q13.3 deletion detected by FISH. *Am J Med Genet* 2000;96(6):839-44
13. Wilson H, Wong A, Shaw S, Tse W, Stapleton G, MC P, et al. Molecular characterisation of the 22q13 deletion syndrome supports the role of haploinsufficiency of SHANK3/PROSAP2 in the major neurological symptoms. *J Med Genet* 2003;40:575–84.
14. Jeffries a R, Curran S, Elmslie F, Sharma a, Wenger S, Hummel M, et al. Molecular and phenotypic characterization of ring chromosome 22. *Am J Med Genet A* 2005;137:139–47.
15. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet* 2007;39:2006–8.
16. Dhar SU, Del Gaudio D, German JR, Peters SU, Ou Z, Bader PI, et al. 22q13.3 Deletion syndrome: Clinical and molecular analysis using array CGH. *Am J Med Genet Part A* 2010;152:573–81.
17. Bonaglia MC, Giorda R, Mani E, Aceti G, Anderlid B, Baroncini A, et al. Identification of a recurrent breakpoint within the SHANK3 gene in the 22q13.3 deletion syndrome. *J Med Genet* 2006;43(10):822–28.

18. Roussignol G, Ango F, Romorini S, Tu JC, Sala C, Worley PF. Shank Expression Is Sufficient to Induce Functional Dendritic Spine Synapses in Aspiny Neurons. *J Neurosci* 2005;25:3560–70.
19. Bozdagi O, Sakurai T, Papapetrou D, Wang X, Dickstein DL, Takahashi N, et al. Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function , social interaction , and social communication. *Mol Autism* 2010;1:15.
20. Peça J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, et al. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* 2011;472:437–42.
21. Wang X, Mccoy PA, Rodriguiz RM, Pan Y, Je HS, Roberts AC, et al. Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3. *Hum Mol Genet* 2011;20:3093–108.
22. Durand CM, Perroy J, Loll F, Perrais D, Fagni L, Bourgeron T, et al. SHANK3 mutations identified in autism lead to modification of dendritic spine morphology via an actin-dependent mechanism. *Mol Psychiatry* 2012;17:71-84.
23. Zwanenburg R, Ruiter SAJ, van den Heuvel ER, Flapper BCT, Van Ravenswaaij CMA. Developmental phenotype in Phelan-McDermid (22q13.3 deletion) syndrome: a systematic and prospective study in 34 children. *Journal of Neurodevelopmental Disorders* 2016;8:16.
24. Figura MG, Coppola A, Bottitta M, Calabrese G, Grillo L, Luciano D, et al. Seizures and EEG pattern in the 22q13.3 deletion syndrome: clinical report of six Italian cases. *Seizure* 2014;23:774–9.
25. Lindquist SG, Kirchhoff M, Lundsteen C, Pedersen W, Erichsen G, Kristensen K, et al. Further delineation of the 22q13 deletion syndrome. *Clin Dysmorphol* 2005;14(2):55–60.
26. Philippe A, Boddaert N, Vaivre-Douret L, Robel L, Danon-Boileau L, Malan V, et al. Neurobehavioral profile and brain imaging study of the 22q13. 3 deletion syndrome in childhood. *Pediatrics* 2008;122:e376–82.
27. Barakat AJ, Pearl PL, Acosta MT, Runkle BP. 22q13 deletion syndrome with central diabetes insipidus: a previously unreported association. *Clinical Dysmorphology* 2004;13:191-194.
28. Manning MA, Cassidy SB, Clericuzio C, Cherry AM, Hudgins L, Enns GM, et al. Terminal 22q Deletion Syndrome: A Newly Recognized Cause of Speech and Language Disability in the Autism Spectrum. *Pediatrics* 2004;114(2):451-7.
29. Denayer A., Van Esch H, De Ravel T, Frijns JP, Van Buggenhout G, Vogels A., et al. Neuropsychopathology in 7 patients with the 22q13 deletion syndrome: Presence of bipolar disorder and progressive loss of skills. *Mol Syndromol* 2012;3:14–20.
30. Holder JL, and Quach MM. The spectrum of epilepsy and electroencephalographic abnormalities due to SHANK3 loss- of- function mutations. *Epilepsia* 2016; In press.
31. Aldinger KA, Kogan J, Kimonis V, Fernandez B, Barkovich AJ, Dobyns WB. Cerebellar and posterior fossa malformations in patients with autism-associated chromosome 22q13 terminal deletion. *Am J Hum Genet* 2013;0:131–36.
32. Verhoeven WM a, Egger JIM, Willemsen MH, de Leijer GJM, Kleefstra T. Phelan-McDermid syndrome in two adult brothers: Atypical bipolar disorder as its psychopathological phenotype. *Neuropsychiatr Dis Treat* 2012;8:175–9.
33. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Association; 2013.
34. Rutter M, Le Couteur A, Lord C. ADI-R Autism Diagnostic Interview - Revised. Los Angeles, CA: Western Psychological Services; 2003.
35. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S. Autism Diagnostic Observation Schedule ™ Second Edition (ADOS TM-2). Torrence CA, Western Psychological Services, 2012.
36. Mullen EM. Mullen Scales of Early Learning: AGS edition. Circle Pines, MN: American Guidance Service; 1995.
37. Sparrow SS, Cicchetti DV, Balla DA. The Vineland Adaptive Behavior Scales, 2nd edition (VABS-II). NCS Pearson Inc. 2005.
38. Frankenburg W, Dodds J, Archer P, et al. The DENVER II: A major revision and restandartization of the Denver Developmental Screening test. *Pediatrics* 1992;89:91–7.

39. Beery KE, Beery NA. Beery-Buktanica Developmental Test of Visual Motor Integration: Administration, Scoring and Teaching Manual, 5th Edition. Parsippany, NJ: Modern Curriculum Press, 2004.
40. Nellhaus G. Composite International & Interracial Graphs. *Pediatrics* 1968;41.
41. Bishop SL, Guthrie W, Coffing M, Lord C. Convergent validity of the Mullen Scales of Early Learning and the Differential Ability Scales in children with autism spectrum disorders. *Am J Intellect Dev Disabil* 2011;116:331–43.
42. Shetreat- Klein M, Shinnar S, Rapin I. Abnormalities of joint mobility and gait in children with autism spectrum disorders. *Brain & development* 2014;36:91 -96.
43. Masdeu JC. Gait and balance disorders. In: *Handbook of Clinical Neurology*. Vol. 136. 3. *Neuroimaging, Part II*, JC Masdeu and RG Gonzalez, Editors, Elsevier BV; 2016:939-955.
44. Ben-Sasson A, Hen L, Fluss R, Cermak SA, Engel-Yeger B, Gal Eynat. A meta-analysis of sensory modulation symptoms in individuals with Autism Spectrum Disorders. *J Autism Dev Disord* 2009;39(1):1-11.
45. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. *JAMA* 2003;290:337-344.
46. Tuchman R, Rapin I. Epilepsy in autism. *Lancet Neurol* 2002;1:352–8.
47. Wong M, Guo D. Dendritic spine pathology in epilepsy: cause or consequence? *Neuroscience* 2013;251:141–50.

Corresponding Author:
Yitzchak Frank, M.D. Seaver Autism Center for Research and Treatment**,** Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1230**,** New York, NY 10029
yitzchak.frank@mountsinai.org