ORIGINAL ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

16p11.2 Deletion and Duplication: Characterizing Neurologic Phenotypes in a Large Clinically Ascertained Cohort

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Manuscript Received: 12 August 2015; Manuscript Accepted: 13 June 2016

Chromosome 16p11.2 deletions and duplications are among the most frequent genetic etiologies of autism spectrum disorder (ASD) and other neurodevelopmental disorders, but detailed descriptions of their neurologic phenotypes have not yet been completed. We utilized standardized examination and history methods to characterize a neurologic phenotype in 136 carriers of 16p11.2 deletion and 110 carriers of 16p11.2 duplication—the largest cohort to date of uniformly and comprehensively characterized individuals with the same 16p copy number variants (CNVs). The 16p11.2 deletion neurologic phenotype is characterized by highly prevalent speech articulation abnormalities, limb and trunk hypotonia with hyporeflexia, abnormalities of agility, sacral dimples, seizures/epilepsy, large head size/macrocephaly, and Chiari I/cerebellar tonsillar ectopia. Speech articulation abnormalities, hypotonia, abnormal agility, sacral dimples, and seizures/epilepsy are also seen in duplication carriers, along with more prominent hyperreflexia; less, though still prevalent, hyporeflexia; highly prevalent action tremor; small head size/microcephaly; and cerebral white matter/corpus callosum abnormalities and ventricular enlargement. The neurologic phenotypes of these reciprocal 16p11.2 CNVs include both shared and distinct features. Reciprocal phenotypic characteristics of predominant hypo- versus hyperreflexia and macro- versus microcephaly may reflect opposite neurobiological abnormalities with converging effects causing the functional impairments shared between 16p11.2 deletion and duplication carriers (i.e., abnormal motor agility and articulation). While the phenotypes exhibit overlap with other genetically-caused neurodevelopmental disorders, clinicians should be aware of the more striking features—such as the speech and motor impairments, growth abnormalities, tremor, and sacral dimples—when evaluating individuals

How to Cite this Article:
Steinman KJ, Spence SJ, Ramocki MB, Proud MB, Kessler SK, Marco EJ, Green Snyder LA, D'Angelo D, Chen Q, Chung WK, Sherr EH, on behalf of the Simons VIP Consortium. 2016. 16p11.2 Deletion and Duplication: Characterizing neurologic phenotypes in a large clinically ascertained cohort.
Am J Med Genet Part A 170A:2943–2955.

Conflicts of interest: none.
Grant sponsor: Simons Foundation Autism Research Initiative (SFARI); Grant number: 198677.
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Article first published online in Wiley Online Library (wileyonlinelibrary.com): 13 July 2016
DOI 10.1002/ajmg.a.37820

with developmental delay, intellectual disability, ASD, and/or language disorders.

Key words: manifestations, neurogical; copy number variants, DNA; associations, genotype–phenotype; 16p11.2 deletion; 16p11.2 duplication; articulation disorders, developmental; hypotonia; sacral dimple; tremor; epilepsy; macrocephaly; microcephaly; incoordination

INTRODUCTION

Deletions and duplications of the recurrent ~600 kb BP4-BP5 region on chromosome 16p11.2 are among the most frequent genetic etiologies of autism spectrum disorder (ASD) and other neurodevelopmental disorders [Kumar et al., 2008; Marshall et al., 2008; Weiss et al., 2008], with a prevalence of approximately 1% of all patients with a diagnosis of ASD [Weiss et al., 2008] and 0.6–0.7% in large series of patients with a variety of other neurodevelopmental diagnoses including developmental delays, intellectual disability, and congenital anomalies [Rosenfeld et al., 2010; Shinawi et al., 2010]. However, a detailed evaluation and careful analysis of their neurologic phenotypes have not yet been completed. Prior reports have identified a range of neurologic abnormalities including hypotonia and other motor abnormalities, speech and language delays or deficits, and seizures. These have relied on case reports, small samples, and mixed methods of subject ascertainment and evaluation (including clinician questionnaires, record review, and literature review) [Ghebranious et al., 2007; Kumar et al., 2008; Marshall et al., 2008; Weiss et al., 2008; Bijlsma et al., 2009; Fernandez et al., 2010; Hanson et al., 2010; Rosenfeld et al., 2010; Shinawi et al., 2010; Zufferey et al., 2012]. Large sample sizes uniformly ascertained and studied in a standardized manner for neurologic differences are needed to better characterize the neurologic phenotypes of the 16p11.2 deletion and duplication.

The goal of this study is to characterize in detail the range and frequency of neurologic variation associated with 16p11.2 deletions and duplications. To do so, we conducted a standardized neurologic history and physical examination assessment on the largest cohort to date of uniformly-ascertained and comprehensively characterized individuals with these genetically well-defined CNVs from the Simons Variation in Individuals Project (Simons VIP).

METHODS

Subjects

Subjects all carry the same recurrent ~600 kb 16p11.2 deletion or duplication—delineated by BP4 and BP5 (29.6–30.2-Hg19)—without other pathogenic CNVs or known genetic diagnoses. Families with a child identified with a deletion or duplication at this locus were referred by their clinician or the testing laboratory to the Simons VIP website to enroll in this clinical and imaging project (Simons VIP Connect; https://simonsvipconnect.org/). Index individuals (i.e., probands) with 16p11.2 deletion or duplication identified through clinical care or prior research studies were recruited to the project without regard to the indication for genetic testing. Biologically related family members were then tested through cascade genetic testing using a custom-designed oligonucleotide array containing genome-wide coverage at a resolution of ~400 kb and targeting known disease-causing CNVs at a resolution of ~50 kb (OGT 60K, Oxford Gene Technologies, Tarrytown, NY) to identify other carriers (here referred to as "familial carriers") and exclude those family members with other pathogenic CNVs [The Simons VIP Consortium, 2012]. The study was approved by each participating site's institutional review board or equivalent.

Participants were assessed at one of five Simons VIP sites (Baylor College of Medicine, Boston Children's Hospital, Children's Hospital of Philadelphia, the University of California—San Francisco, and the University of Washington) for a comprehensive evaluation which included a standardized neurologic examination, neurologic history, and neurologic record review. These evaluations were conducted by a board-certified pediatric neurologist (KJS, SJS, MBR, MBP, SKK, EJM, or, rarely, a substitute neurologist). Detailed cognitive and behavioral phenotypes of the Simons VIP 16p11.2 deletion and duplication cohorts have been described elsewhere [Hanson et al., 2014; D'Angelo et al., 2016]. Full-scale IQs (FSIQ; mean ± standard deviation) for the subset of subjects examined in the current analysis are presented here.

Neurologic Examination

A standardized neurologic examination included assessment of speech articulation, cranial nerve functions, muscle bulk, limb and truncal tone, limb power, deep tendon reflexes, adventitial movements, cerebellar function (truncal ataxia, dysmetria, and dysrhythmia), casual and stressed (toe, heel, and tandem) gaits, jumping, and one-foot skills (balance and hopping), as well as examination for sacral dimples and neurocutaneous abnormalities (Table I). Each item was rated as normal versus abnormal or present versus absent. Jumping (one jump), hopping on each foot (one hop), one-foot balance (for 5 sec), and tandem gait were only assessed for individuals 3, 5, 6, and 6 years of age or older, respectively. For this analysis, a measure of "abnormal agility" was defined as exhibiting one or more of the following: a wide-based, waddling, or shuffling gait; toe-walking during casual gait; or inability to perform toe walk, heel walk, tandem walk, jumping, hopping, or one-foot balance. To examine for associations between abnormal agility and other neurologic examination findings (see Data Analysis section), the prevalences of four lower extremity (LE)-specific findings were calculated: symmetric LE hypotonia, symmetric LE weakness, LE dysrhythmia, and LE dysmetria. If an exam item was unable to be assessed, the subject was excluded from frequency calculation for that item for the purposes of this analysis. Photographs were taken of a subset of sacral dimples with atypical features associated with higher risk of underlying neurologic abnormalities (e.g., non-visualizable bottom, multiple dimples, dimples not on the midline; Figs. 1 and 2) [Kriss and Desai, 1998]. Prevalence estimates of atypical features are not available as this was performed ad hoc.

TABLE I. Frequencies of Neurologic Examination Findings of 16p11.2 Deletion and Duplication Carriers

Exam finding	Deletion carriers (total N = 136)		Duplication carriers (total N = 110)		Del vs. dup
	% (n)[a]	N	% (n)[a]	N	*P*-value[b]
Skin					
Café-au-lait spots	**30 (40)**	134	**31 (34)**	109	0.9
Patterned skin changes	4 (5)	134	3 (3)	109	0.9
Sacral dimple	**34 (43)**	127	**28 (29)**	105	0.2
Cranial nerves					
Articulation abnormality	**79 (93)**	117	**30 (30)**	100	**<0.001**
Nystagmus	5 (6)	127	1 (1)	103	0.2
Extraocular muscle weakness	8 (11)	132	10 (11)	110	0.6
Difficulty crossing midline	0 (0)	129	0 (0)	107	n.a.[c]
Difficulty with eye convergence	11 (10)	89	**20 (16)**	80	0.3
Eso/exotropia	11 (14)	132	10 (11)	108	0.8
Abnormal eye saccades	2 (2)	113	2 (2)	100	0.9
Abnormal smooth visual pursuit	9 (12)	130	10 (11)	108	0.8
Facial diplegia/hypotonia/drooling	6 (7)	123	3 (3)	94	0.4
Soft palate weakness	4 (5)	127	0 (0)	102	0.07[d]
Tongue weakness	0 (0)	128	1 (1)	107	0.5[d]
Motor					
Diffuse low bulk	4 (5)	135	2 (2)	110	0.4
Symmetric hypotonia	**49 (63)**	129	**46 (45)**	98	0.6
Truncal hypotonia	**20 (26)**	133	**15 (16)**	106	0.5
Symmetric weakness	7 (10)	136	5 (6)	110	0.6
Hyporeflexia	**48 (63)**	130	**31 (33)**	108	**0.003**
Hyperreflexia/clonus	13 (17)	130	**32 (35)**	108	**0.003**
Abnormal movements/coordination/gait					
Dystonia	1 (1)	135	0 (0)	110	0.6[d]
Tic	1 (1)	135	5 (5)	110	0.09
Tremor	13 (18)	135	**43 (47)**	110	**<0.001**
Truncal ataxia	1 (1)	136	3 (3)	110	0.3
Upper or lower extremity dysrhythmia (tapping)	9 (9)	97	**19 (17)**	90	0.07
Dysmetria (finger-nose-finger and/or heel-knee-shin)	8 (8)	106	6 (6)	94	0.8
Abnormal agility[e]	**47 (61)**	129	**25 (26)**	105	**0.001**

n, number of carriers in whom the finding was observed; N, number of carriers examined for the finding; total N, total number of carriers examined.
[a]Exam findings observed in ≥15% of carriers are in bold.
[b]*P*-values from GEE analyses comparing frequencies between del and dup for neurologic findings. Significant *P*-values are in bold.
[c]*P*-value is not applicable (n.a.) when 0% prevalence in both groups.
[d]Fisher's exact test *P*-value when prevalence in one group is 0%.
[e]See text for definition.

Head Circumference

Head circumference (HC) was measured by the examining neurologist and by each site's research staff. The neurologist's HC measurement was used for this analysis. When the neurologist's assessment was not available, the site staff's measurement was used. HC measurements were converted to HC z-scores using the World Health Organization norms for children 2 years old and under and Simulconsult Head Circumference Calculator (Segal and Rapin, 2014, http://www.simulconsult.com/resources/head.html) for individuals older than 2 years.

Best Available Neurologic History (BANH)

A standardized "best available neurologic history" (BANH) was obtained by each site's neurologist for a majority of the deletion and duplication carriers. A medical history interview was initially performed by a genetic counselor, which included assessment for the following neurologic features: head size abnormalities, cranial nerve disorders, tone abnormalities, weakness, neuropathy, myopathy, abnormal movements, seizures, and brain imaging and EEG findings. During the on-site clinical evaluation, these neurologic components of the medical history interview were reviewed by the site neurologist with the family historian (typically, the parent(s) of pediatric subjects or the carrier him/herself for adult subjects). All available clinical neurologic records were obtained for each subject and reviewed by the site neurologist. These neurologic records consisted of clinic and consultation notes by neurologists, developmental pediatricians, and neurosurgeons as well as brain imaging (head CT, brain MRI) and EEG reports. Head CT and brain MRIs were considered abnormal if

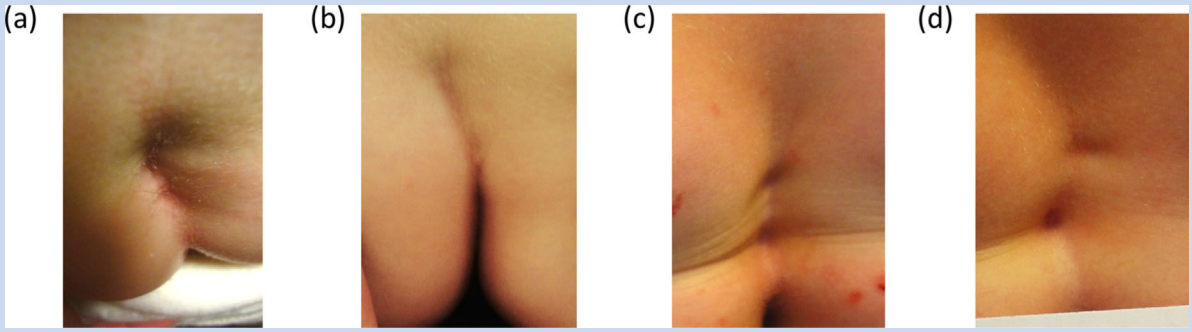

FIG. 1. Examples of atypical sacral dimples seen in 16p11.2 deletion carriers: (a) off-midline, curvilinear indentation; (b) off-midline, curvilinear; (c) double dimple; (d) double dimple, off-midline. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

there were abnormalities identified of the ventricles, brain parenchyma, or extra-axial spaces; abnormalities of the bones and sinuses were not considered. Combining information obtained from expert interview and his/her own review of records, the site neurologist determined for each symptom/sign/diagnosis (SSDx) on the BANH (Table II): (i) if it was ever present for the subject (now or in the past); (ii) if it was only suspected by the family or was diagnosed by a trained professional (e.g., physician, physical therapist, occupational therapist); and (iii) age of onset or diagnosis. After review of all available information, if the neurologist was uncertain whether a SSDx was ever present (i.e., suspected or diagnosed), the subject was excluded from frequency calculation for that item for the purposes of this analysis. For the purposes of the BANH, macrocephaly and microcephaly were defined as HC $\geq$ 2SD or ≤ -2SD from population norm mean, respectively, if based on record review or "above the range of normal" or "below the range of normal," respectively, if based on family report alone.

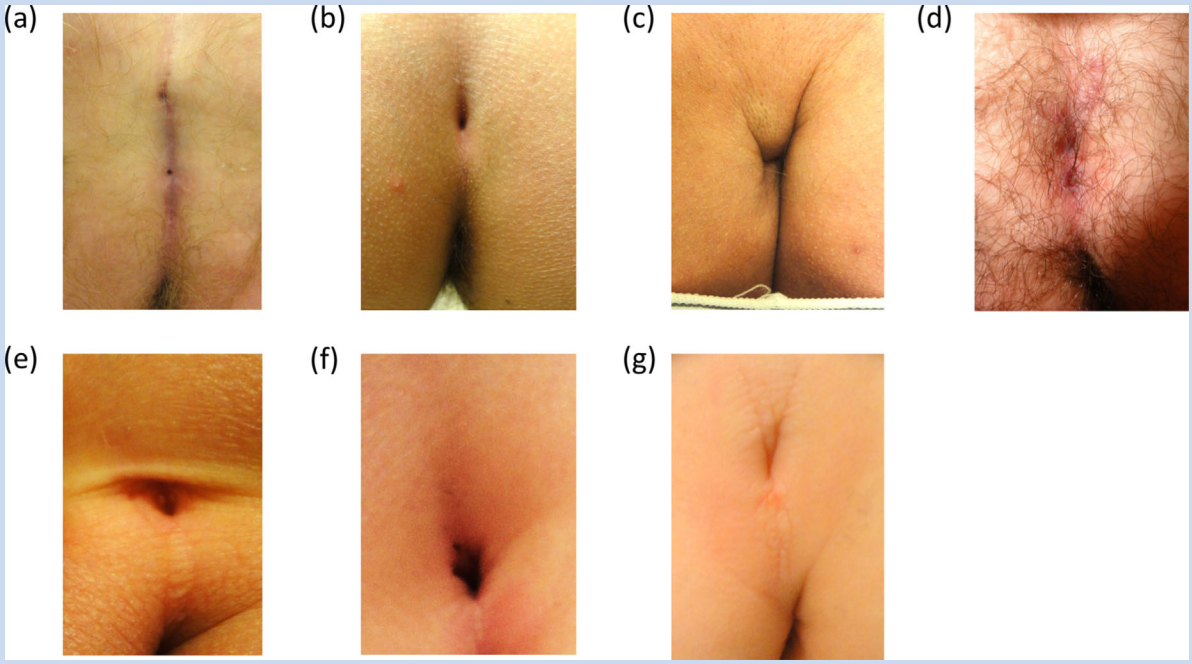

FIG. 2. Examples of atypical sacral dimples seen in 16p11.2 duplication carriers: (a) double dimple, deep without visualizable base; (b) deep without visualizable base; (c) unusual shape; (d) double dimple; (e) deep; (f) deep; (g) above gluteal cleft, off-midline, linear. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

TABLE II. Frequencies of Neurologic Signs/Symptoms/Diagnoses (SSDx) on Best Available Neurologic History (BANH)

	Deletion carriers (total N = 83)			Duplication carriers (total N = 76)			Del vs. dup *P*-values[c]	
	Present[a]	Formally diagnosed		Present[a]	Formally diagnosed		Present[a]	Formally diagnosed
BANH SSDx	**% (n)[b]**	**% (n)[b]**	**N**	**% (n)[b]**	**% (n)[b]**	**N**		
Head								
Macrocephaly[d]	**17 (14)**	**17 (14)**	81	3 (2)	1 (1)	72	**0.01**	**0.01**
Microcephaly[d]	5 (4)	5 (4)	83	**17 (13)**	13 (10)	75	**0.02**	0.07
Cranial nerve disorder	7 (6)	7 (6)	83	6 (4)	6 (4)	72	0.7	0.7
Motor								
Hypotonia	**54 (44)**	**50 (41)**	82	**44 (32)**	**38 (28)**	73	0.3	0.2
Hypertonia	9 (7)	6 (5)	81	8 (6)	5 (4)	75	0.9	0.8
Weakness	**22 (18)**	**17 (14)**	82	**32 (24)**	**23 (17)**	74	0.2	0.3
Neuropathy	4 (3)	0 (0)	81	3 (2)	1 (1)	74	0.7	0.5[e]
Myopathy	1 (1)	0 (0)	77	1 (1)	1 (1)	74	1.0	0.7[e]
Abnormal movements								
Ataxia	5 (4)	1 (1)	83	5 (4)	3 (2)	76	0.9	0.5
Tremor	8 (7)	5 (4)	83	**28 (21)**	**18 (14)**	76	**0.004**	**0.01**
Dystonia	1 (1)	1 (1)	83	3 (2)	3 (2)	76	0.5	0.5
Chorea	0 (0)	0 (0)	83	0 (0)	0 (0)	75	n.a.[f]	n.a.[f]
Tics/Tourette	12% (10)	5% (4)	80	**16 (12)**	3 (2)	76	0.6	0.5
Seizures (Sz)								
Febrile Sz	7 (6)	7 (6)	83	12 (9)	9 (7)	76	0.3	0.6
Unprovoked Sz/Epi	**27 (22)**	**22 (18)**	82	**29 (22)**	**26 (20)**	76	0.9	0.7

SSDx, sign/symptom/diagnosis; Sz, seizure; Epi, epilepsy; n, number of carriers in whom SSDx was present or formally diagnosed; N, number of carriers with data available about the SSDx; total N, total number of carriers in whom BANH was conducted.
[a]Includes suspected or formally diagnosed.
[b]SSDx present or formally diagnosed in ≥15% of carriers are in bold.
[c]*P*-values from GEE analyses comparing frequencies between del and dup for neurologic findings. Significant *P*-values are in bold.
[d]Macrocephaly and microcephaly were defined as HC ≥ 2SD or ≤−2SD from population norm mean, respectively, if based on record review or "above the range of normal" or "below the range of normal," respectively, if based on family report alone.
[e]Fisher's exact test *P*-value when prevalence in one group is 0%.
[f]*P*-value is not applicable (n.a.) when 0% prevalence in both groups.

Data Analysis

We examined the frequency of neurologic exam findings in 16p11.2 deletion and duplication carriers (Table I). A finding with a frequency greater than or equal to 15% was considered a noteworthy component of a 16p deletion or duplication neurologic phenotype. To evaluate differences in the 16p deletion and duplication neurologic exam phenotypes, we used generalized estimating equations (GEEs) with a compound symmetric correlation structure and logit link function to assess for differences between the deletion and duplication groups in frequency of exam findings, while accounting for correlation within families (potentially resulting from other familial genetic or environmental factors influencing neurologic phenotype). When prevalence of an abnormality was 0% in one group, Fisher's exact test was used instead of a GEE. We also used GEEs to assess for differences in exam finding frequencies between probands and non-proband familial CNV carriers within each CNV group (Supplementary Tables SIA and SIB). Fisher's exact test was again used instead of a GEE when prevalence of an abnormality was 0% among either probands or familial carriers or, in rare circumstances, when frequency of a finding was small and the GEE model did not converge.

To further characterize functional impairments observed on neurologic examination (i.e., articulation and agility abnormalities), we examined frequencies in children (under 18 years old) and adults (18 years old and above). We used GEEs to examine associations between functional impairments (abnormal articulation and agility) and potential underlying neurologic abnormalities in anatomically-related body parts that were present in 15% or more subjects and, in secondary analyses, those present in less than 15% of subjects. Potential underlying abnormalities were facial hypotonia/diplegia/drooling, soft palate weakness, or tongue weakness for articulation and symmetric LE hypotonia, symmetric LE weakness, LE dysrhythmia, LE dysmetria, truncal ataxia, or sacral dimple (given possible underlying spina bifida occulta) for agility. When prevalence of an abnormality was 0% in one group, Fisher's exact test was used instead of a GEE.

Deletion and duplication carriers' HC z-scores were analyzed using GEEs with an identity link function to determine whether each group's mean z-score differed significantly from population norms (mean HC z-score = 0) and whether deletion and duplication HC z-scores differed while accounting for correlation within family. Frequency of macrocephaly and microcephaly on examination were calculated using definitions of HC ≥ 2SD and ≤−2SD from population norm mean, respectively.

The frequency of neurologic SSDx by BANH and the frequency with which they have been formally diagnosed were examined in

deletion and duplication carriers (Table II). A SSDx with a frequency at or above 15% was considered a clinically noteworthy component of the 16p deletion or duplication neurologic phenotype. We calculated median (and range) age of onset or diagnosis for SSDx present in 15% or more of subjects. To examine similarities and differences in the neurologic history phenotypes between 16p deletion and duplication carriers, we used GEEs to assess for differences between groups while accounting for familial correlation. We also used GEEs to assess for differences in frequency of SSDx between probands and familial carriers within each CNV group (Supplementary Tables SIIA and SIIB). Fisher's exact test was used instead when frequency of a SSDx was 0% amongst either probands or familial carriers or, rarely, when the GEE model did not converge. Since no participants with seizures were from the same family, we used the appropriate non-parametric test (χ^2 or Fisher's exact test) to assess for differences in seizure type frequency between groups.

RESULTS

Neurologic Examination

A neurologic examination was performed on 136 deletion carriers (114 probands [112 children, 2 adults] and 22 family member carriers [10 children, 12 adults]) and 110 duplication carriers (53 probands [49 children, 4 adults] and 57 family member carriers [17 children, 40 adults]). Median [and range] age of deletion carriers was 8.2 years [0.9–48.0] and of duplication carriers was 11.2 years [0.7–63.1]. Each group had 53% males and 47% females. Mean $\pm$ standard deviation FSIQ was 84 ± 16 for deletion carriers (probands 83 ± 16; familial carriers 88 ± 13) and 86 ± 22 for duplication carriers (probands 76 ± 22; familial carriers 95 ± 17). Among deletion carriers, 74 were de novo (71 probands, 3 familial carriers [1 monozygotic twin of proband, 2 parents of a proband whose own parents were non-carriers]), 23 were inherited (15 probands, 8 familial carriers), 3 were inherited from parents who were presumed gonadal mosaic (1 proband, 2 familial carriers), and 36 were unknown (27 probands, 9 familial carriers). Among duplication carriers, 13 were de novo (10 probands, 3 familial carriers [all parents of a proband whose own parents were non-carriers]), 63 were inherited (33 probands, 30 familial carriers), and 34 were unknown (10 probands, 24 familial carriers).

Deletion carriers. In deletion subjects, seven exam findings were found to be prevalent in 15% or more of the subjects (Table I): speech articulation abnormalities (79%); symmetric limb hypotonia (49%); hyporeflexia (48%); abnormal agility (47%); sacral dimples (34%; multiple with atypical features [Fig. 1]); café-au-lait (CAL) spots (30%); and truncal hypotonia (20%). Of these findings, only articulation abnormalities differed significantly between proband and familial carriers—present in 86% of probands and 48% of familial carriers (Supplementary Table SIA).

Articulation abnormalities were observed in 86% (89/103) of pediatric deletion carriers and 29% (4/14) of adult deletion carriers. No association was observed between articulation abnormalities and soft palate weakness (Fisher's exact test, $p = 0.6$) or any facial findings of hypotonia, diplegia, or excessive drooling (Fisher's exact test, $P = 0.6$), though all individuals with palate weakness ($n = 5$) or facial hypotonia ($n = 5$) had abnormal articulation. No deletion carriers had tongue weakness. Abnormal agility was seen in 49% (56/115) of pediatric deletion carriers and 29% (4/14) of adult deletion carriers. Frequencies of LE-specific findings were: symmetric LE hypotonia in 35% (36/102), symmetric LE weakness in 5% (7/129), LE dysrhythmia in 10% (9/86), and LE dysmetria in 10% (8/77). Abnormal agility was associated with truncal hypotonia (GEE, $P = 0.05$) but not with the presence of sacral dimple (GEE, $P = 0.8$) or LE hypotonia (GEE, $P = 0.07$). Among findings present in less than 15% of deletion carriers, agility was associated with the presence of LE dysmetria (GEE, $P = 0.03$), but not LE weakness (GEE, $P = 0.3$), LE dysrhythmia (GEE, $P = 0.8$), or truncal ataxia (Fisher's exact, $P = 1.0$).

Duplication carriers. For duplication carriers, 11 neurologic examination findings were observed in 15% or more of the subjects (Table I): symmetric limb hypotonia (46%); tremor (43%); hyperreflexia (32%); hyporeflexia (31%); CAL spots (31%); articulation abnormalities (30%); sacral dimples (28%; many with atypical features [Fig. 2]); abnormal agility (25%); abnormalities of eye convergence (20%); dysrhythmia (19%); and truncal hypotonia (15%). Of those with tremor ($n = 47$), postural tremor was observed in 36 (77%), intention tremor in 24 (51%), and resting tremor in 2 (4%). Tremors were described as fine and rapid in all but three (slow and coarse in one with intention tremor and two with postural tremor). Articulation abnormalities, limb hypotonia, truncal hypotonia, and abnormal agility were seen significantly less frequently in familial carriers than probands ($P \leq 0.05$)—still prevalent in over 15% of familial carriers for limb hypotonia and abnormal agility, but not articulation abnormalities (11%) or truncal hypotonia (4%). Tremor and hyperreflexia were seen more frequently in familial carriers than probands, though both findings were seen in over 15% in each group (Supplementary Table SIB).

Articulation abnormalities were seen in 47% (27/57) of pediatric duplication carriers and 7% (3/43) of adult duplication carriers. No association was observed between articulation abnormalities and tongue weakness (Fisher's exact test, $P = 0.3$) or facial findings of hypotonia, diplegia, or excessive drooling (Fisher's exact test, $P = 0.1$), but all individuals with facial hypotonia ($n = 2$) and the individual with tongue weakness ($n = 1$) had abnormal articulation. No duplication carriers had soft palate weakness. Abnormal agility was seen in 29% (18/62) of pediatric duplication carriers and 19% (8/43) of adult duplication carriers. Frequencies of LE-specific findings were: symmetric LE hypotonia in 33% (26/78), symmetric LE weakness in 2% (2/105), LE dysrhythmia in 13% (10/79), and LE dysmetria in 8% (6/75). Abnormal agility was associated with truncal hypotonia (GEE, $P = 0.001$), and sacral dimple (GEE, $P = 0.05$), but not symmetric LE hypotonia (GEE, $P = 0.8$). For findings prevalent in less than 15% of duplication carriers, abnormal agility was associated with LE dysrhythmia (GEE, $P < 0.001$). No associations were found between impaired agility and the presence of LE dysmetria (GEE, $P = 0.2$), truncal ataxia (GEE, $P = 0.4$), or symmetric LE weakness (Fisher's exact test, $P = 0.06$), though the individuals with symmetric LE weakness ($n = 2$) had abnormal agility.

Deletion versus duplication carriers. Overall, exam findings more commonly observed in deletion carriers than duplication

carriers (GEE, $P \leq 0.05$) were abnormal articulation, hyporeflexia, and abnormal agility while duplication carriers more frequently demonstrated hyperreflexia and tremor.

Head Circumference

Deletion carriers (n = 127) had a mean ±standard deviation HC z-score of 1.4 ±1.3, significantly greater than the normal population mean z-score of 0.0 (GEE, $P < 0.001$). At the time of HC measurement, 36% were macrocephalic and 2% were microcephalic. Mean ±standard deviation HC z-score for duplication carriers (n = 103) was −0.3 ±1.4, which demonstrated a trend toward smaller head size compared to the normal population HC (GEE, $P = 0.08$), and was significantly smaller than deletion carriers (GEE, $P < 0.001$). Macrocephaly was seen in 5% of duplication carriers and microcephaly was present in 10% of duplication carriers.

Best Available Neurologic History (BANH)

BANH was completed on 83 deletion carriers (71 probands and 12 family member carriers) and 76 duplication carriers (39 probands and 37 family member carriers). Median [and range] age for these deletion carriers was 6.6 years [0.8–44.7] and for these duplication carriers was 10.1 years [0.7–63.1]. Mean ± standard deviation full-scale IQ was 85 ± 16 for these deletion carriers (probands 84 ± 17; familial carriers 90 ± 11) and 84 ± 22 for these duplication carriers (probands 75 ± 22; familial carriers 93 ± 18).

Deletion carriers. SSDx present in at least 15% of deletion carriers were: hypotonia (54%), unprovoked seizures/epilepsy (27%), weakness (22%), and macrocephaly (17%). Most findings suspected by families were diagnosed/confirmed by a medical professional (hypotonia 50%, seizures 22%, weakness 17%, and macrocephaly 17% of all deletion carriers) (Table II). Only hypotonia differed significantly between probands and familial carriers in the frequencies of its suspected presence by family (probands 61%, familial carriers 8%) and its formal diagnosis by a clinician (probands 57%, familial carriers 8%; Supplementary Table SIIA). The most common seizure types amongst those in whom unprovoked seizures were diagnosed were generalized tonic-clonic (61%), focal seizures with impairment of consciousness or awareness (or "focal dyscognitive seizures," previously called complex partial; 44%), and absence seizures (33%) (Table III). The median [and range] ages of onset/diagnosis for these high-prevalence SSDx were: hypotonia (12 months [birth—5 years]), weakness (10 months [birth—11 years]), macrocephaly (6 months [birth—8 years]), and unprovoked seizures/epilepsy (13 months [1 month—7 years]).

Of the 41 deletion carriers who had clinical EEGs, 54% (21/39) had at least one abnormal EEG (Table IV; 2 had unknown results). Various EEG abnormalities were described though no specific abnormality was prominent (all observed in less than 15% of those with EEGs). Clinical head CTs had been performed in 27 and brain MRIs in 44 of the deletion carriers. Of deletion carriers with neuroimaging, 28% of subjects with a CT and 26% of subjects with brain MRIs had at least one abnormality (results unknown for two subjects with CT scans and one with MRI). The only abnormality seen on CT in more than a single individual was enlargement of subarachnoid spaces (n = 2) while the most common MRI abnormality was cerebellar tonsillar ectopia/Chiari I (n = 5). Isolated findings included: right frontal gray matter heterotopia (n = 1); mega cisterna magna vs. posterior fossa arachnoid cyst (n = 1); and "variant Chiari II" with crowding of the posterior fossa, tectal flattening and narrowing of the pons, inferior displacement of cerebellar hemispheres, partial absence of the falx with gyral interdigitation and some gyral midline crossing and fusion, foreshortened and angulated anterior corpus callosum, bilateral frontal gray matter heterotopia, left occipital

TABLE III. Prevalence of Seizure Types Among Those Diagnosed With Unprovoked Seizures/Epilepsy

	Deletion carriers with seizures (total N = 18)	Duplication carriers with seizures (total N = 20)	
Seizure type	**% (n)[a]**	**% (n)[a]**	***P*-values[b]**
Focal			
Focal motor (simple partial)	6 (1)	15 (3)	0.6
Focal dyscognitive (complex partial)	**44 (8)**	**50 (10)**	1.0
Generalized			
Generalized tonic-clonic	**61 (11)**	**45 (9)**	0.4
Absence	**33 (6)**	5 (1)	**0.03**
Atonic	11 (2)	5 (1)	0.6
Infantile spasms	0 (0)	0 (0)	n.a.[c]
Other	**22 (4)**	**20(4)**	0.5
Unable to discern	11 (2)	**15 (3)**	1.0
Status epilepticus	0 (0)	5 (1)	1.0

n, number of carriers with seizures in whom the seizure type was identified; total N, number of carriers with seizures.
[a]Seizure types occurring in ≥15% of carriers with seizures are in bold.
[b]*P*-values from GEE analyses comparing frequencies between del and dup for neurologic findings. Significant *P*-values are in bold.
[c]*P*-value is not applicable (n.a.) when 0% prevalence in both groups.

TABLE IV. Lifetime Prevalence of Abnormal EEGs and Neuroimaging in Those who Had Results Available for Review

	Deletion carriers		Duplication carriers	
Study abnormalities	**% (n)[a]**	**N**	**% (n)[a]**	**N**
EEG abnormal[b]	**54 (21)**	39	**40 (12)**	30
Focal sharps	8 (3)		**27 (8)**	
Generalized sharps	8 (3)		7 (2)	
Multifocal sharps	8 (3)		13 (4)	
Focal slowing	8 (3)		13 (4)	
Generalized slowing	8 (3)		13 (4)	
Other abnormalities	**15 (6)**		3 (1)	
Abnormalities not described	**15 (6)**		7 (2)	
Head CT scan abnormal[c]	**28 (7)**	25	**31 (5)**	16
Brain MRI abnormal[d]	**26 (11)**	43	**55 (18)**	33

n, number of carriers in whom abnormality was present; N, number of carriers with study results available for review.
[a]Abnormalities present in ≥15% of carriers are in bold.
[b]An additional two deletion carriers and six duplication carriers had EEGs but results are unknown.
[c]An additional two deletion carriers and one duplication carrier had CT scans but results are unknown.
[d]An additional one deletion carrier and three duplication carriers had MRIs but results are unknown.

closed-lip schizencephaly, and hydrocephalus with ventricular drain placement (n = 1).

Duplication carriers. The most common neurologic SSDx in duplication carriers were: hypotonia (44%), weakness (32%), unprovoked seizures/epilepsy (29%), tremor (28%), microcephaly (17%), and tics (16%) (Table II). Formal diagnosis had been made in the majority of subjects in whom hypotonia, weakness, seizures, and microcephaly were suspected (38%, 23%, 26%, and 13% of duplication carriers, respectively) and at somewhat lower frequency than suspected by families for tremor (18%). Though tics were noted by families with relatively high frequency, formal diagnosis occurred in only 3%. Microcephaly, hypotonia, weakness, febrile seizures, and unprovoked seizures/epilepsy were all clinically diagnosed less frequently in familial carriers (all with less than 15% prevalence) than probands (Supplementary Table SIIB). The most common seizure types were focal dyscognitive seizures (in 50% of those with diagnosed unprovoked seizures) and generalized tonic-clonic (45%) (Table III). The median [and range] age of onset/diagnosis for SSDx seen in at least 15% of duplication subjects were: hypotonia (9 months [birth—6.5 years]), weakness (15 months [birth—59 years]), microcephaly (12 months [birth—3 years]), tics (7 years [1–18 years]), tremor (9 years [2 months—40 years]), and unprovoked seizures/epilepsy (22 months [3 months—4.5 years]).

Amongst the 36 duplication carriers who had undergone EEGs, 43% (13/30) had at least one abnormal EEG finding (Table IV; six had unknown results). Focal sharp activity was the most common abnormality (27% of those with known EEGs results). Clinical head CTs had been obtained in 17 of duplication carriers and brain MRIs in 36. Among duplication carriers, 31% with a CT and 55% with an MRI had at least one that exhibited an abnormality (results unknown for one subject with CT scan and three with MRI). The most common abnormality on head CT was ventriculomegaly (n = 2) and MRI abnormalities included white matter and/or corpus callosum abnormalities (n = 10) and ventricular enlargement (n = 5). Isolated findings included: a large posterior fossa cyst exerting mass effect on the cerebellum and occipital lobes, two small thin-walled cystic structures to left of the tentorium and between the vermis and right cerebellar hemisphere, and parietooccipital and midvertex scalp lesions (identified as encephaloceles s/p resection; n = 1); a T2-hyperintense focus in the left basal ganglia (n = 1); and mild inferior vermis hypoplasia (n = 1).

Deletion versus duplication carriers. Comparing BANH SSDx in deletion and duplication carriers, macrocephaly was both present and formally diagnosed more commonly in deletion carriers. In duplication carriers, microcephaly and tremor were both present more frequently, but only tremor had more frequently been formally diagnosed (Table II). Absence seizures were the only seizure type that differed in frequency between CNV groups, being diagnosed in 33% (6/18) deletion carriers with unprovoked seizures but only one (of 20) duplication carriers with unprovoked seizures (Fisher's exact test, $P = 0.03$; Table III).

DISCUSSION

This study is the largest to date to characterize neurologic phenotypes in 16p11.2 deletion and duplication carriers using a comprehensive standardized method of neurologic evaluation. Our analyses indicate that the 16p11.2 deletion neurologic phenotype is characterized by highly prevalent (>75%) speech articulation abnormalities, hypotonia with hyporeflexia, poor agility, sacral dimples, seizures/epilepsy, and large head size with the relatively common brain imaging finding of Chiari I/cerebellar tonsillar ectopia. Hypotonia, macrocephaly, and seizures are often present within the first year of life. The 16p11.2 duplication neurologic phenotype shares many features with the deletion neurologic phenotype, including speech articulation abnormalities, hypotonia, abnormal agility, sacral dimples, and seizures/epilepsy — though the frequencies of the identified functional motor abnormalities (i.e., oromotor articulation and agility) are significantly lower in duplication than deletion carriers. Additional distinguishing characteristics of the duplication phenotype are more prominent hyperreflexia (and less hyporeflexia), highly prevalent tremor, and a trend toward small (rather than large) head size on average, though duplication carriers exhibit variability in this regard with increased prevalence of both microcephaly and macrocephaly. Motor dysrhythmia is a less common component of the phenotype. As in deletion carriers, hypotonia and head size abnormality are often noted in the first year of life amongst duplication carriers, while average seizure onset occurs closer to 2 years old and tremor is commonly not noted until school age or later. White matter and/or corpus callosum abnormalities and ventricular enlargement are seen most commonly on brain imaging and focal sharp/epileptiform activity is commonly observed on EEG.

Speech and/or language problems have been identified with high frequency in both 16p deletion and duplication carriers in prior case series [Marshall et al., 2008; Weiss et al., 2008; Bijlsma et al.,

2009; Fernandez et al., 2010; Hanson et al., 2010; Rosenfeld et al., 2010; Zufferey et al., 2012]. These studies have largely documented delays in speech/language development by history rather than direct examination. Further, the distinction between speech articulation impairments (i.e., the oromotor component) and language impairments (i.e., the cognitive component) has been made in only case reports of one 16p deletion and one 16p duplication subject [Marshall et al., 2008] and one case series based on history [Hanson et al., 2010]. The current analysis now provides strong evidence for highly prevalent speech articulation abnormalities (irrespective of language impairment) on direct neurologic examination in both pediatric and adult 16p deletion and duplication populations. In a subset of the deletion carriers from the current Simons VIP cohort, Hanson et al. [2014] identified DSM-IV "phonologic processing disorder" in 56% (44/77) of pediatric 16p deletion carriers, yet in none (0/7) of the adult carriers. We attribute the much higher frequency of abnormal articulation found via neurologic examination − 86% (89/103) of pediatric deletion carriers and 28% (4/14) of adult deletion carriers—to (i) the careful evaluation of articulation during the neurologic examination and (ii) to the more restrictive DSM-IV requirement—used by psychologists and not for the neurologic examination—for significant functional impact on academic, occupational, or social communication abilities. Further, we suspect that frequencies of articulation abnormalities identified with each CNV are likely underestimates since articulation could not be assessed in individuals with limited or no verbalizations during the evaluation.

We conceptualize the oromotor/articulatory dysfunction as part of a broader array of motor abnormalities identified in our 16p11.2 deletion or duplication cohorts. High frequencies of hypotonia (diagnosed in clinical care and seen on direct examination) and frequent agility abnormalities in both groups are consistent with previous reports of low tone and delays in motor development [Kumar et al., 2008; Weiss et al., 2008; Bijlsma et al., 2009; Rosenfeld et al., 2010; Shinawi et al., 2010]. However, weakness was identified much less commonly on our clinical examinations, despite its frequent diagnosis in subjects' personal medical care. We hypothesize that this difference may result from resolution of weakness between the time of diagnosis and our neurologic exam in many individuals or from potentially more restricted use of the term "weak" (vs "low tone" or "coordination difficulties") by neurologists compared to other professional specialties (e.g., developmental pediatrician, occupational therapist, physical therapist), especially in early childhood, when that differentiation is more difficult to make. Though the motor delays or impairments can be mild [Shinawi et al., 2010], our identification of agility abnormalities in 29% of adult deletion carriers and 19% of adult duplication carriers and high prevalence of agility abnormalities even amongst non-proband familial carriers in each CNV group (33% deletions, 16% duplications) suggests the possibility that motor impairments could potentially persist after perceived resolution of delays and/or that more subtle motor impairments occur even in those who do not report functional motor impairments. "Sub-clinical" motor impairments of this nature parallel the unrecognized cognitive sequelae recently found in unselected, presumed "healthy" adult populations with 16p11.2 CNVs [Stefansson et al., 2014; Männik et al., 2015].

While frank weakness does not appear to be responsible for the majority of agility abnormalities seen, our analysis indicates different associations with agility problems between the deletion and duplication groups. The association of abnormal agility with LE dysrhythmia and sacral dimples in duplication carriers but with LE dysmetria (though not commonly seen) in deletion carriers suggest the possibility that different underlying mechanisms in the two groups converge toward the common phenotype of functional movement abnormalities.

When considering possible underlying mechanisms for agility abnormalities, the high prevalence of sacral dimples in both groups is of particular interest. To the best of our knowledge, sacral dimples have been reported previously in only two individuals with the 16p11.2 duplication [Rosenfeld et al., 2010] and in none with the 16p11.2 deletion, but it is unknown how often this has been evaluated in prior studies. Though sacral dimples are observed as a normal variant in approximately 4% of the general population and are not typically associated with neurologic abnormalities, the risk of abnormalities is higher with atypical features seen in a number of our subjects, such as non-visualizable bottom, multiple dimples, and dimples not on the midline (see Figs. 1 and 2) [Kriss and Desai, 1998]. The high prevalences of sacral dimples, along with some of the aforementioned atypical features seen amongst 16p11.2 deletion and duplication carriers, raise the question as to whether dimples may be accompanied by occult spinal dysraphism. Further, the identified association between sacral dimples and LE agility in the 16p duplication carriers—along with the presence of tethered cord and syringomyelia in some individuals in the current study and observed by Shinawi et al. [2010] amongst their duplication subjects and meningocele/spina bifida occulta identified by Zufferey et al. [2012] in two deletion carriers—emphasize the need for future studies to include MR imaging of the lumbosacral spine to answer this question, particularly given the significant impact this could have on clinical management/intervention.

The prominence of tremor as another motor-related feature of the 16p11.2 duplication phenotype was unanticipated, and was noted in only two previously studied subjects [Rosenfeld et al., 2010]. Despite being commonly experienced by duplication carriers in our cohort (28%) and seen in a high proportion on standardized examination (43%), it appears to have been sufficiently mild that it has warranted prior clinical attention in only a subset (18%) which may explain its near-absence in prior phenotypic descriptions.

Copy number variation at 16p11.2 has previously been linked to epilepsy [Mefford et al., 2010; Shinawi et al., 2010; Zufferey et al., 2012; Mullen et al., 2013; Olson et al., 2014], and mutations of *PRRT2*, a gene within this region, has been linked with benign infantile epilepsy syndromes [Scheffer et al., 2012]. In the current cohort, both deletion and duplication carriers exhibit an elevated frequency of diagnosed unprovoked seizures/epilepsy compared to the 8% lifetime incidence of a seizure (provoked or unprovoked) in the general population [So, 1995]. While both phenotypes include localization-related (partial) and generalized tonic-clonic seizures, absence seizures—previously reported in both 16p11.2 deletion and duplication patients [Mullen et al., 2013]—are more specific to deletion carriers (33%) than duplication carriers (5%). More detailed examination of seizures and epilepsy in this cohort will

be important to more comprehensively characterize their epilepsy and possibly epilepsy syndromes.

A common theme emerging from the growing literature on CNVs is that of contrasting (or what have been called "mirrored") phenotypes when comparing deletion and duplication carriers of the same genomic region [Crespi et al., 2009; reviewed in Golzio and Katsanis, 2013]]. Reciprocal 16p11.2 CNVs exhibit mirrored phenotypes of obesity versus underweight and increased versus decreased brain volume in deletion and duplication carriers, respectively [Jacquemont et al., 2011; Qureshi et al., 2014; Maillard et al., 2015]. Mirrored phenotypes are more evident in features that exhibit bidirectional variation from the norm (i.e., "too much" or "too little"; e.g., anthropometric traits). Contrasting phenotypes are less commonly seen in neurologic function where deviation from normal is typically unidirectional—for example, abnormal (i.e., decreased) agility and abnormal (i.e., poorer) articulation. The presence of more common hyporeflexia in deletion carriers and hyperreflexia in duplication carriers, however, may be a subtle indication of opposite underlying neurobiologic mechanisms resulting in the hypotonia and functional motor impairments shared between these CNVs. Opposite head size phenotypes may similarly serve as anatomic markers of differing neurobiologic abnormalities with converging functional effects on articulation and agility in 16p11.2 deletion and duplication carriers. Recent work by Golzio et al. [2012] identified the *KCTD13* gene—included in the 600 kb region of recurrent 16p11.2 copy number variation—as a principal driver of the neurodevelopmental deletion and duplication phenotypes. Overexpression of *KCTD13* yielded microcephalic zebrafish embryos, with decreased neuronal proliferation and increase in cell apoptosis. Conversely, reduced *KCTD13* expression resulted in an increase in proliferating cells in the brains of zebrafish embryos accompanied by macrocephaly. Recent evidence of opposing measures of global increased brain size in human 16p11.2 deletion carriers and reduced size in duplication carriers [Qureshi et al., 2014] suggests that similar neurobiological mechanisms may underlie some of the human 16p11.2 CNV phenotypic features. We suggest that a predominant effect on inhibitory components of the cerebral pyramidal motor system could result in hyperreflexia in duplication carriers (insufficient upper motor neuron inhibition) and hyporeflexia in deletion carriers (excessive upper motor neuron inhibition). That excessive or insufficient inhibition would alter the normal balance of excitatory and inhibitory output and therefore both impair motor function seems straightforward. How both would result in hypotonia is not as clear, though the same phenomenon is also seen in other reciprocal CNVs, such as the Smith-Magenis and Potocki-Lupski syndromes caused by deletion and duplication at chromosome 17p11.2 [Smith et al., 2012; Magoulas et al., 2014]. Gains or losses of a given chromosome segment—including *MECP2*, *SHANK3*, and 15q11-13—can cause a range of overlapping phenotypes, including intellectual disability, autism, seizures, and motor abnormalities [Magenis et al., 1987; Cook et al., 1997; Ohta et al., 1999; Huppke et al., 2000; Lossie et al., 2001; Anderlid et al., 2002; Wilson et al., 2003; Van Esch et al., 2005; Sahoo et al., 2006; Durand et al., 2007; Okamoto et al., 2007; Christian et al., 2008; Ramocki et al., 2009; Dhar et al., 2010; Christodoulou and Ho, 2012; Han et al., 2013]. Overlapping phenotypic features in these disorders are proposed to result from "failure of homeostatic regulation of synaptic function." Zoghbi and Bear [2012] suggest that "optimal synaptic function occurs within a limited dynamic range" and that synaptic pathophysiology at both ends of this range can cause a given phenotype.

Among reciprocal CNVs, duplications tend to exhibit lower penetrance and greater variability in expressivity than deletions [Golzio and Katsanis, 2013]. Among 16p deletion carriers, only articulation abnormalities on examination and prior diagnosis of hypotonia differed between probands and non-proband familial carriers and articulation abnormalities were still quite common amongst non-probands. By contrast, proband and familial carriers of the 16p duplication differed in their frequency of articulation, hypotonia, agility, tremor, and hyperreflexia on exam, as well as diagnoses of microcephaly, hypotonia, weakness, and seizures (febrile and unprovoked), demonstrating the greater phenotype variability of neurologic features among 16p duplication carriers than among deletion carriers.

While strengths of this study include the large sample size and the standardized neurologic phenotyping methods, there are still some limitations that should be acknowledged. Though standardized, the BANH was based on clinical care and on different amounts and types of historical information across subjects. Variability in the historical information provided depended on the quality of the historian, ability to acquire and extent of medical records, and the age of the subject (less information recalled or known about earlier life in older subjects). Also notable is that age of onset or diagnosis of SSDx differs in meaning depending on whether the SSDx was merely suspected by family (age of onset obtained) or diagnosed by a professional (age of diagnosis documented). These ages, therefore, should be interpreted as the latest age at which a SSDx emerged. Despite inherent subjectivity of certain neurologic examination measures (e.g., hypotonia, hyper-/hyporeflexia, tremor), our findings are supported by similar symptoms/diagnoses on BANH. Adult subjects in the study are typically familial carriers identified through the cascade genetic testing in the family after the proband has been genetically diagnosed. Therefore, they often have fewer signs and symptoms as they have not come to clinical attention. Because of this, as well as the cross-sectional nature of the study, caution is advised in making definitive conclusions about age-specific prevalence of neurologic abnormalities or longitudinal neurologic course of individuals with 16p CNVs. Finally, regarding statistical methods, we note that even if one group has 0% frequency, individuals from the same family are still correlated, which is not accounted for by the Fisher's exact test. However, when a group has a frequency of 0%, GEE model convergence becomes a significant issue and the large sample assumption underlying the model is not reasonable. We therefore believe that Fisher's exact test is the most appropriate statistical method to use in these circumstances.

In summary, 16p deletion and duplication carriers exhibit a variety of neurologic abnormalities, some shared and some distinct. When seen in the clinical setting, various combinations of speech abnormalities, low tone, abnormalities of agility without frank weakness, tremor, sacral dimples, and seizures—especially when accompanied by ASD, ADHD, speech and/or language disorders, intellectual disability, or other neurocognitive

disorders—should remind clinicians to consider genetic evaluation with a chromosomal microarray. Identification of 16p CNVs provide families with an explanation for the neurodevelopmental challenges faced by their children, limit the diagnostic odysseys often embarked on for these patients, and provide families with a sense of community by enabling them to connect with other families who, in sharing the same genotype, also share some of the same challenges and struggles.

ACKNOWLEDGMENTS

This study was supported by Simons Foundation Autism Research Initiative (SFARI) award #198677 from the Simons Foundation to WKC and EHS. We are grateful to all of the families at the participating Simons Variation in Individuals Project (Simons VIP) sites as well as the Simons VIP working group (Simons VIP consortium, Neuron, 73:1063–1067, 2012). We thank Drs. Nigel Bamford; William Dobyns; Sidney Gospe, Jr.; and Kiran Maski for their help as substitute neurologists. We appreciate obtaining access to phenotypic data on the Simons Foundation Autism Research Initiative Base. Approved researchers can obtain the Simons VIP population data set described in this study by applying at https://base.sfari.org. Dr. Sherr is on the advisory board of InVitae and consults for Personalis. He has stock in Chemocentryx and has consulted for medicolegal cases. Dr. Sherr receives support for his research from the NIH, the Simons Foundation, the Marsha and John Goldman Foundation and the CURE Foundation.

Contributors to the Simons VIP Consortium include the following: B Aaronson, S Ackerman, H Alupay, K Ankenmann, C Atwell, E Aylward, A Beaudet, M Benedetti, J Berman, R Bernier, A Bibb, L Blaskey, C Brewton, R Buckner, P Bukshpun, J Burko, B Cerban, Q Chen, M Cheong, Z Chu, W Chung, C Dale, A Dempsey, J Elgin, J Olson, Y Evans, WA Faucett, G Fischbach, S Garza, J Gerdts, S Gobuty, R Goin-Kochel, PE Grant, L Green Snyder, M Greenup, E Hanson, K Hines, L Hinkley, J Hunter, R Jeremy, K Johnson, S Kanne, S Kessler, S Khan, A Laakman, M Lasala, D Ledbetter, H Lee, C Lese Martin, A Lian Cavanagh, A Llorens, T Luks, E Marco, A Martin, G Marzano, K McGovern, R McNally Keehn, D Miller, F Miller, T Moss, P Mukherjee, S Nagarajan, K Nowell, J Owen, A Paal, A Packer, P Page, B Paul, N Pojman, M Proud, S Qasmieh, M Ramocki, B Reilly, T Roberts, D Shaw, E Sherr, T Sinha, B Smith-Packard, A Snow, S Spence, J Spiro, K Steinman, V Swarnakar, J Tjernagel, C Triantafallou, R Vaughan, N Visyak, M Wakahiro, A Wallace, T Ward, and J Wenegrat.

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