Brain MR Imaging Findings and Associated Outcomes in Carriers of the Reciprocal Copy Number Variation at 16p11.2[1]

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Purpose: To identify developmental neuroradiologic findings in a large cohort of carriers who have deletion and duplication at 16p11.2 (one of the most common genetic causes of autism spectrum disorder [ASD]) and assess how these features are associated with behavioral and cognitive outcomes.

Materials and Methods: Seventy-nine carriers of a deletion at 16p11.2 (referred to as deletion carriers; age range, 1–48 years; mean age, 12.3 years; 42 male patients), 79 carriers of a duplication at 16p11.2 (referred to as duplication carriers; age range, 1–63 years; mean age, 24.8 years; 43 male patients), 64 unaffected family members (referred to as familial noncarriers; age range, 1–46 years; mean age, 11.7 years; 31 male participants), and 109 population control participants (age range, 6–64 years; mean age, 25.5 years; 64 male participants) were enrolled in this cross-sectional study. Participants underwent structural magnetic resonance (MR) imaging and completed cognitive and behavioral tests. MR images were reviewed for development-related abnormalities by neuroradiologists. Differences in frequency were assessed with a Fisher exact test corrected for multiple comparisons. Unsupervised machine learning was used to cluster radiologic features and an association between clusters and cognitive and behavioral scores from IQ testing, and parental measures of development were tested by using analysis of covariance. Volumetric analysis with automated segmentation was used to confirm radiologic interpretation.

Results: For deletion carriers, the most prominent features were dysmorphic and thicker corpora callosa compared with familial noncarriers and population control participants (16%; $P < .001$ and $P < .001$, respectively) and a greater likelihood of cerebellar tonsillar ectopia (30.7%; $P < .002$ and $P < .001$, respectively) and Chiari I malformations (9.3%; $P < .299$ and $P < .002$, respectively). For duplication carriers, the most salient findings compared with familial noncarriers and population control participants were reciprocally thinner corpora callosa (18.6%; $P < .003$ and $P < .001$, respectively), decreased white matter volume (22.9%; $P < .001$, and $P < .001$, respectively), and increased ventricular volume (24.3%; $P < .001$ and $P < .001$, respectively). By comparing cognitive assessments to imaging findings, the presence of any imaging feature associated with deletion carriers indicated worse daily living, communication, and social skills compared with deletion carriers without any radiologic abnormalities ($P < .005$, $P < .002$, and $P < .004$, respectively). For the duplication carriers, presence of decreased white matter, callosal volume, and/or increased ventricle size was associated with decreased full-scale and verbal IQ scores compared with duplication carriers without these findings ($P < .007$ and $P < .004$, respectively).

Conclusion: In two genetically related cohorts at high risk for ASD, reciprocal neuroanatomic abnormalities were found and determined to be associated with cognitive and behavioral impairments.

Online supplemental material is available for this article.

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Study supported by Simons Foundation (SFARI A118077).

Autism spectrum disorder (ASD) affects one in 68 children and presents a major public health concern (1). Determining an association between neuroanatomical deviations and cognitive and behavioral sequelae in ASD has been difficult because of its heterogeneous etiologic causes. By focusing on individuals who share a causative genetic event, such as a single copy number variant (CNV) linked to ASD phenotypes, understanding of the linkage between brain and behavior in ASD could be improved. Imaging studies of genetically homogeneous cohorts linked to ASD reveal consistent alterations in cortical white matter and in the morphologic structure of the corpus callosum, among other anatomic abnormalities (2–9).

A recurrent and reciprocal 593-kb deletion and duplication CNV pair at the 16p11.2 chromosome is associated with global developmental delay, intellectual disability, and schizophrenia and is perhaps the most common CNV associated with ASD (10–16). Approximately 0.6% of individuals with ASD have a 16p11.2 CNV (13). Early case studies of patients also suggested anatomic abnormalities, such as syringomyelia, Chiari I malformation, and ventriculomegaly (17,18). These preliminary studies provide a rationale for a detailed large-scale study of individuals with 16p11.2 CNVs to obtain magnetic resonance (MR) imaging data independent of clinical symptoms and to then directly assess associations between neuroanatomical changes and clinical outcomes.

The Simons Variation in Individuals Project (VIP) (19) examined a large cohort of individuals with a range of clinical phenotypes who all had a 16p11.2 CNV. Unaffected family members and population control participants were included for comparison. Several articles were published from the extensive neuropsychologic and imaging data collected as part of the Simons VIP (13,20–25). The purpose of our study was to identify the salient neuroradiologic findings in a large cohort of 16p11.2 deletion and duplication carriers by using well-established clinical radiologic assessments from over 300 participants and to assess how these features are associated with behavioral and cognitive outcomes. Our central study hypotheses were that carriers of deletions and duplications at 16p11.2 (referred to here as deletion carriers and duplication carriers, respectively) would differ in the frequency of radiologic findings compared with both familial noncarriers and population control participants, and the presence of neuroradiologic abnormalities in the carriers would be associated with cognitive or behavioral deficits. Unsupervised machine learning extracted a nonoverlapping constellation of features associated with the deletion and duplication carriers, which provided imaging features that could constitute a radiologic syndrome for 16p11.2 deletion and duplication carriers.

Advances in Knowledge

- Compared with unaffected familial noncarriers and population control participants, carriers of deletion at 16p11.2 have abnormally shaped and thicker corpora callosa (16.0%) and also show signs of overgrowth in the posterior fossa, with a high likelihood of cerebellar tonsillar ectopia (30.7%) and Chiari I malformations (9.3%).
- Compared with unaffected familial noncarriers and population control participants, carriers of duplication at 16p11.2 have reciprocally thinner corpora callosa (18.6%), decreased white matter volume (22.9%), and increased ventricular volume (24.3%).
- By using data-driven hierarchical clustering, we identified groups of covariant radiologic features, which provided evidence for a radiologic syndrome for both the 16p11.2 deletion and duplication carriers.
- We demonstrated associations between the presence of these abnormal radiologic findings and impaired performance for measures of social function, communication, overall developmental delay, and cognitive ability; the presence of any feature associated with the deletion carriers indicated worse daily living, communication, and social skills, and for the duplication carriers, the presence of decreased white matter and callosal volume and increased ventricle size was associated with decreased full-scale and verbal IQ scores.

Implications for Patient Care

- This study assessed the clinical neuroradiologic abnormalities in a large cohort of 16p11.2 deletion and duplication carriers, which will further inform neuroradiologists about the radiologic features of this copy number variant (CNV) and increase general awareness of this genetic disorder.
- The identification of the radiologic features associated with cognitive and behavioral deficits may inform neurologists who see 16p11.2 CNV carriers in a clinical setting.

https://doi.org/10.1148/radiol.2017162934

Content code: NR

Radiology 2018; 286:217–226

Abbreviations:
ASD = autism spectrum disorder
CNV = copy number variant
VIP = Variation in Individuals Project

Author contributions:
Guarantors of integrity of entire study, J.P.O., P.B., P.M.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, J.P.O., J.V.H., T.P.R., S.S.N., P.M., E.H.S.; clinical studies, J.P.O., P.B., T.T., O.A.G., J.V.H., T.P.R., E.H.S.; experimental studies, N.P., T.T., J.I.B., S.S.N., E.H.S.; statistical analysis, P.B., Q.C., J.L., D.D., S.S.N.; and manuscript editing, J.P.O., P.B., Q.C., J.L., O.A.G., T.P.R., R.B., S.S.N., P.M., E.H.S.

Conflicts of interest are listed at the end of this article.

See also the editorial by Rollins in this issue.

Materials and Methods

Participants

Institutional review board approval was obtained at each participating university. All data were collected and stored in accordance with the Health Insurance Portability and Accountability Act. Families with at least one duplication or deletion carrier were invited to participate in this retrospectively conducted cross-sectional study; informed consent was obtained from all participants. Structural brain imaging was performed at the University of Washington (Seattle, Wash), Baylor College of Medicine (Houston, Tex), or Harvard School of Medicine (Cambridge, Mass) as part of the advanced neuroimaging protocol of the Simons VIP Project from 2011 to 2015 (19). Seventy-nine participants who were 16p11.2 deletion carriers (age range, 1–48 years; mean age, 12.3 years; 42 male participants), 79 participants who were 16p11.2 duplication carriers (age range, 1–63 years; mean age, 24.8 years; 43 male participants), and 64 unaffected noncarrier family members (age range, 1–46 years; mean age, 11.7 years; 31 male participants) were imaged. We recruited and imaged 109 people in a population control group matched for age and sex (age range, 6–64 years; mean age, 25.5 years; 64 male participants) at the University of California (San Francisco, Calif) and the Children's Hospital of Philadelphia (Philadelphia, Pa). Carriers and family members were screened for recurrent breakpoints at BP4–BP5 of 16p11.2 without other pathogenic CNVs, fluency in English, and no history of environmental insults. Population control participants were screened for a major DSM-IV diagnosis, dysmorphic features, and family history of genetic abnormalities, and had a chromosome microarray. For a complete description of inclusion and exclusion criteria, see Appendix E1 (online).

Image Acquisition

We performed thin-section three-dimensional structural MR sequences, including T1-weighted, T2-weighted, and fluid-attenuated inversion recovery sequences. Standard clinical imaging protocols were standardized and used across all sites (Appendix E1 [online]).

Radiologic Interpretation and Volumetric Analysis

Structural images were independently reviewed (blinded to genetic status) by at least two board-certified neuroradiologists (O.A.G., J.V.H., and P.M., each with more than 14 years of experience as faculty at major medical centers). A comprehensive review checklist was used with 16 categories designed to identify congenital and developmental malformations applicable to both the healthy and the developmentally abnormal brain, including abnormalities of the following: corpus callosum, white matter, cortex, cerebellum, basal ganglia, thalamus, hippocampi, pituitary, brainstem, skull base, and ventricles. More details of the radiologic review process and checklist can be found in Appendix E1 (online). Each item in the checklist was coded as the following: normal; abnormal in size or shape, or abnormality present; or indeterminate. The independent qualitative reviews of MR imaging were compared for consistency and to prevent individual bias. Discrepant interpretations were reviewed by the three neuroradiologists (O.A.G., J.V.H., and P.M.) and a child neurologist (E.H.S., with 15 years of experience as faculty at a major medical center), all of whom were blinded to genetic status, until consensus was reached. Four deletion carriers (four of 75 [5.3%]), eight duplication carriers (seven of 71 [9.8%]), and six familial noncarriers (five of 58 [8.6%]) were excluded for excessive motion deemed by visual inspection of images and by having 50.0% or more indeterminate reads. No population control participants were excluded. A secondary analysis was performed in which the T1-weighted structural images were passed through a quality control pipeline and were analyzed by using the software package FreeSurfer, similar to that in Qureshi et al (20), to obtain volumes of white matter and cerebellum. The T1-weighted images from 25 deletion carriers, 17 duplication carriers, and 62 population control participants were previously analyzed by using automatic segmentation performed with software (FreeSurfer version 4.5.0; *surfer.nmr.mgh.harvard.edu*) (20), and preliminary radiologic findings from these carriers (20) and 34 deletion carriers in the Simons VIP cohort (25) were previously reported.

Neuropsychologic Assessment

Previous publications have detailed the cognitive and behavioral phenotype of 16p11.2 CNV carriers in the Simons VIP cohort (13,25). Here, we focus on the association between radiologic abnormalities and measures of general cognition and/or behavioral function. A board-certified neuropsychologist with more than 15 years of experience and research validity administered the assessments on the same day that the MR images were collected. General measures of cognitive abilities were measured with the Differential Ability Scales–Second Edition, Mullen Scales of Early Learning, and Wechsler Abbreviated Scale of Intelligence standard scores, which were combined to report full-scale IQ, verbal IQ, and nonverbal IQ. Measures of social and language function consisted of the Social Responsiveness Scale, Vineland-II Socialization scale, and Vineland-II Communication scale. The Vineland-II Adaptive Behavior Composite scale was used to measure overall developmental delay.

Statistical Analysis

An analysis of covariance was used to test for group differences of age and cognitive and behavioral scores between deletion or duplication carriers and the familial noncarriers and the population control participants. The analysis of covariance included adjustments for age and sex in the models for the behavioral and cognitive scores, and adjustments for sex in the model for age. The P values were corrected by using the Bonferroni method to account for the eight demographic and neuropsychologic measures compared at Q less than 0.05 ($P < .0063$). A Fisher exact test was used to detect differences in frequencies of the

radiologic findings between deletion or duplication carriers and the familial noncarriers and the population control participants. The analysis only included subjects for which there was a determinate interpretation. Bonferroni correction was used to adjust for the multiple comparisons across all the radiologic features, and Q less than 0.05 ($P < .0031$) was used to determine statistical significance.

Data-driven hierarchical clustering was performed by using the hclust function in the statistical computing language R v2.13.1 (R Foundation for Statistical Computing, Vienna, Austria). A binary distance was used and the Ward minimum variance method was specified for the clustering method. Only data from the deletion and duplication carriers were used for the clustering. The branches of the dendrogram were determined by the hclust algorithm independently of the behavioral and cognitive data. Because the distance metric only accounts for the overlap in abnormal findings, to maximize the sample size, so-called indeterminate reads were artificially set to indicate "normal." The results from the clustering were used to group deletion and duplication carriers by presence or absence of radiologic findings. To assess statistical differences in cognitive and behavioral measures between these subgroups within the deletion and duplication carriers defined after clustering, an analysis of covariance model that was adjusted for age and sex was performed. The P values were corrected by using the Bonferroni method to account for the multiple tests across the six cognitive and behavioral measures; Q less than 0.05 ($P < .008$) was used to determine statistical significance.

Statistical analysis of the volumetric analyses with FreeSurfer followed the methods used for the age and cognitive and behavioral scores: An analysis of covariance adjusted for age and sex was performed, and P values were corrected for multiple comparisons by using the Bonferroni method ($Q < 0.05$).

Throughout the Results section, P values that compare deletion and duplication carriers to population control participants are indicated by P_{delvpc} and P_{dupvpc}, respectively, and P values that compare deletion and duplication carriers to familial noncarriers are indicated by P_{delvfc} and P_{dupvfc}, respectively.

Table 1

Sample Demographics and Neuropsychologic Testing Scores for 16p11.2 Deletion and Duplication Carriers, Familial Noncarriers, and Population Control Participants

Parameter	Deletion Carriers ($n = 75$)	Duplication Carriers ($n = 71$)	Familial Noncarriers ($n = 58$)	Population Control Participants ($n = 109$)
Age	12.3 ± 9.6 (1.1–48.0)*	24.8 ± 17.2 (1.4–63.1)†	11.7 ± 8.3 (1.5–46.1)	25.5 ± 15.1 (6.5–63.5)
Sex				
M/F ratio	38:37	38:33	28:30	64:45
IQ				
FSIQ	85 ± 15*†	90 ± 19*†	108 ± 11	106 ± 14
VIQ	83 ± 18*†	91 ± 18*†	107 ± 12	107 ± 15
NVIQ	88 ± 15*†	90 ± 19*†	105 ± 13	107 ± 11
Vineland scale				
Adaptive Behavior Composite	81 ± 12	84 ± 15	105 ± 12	103 ± 12
Communication	79 ± 12†	86 ± 18	105 ± 12	102 ± 13
Socialization	83 ± 15	89 ± 15	104 ± 12	106 ± 14
SRS	71 ± 36*†	57 ± 35*†	21 ± 20	19 ± 12

Note.—Data are means ± standard deviation unless otherwise mentioned. Data in parentheses are range. Comparisons between carriers and the familial noncarriers and the population control participants were assessed by using an analysis of covariance that was adjusted for age and sex for IQ, Vineland, and Social Responsiveness Scale, and age was adjusted for sex. FSIQ = full-scale IQ, NVIQ = nonverbal IQ, SRS = Social Responsiveness Scale, VIQ = verbal IQ.

* Denotes statistically significant difference from population control participants, corrected by using Bonferroni correction to account for the eight demographic and neuropsychologic measures compared.

† Denotes statistically significant difference from familial noncarriers.

Results

In Table 1, we presented demographics and cognitive and behavioral scores for the 16p11.2 deletion and duplication carriers, the familial noncarriers, and population control participants. There was a statistically significant difference in age between deletion carriers and population control participants ($P_{delvpc} <$ 4e-10; $P_{delvfc} < .72$) and between duplication carriers and familial noncarriers ($P_{dupvpc} < .75$; $P_{dupvfc} <$ 5e-7). Both carrier groups had statistically significantly different IQ and Social Responsiveness Scale scores compared with both control groups: full-scale IQ ($P_{delvpc} <$ 5e-6, $P_{delvfc} <$ 1e-16, $P_{dupvpc} <$ 1e-9, and $P_{dupvfc} <$ 5e-6), verbal IQ ($P_{delvpc} <$ 3e-6, $P_{delvfc} <$ 5e-15, $P_{dupvpc} <$ 6e-9, and $P_{dupvfc} <$ 1e-5), nonverbal IQ ($P_{delvpc} <$ 1e-11, $P_{delvfc} <$ 6e-14, $P_{dupvpc} <$ 8e-10, and $P_{dupvfc} <$ 1e-11), and Social Responsiveness Scale ($P_{delvpc} <$ 6e-24, $P_{delvfc} <$ 2e-13, $P_{dupvpc} <$ 7e-14, and $P_{dupvfc} <$ 1e-5). After corrections for multiple comparisons, the only Vineland score that was statistically significantly different was the Vineland-II Communication scale for the deletion carriers compared with the familial noncarriers: Vineland-II Adaptive Behavior Composite scale ($P_{delvpc} < .02$, $P_{delvfc} < .02$, $P_{dupvpc} < .22$, and $P_{dupvfc} <$ .42), Vineland-II Communication scale ($P_{delvpc} < .03$, $P_{delvfc} <$ 2e-4, $P_{dupvpc} < .02$, and $P_{dupvfc} < .09$), and Vineland-II Socialization scale ($P_{delvpc} < .009$, $P_{delvfc} <$ .008, $P_{dupvpc} < .82$, and $P_{dupvfc} < .23$).

In Figure 1, example axial and sagittal MR images for a control population, deletion carrier, and duplication carrier are shown along with the key imaging features. Table 2 provides the frequency of the radiologic features in each group, a tabulation of missing data, and results of statistical comparisons. For deletion carriers, we found an increased frequency of a dysmorphic

Figure 1

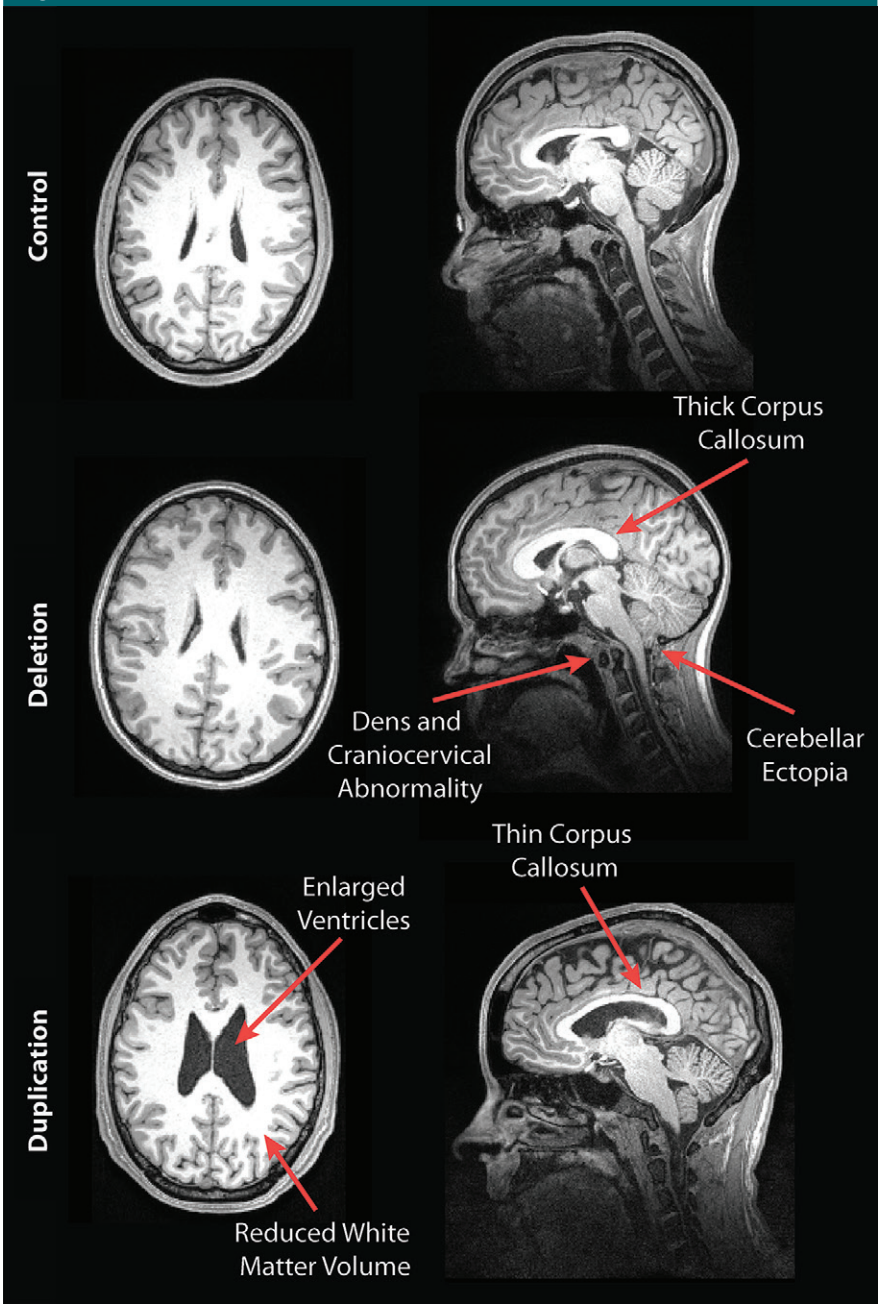

Figure 1: Example images for a control participant , a deletion carrier, and a duplication carrier. In the sagittal image of the deletion carrier, the thick corpus callosum, dens and craniocervical abnormality, and cerebellar ectopia are shown. For the duplication carrier, the sagittal image shows the thin corpus callosum and the axial image shows the increased ventricle size and decreased white matter volume.

and thick corpus callosum, referred to as a thick corpus callosum (frequency, 16%; $P_{delvfc} < .001$, $P_{delvpc} < .001$), Chiari type I malformation (frequency, 9.3%; $P_{delvfc} < .30$, $P_{delvpc} < .002$), and the anatomically milder cerebellar tonsillar ectopia (frequency, 30.7%; $P_{delvfc} < .002$, $P_{delvpc} < .001$), craniocervical junction abnormality (frequency, 26.4%; $P_{delvfc} < .001$, $P_{delvpc} < .001$), and dens abnormality (frequency, 19.7%; $P_{delvfc} < .11$, $P_{delvpc} < .001$). These radiologic features occurred with relatively low frequency in the duplication carriers.

For the duplication carriers, the most prevalent radiologic features were a dysmorphic and thinned corpus callosum, referred to as a thin corpus callosum (frequency, 18.6%; $P_{dupvfc} < .003$, $P_{dupvpc} < .001$), enlarged ventricles (frequency, 24.3%; $P_{dupvfc} < .001$, $P_{dupvpc} < .001$), and decreased white matter volume (frequency, 22.9%; $P_{dupvfc} < .001$, $P_{dupvpc} < .001$). An increase in ventricular size was found concordant with a decrease in white matter volume in the majority of duplication carriers. Whereas decreased cerebellar hemisphere volume (frequency, 8.6%; $P_{dupvfc} < .30$, $P_{dupvpc} < .003$), cerebellar vermis volumes (frequency, 13.2%; $P_{dupvfc} < .03$, $P_{dupvpc} < .001$), and hippocampal abnormalities (frequency, 9.1%; $P_{dupvfc} < .04$, $P_{dupvpc} < .001$) were detected by comparing with the population control participants, these differences did not survive multiple comparison correction when aligned to the familial noncarriers.

Two of the group comparisons were not matched for age: the deletion carriers and population control participants and the duplication carriers and the familial noncarriers. To address this, we performed a post hoc analysis in which we removed participants from all four groups until age and sex were matched ($P > .05$). The results of this analysis are presented in Table E2 (online). The core of the statistically significant findings in Table 2 were found to be statistically significant in this subgroup analysis, which suggested that differences in frequency found were not because of age-related differences in the larger unmatched cohorts. To corroborate the qualitative reads, we used automatic segmentation of the white matter and cerebellar volumes from FreeSurfer in the duplication carriers with sufficient data quality. The duplication carriers have decreased white matter ($P_{dupvfc} < 2e\text{-}6$, $P_{dupvpc} < 5e\text{-}13$) and cerebellar volumes ($P_{dupvfc} < 8e\text{-}3$, $P_{dupvpc} < 1e\text{-}7$) (Table E3 [online]).

Table 2

Frequency and Percentage of Radiologic Findings

Parameter	Deletion Carriers	Duplication Carriers	Familial Noncarriers	Population Control Participants	*P* Value, Deletion vs Familial Noncarrier	*P* Value, Deletion vs Population Control	*P* Value, Duplication vs Familial Noncarrier	*P* Value, Duplication vs Population Control
Thick corpus callosum	12/75 (16.0)	1/70 (1.4)	0/56 (0)	0/109 (0)	.001*	<.001*	>.999	.391
Thin corpus callosum	2/75 (2.7)	13/70 (18.6)	1/56 (1.8)	1/109 (0.9)	>.999	.568	.003*	<.001*
Pituitary abnormality	2/73 (2.7)	2/66 (3.0)	0/54 (0)	3/109 (2.8)	.507	>.999	.501	>.999
Brainstem abnormality	1/74 (1.4)	0/69 (0)	0/58 (0)	1/109 (0.9)	>.999	>.999	…	>.999
Cerebellar tonsillar ectopia	23/75 (30.7)	3/70 (4.3)	5/57 (8.8)	6/109 (5.5)	.002*	<.001*	.466	>.999
Chiari I malformation	7/75 (9.3)	0/70 (0)	2/57 (3.5)	0/109 (0)	.299	.002*	.2	…
Decreased cerebellar hemisphere volume	1/75 (1.3)	6/70 (8.6)	2/57 (3.5)	0/109 (0)	.578	.408	.295	.003*
Decreased cerebellar vermis volume	4/74 (5.4)	9/68 (13.2)	1/56 (1.8)	0/109 (0)	.39	.025	.022	<.001*
Craniocervical junction abnormality	19/72 (26.4)	4/69 (5.8)	2/53 (3.8)	1/109 (0.9)	.001*	<.001*	.696	.075
Platybasia	7/68 (10.3)	0/71 (0)	2/55 (3.6)	1/109 (0.9)	.186	.006	.189	>.999
Dens abnormality	13/66 (19.7)	5/69 (7.3)	4/52 (7.7)	1/109 (0.9)	.111	<.001*	>.999	.033
Cortical malformations	3/71 (4.2)	2/64 (3.1)	0/56 (0)	0/108 (0)	.255	.061	.498	.137
Enlarged ventricles	1/75 (1.3)	17/70 (24.3)	1/58 (1.7)	0/109 (0)	>.999	.408	<.001*	<.001*
Basal ganglia/thalamus abnormality	2/75 (2.7)	1/71 (1.4)	0/57 (0)	1/109 (0.9)	.506	.568	>.999	>.999
Hippocampus abnormality	5/69 (7.2)	6/66 (9.1)	0/51 (0)	0/109 (0)	.071	.008	.035	.003*
Decreased white matter volume	2/74 (2.7)	16/70 (22.9)	0/58 (0)	0/109 (0)	.504	.162	<.001*	<.001*

Note.—Data are numerator and denominator unless otherwise indicated; the numerator is the frequency and the denominator is the number of participants with sufficient image quality to make a radiologic read for that feature. Data in parentheses are percentage. By using a Fisher exact test, *P* values were calculated to assess differences in frequency between the carrier and noncarrier groups.

* *P* value survived a Bonferroni correction across radiologic categories ($\rho < 0.05$).

We then analyzed the imaging findings from the cohort by using data-driven hierarchical clustering, which identified distinct groups of radiologic features that cluster uniquely within the duplication and deletion carriers (Fig 2). In Figure 2, the branch to the far left (designated *A* and *B*) segregated the main radiologic features associated with duplication carriers, the middle branch (designated as *C*) contained the nonbrain features tied to abnormalities of the skull base that were present in the deletion carriers, and the branch to the right (*D*, *E*, and *F*) was composed of brain features associated with the deletion carriers (*D* and *E*) and the brain features that are relatively infrequent in occurrence (designated as *F*).

We used the branches defined by cluster analysis to test the hypothesis that carriers with these radiologic findings would be more cognitively and behaviorally impaired than those without these specific combinations of anatomic abnormalities (Table 3). Duplication carriers with at least one finding from *A* in Figure 2 had both low full-scale IQ ($P < .007$) and verbal IQ ($P < .004$), and duplication carriers with at least one of the findings in *B* had impaired Vineland-II Adaptive Behavior Composite scale ($P < .017$) and Vineland-II Socialization scale scores ($P < .006$) compared with duplication carriers without these findings. Interestingly, deletion carriers with a skull base abnormality from *B* did not differ from deletion carriers without skull base abnormalities in any cognitive or behavioral score, which suggested that not every anatomic phenotype associated with either CNV has a clear cognitive or behavioral association. Deletions with a thick corpus callosum, group *D*, had more impaired Social Responsiveness Scale scores ($P < .016$) and lower Vineland-II Communication scale ($P < .049$) and Vineland-II Socialization scale scores ($P < .011$) than did deletion carriers with a seemingly normal corpus callosum. Deletion carriers with either cerebellar tonsillar ectopia or a Chiari type I malformation from E had lower scores on the Vineland-II Communication scale scores ($P < .008$) compared with deletion carriers without these findings.

Finally, we used the presence of any brain-based radiologic abnormality to segregate the duplication and deletions into subgroups. Because of the lack of association between the skull base abnormalities and cognitive and/or behavioral deficits, we excluded platybasia, craniocervical junction, and dens abnormalities from this calculation. Duplication carriers with at least one radiologic finding in the brain were more impaired than

Figure 2

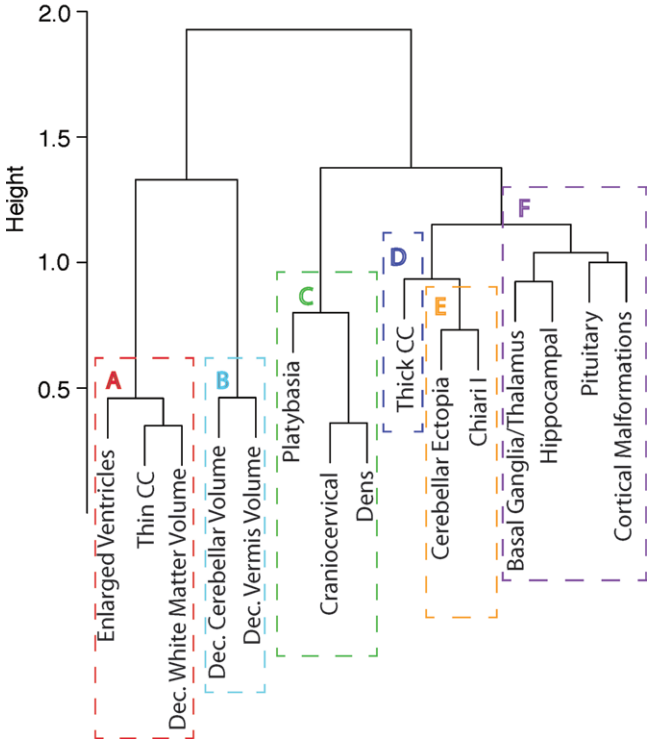

Figure 2: Dendrogram obtained from hierarchical clustering of the radiologic findings in the duplication and deletion carriers. *A* and *B* represent radiologic features of the duplication carriers; *C*, *D*, and *E* contain the features associated with the deletion carriers; and *F* is composed of the radiologic features that were relatively low in frequency in both groups and not more strongly associated with deletion or duplication carriers. *CC* = corpus callosum; *Dec.* = decreased.

were duplication carriers without any findings on all measures except the Social Responsiveness Scale: full-scale IQ ($P < .046$), verbal IQ ($P < .014$), Vineland-II Adaptive Behavior Composite scale ($P < .022$), Vineland-II Communication scale ($P < .044$), and Vineland-II Socialization scale ($P < .029$). The deletion carriers with at least one radiologic finding in the brain were more impaired than deletion carriers without any findings on the Social Responsiveness Scale ($P < .028$), Vineland-II Adaptive Behavior Composite scale ($P < .005$), Vineland-II Communication scale ($P < .002$), and Vineland-II Socialization scale ($P < .004$). We performed a secondary analysis in which we controlled for full-scale IQ in all subgroup comparisons in Table 3 for the Vineland and Social Responsiveness Scale scores. The results (not shown) were strikingly similar to those obtained without full-scale IQ correction, which provided evidence that differences in full-scale IQ alone do not drive the differences between subgroups.

Discussion

This study assesses neuroradiologic abnormalities in a large cohort (with over 300 participants) of 16p11.2 deletion and duplication carriers. In 16p11.2 deletion and duplication carriers, both at high risk for ASD, we found reciprocal neuroanatomic abnormalities and determined that these abnormalities were associated with cognitive and behavioral impairments. Data-driven hierarchical clustering was used to identify salient groups of radiologic features in a neurodevelopmental disorder. Because of relatively recent awareness that large and often de novo CNVs are associated with ASD, there are few large-scale radiologic studies that address this correlation. Only two previous studies (26,27) of deletions at 22q11.2 were on the same scale as our study. In 45 children with deletions at 22q11.2, the presence of cavum septum pellucidum was reported, but no association between cavum septum pellucidum presence and IQ or other cognitive measures was found (26). In a second study (27) with 58 children and adults with 22q11.2 deletions, a high prevalence of cavum septum pellucidum and additional white matter abnormalities were also reported, with an association between the presence of these abnormalities and the diagnosis of psychosis. Our findings in 16p11.2 reveal a more extensive set of neuroradiologic abnormalities than what has been reported in 22q11.2 deletions and multiple associations with cognitive performance and behavioral deficits.

The radiologic features that were most prevalent in the duplication carriers were mostly nonoverlapping with those prevalent in the deletion carriers, which demonstrated a differential effect of gene dosage on the brain anatomy in 16p11.2 CNVs despite the fact that both deletion and duplication carriers have an equivalent 20%–25% risk of ASD. Because the radiologic abnormalities are present in even the youngest of the duplication carriers (half with decreased white matter, a thin corpus callosum, or increased ventricle size were under age 10 years), these decreases in volume do not manifest solely after puberty or in advanced aging. Decreased cerebellar volume was associated with more impaired performance on the Vineland scales. There is evidence in the literature (28–30) that ASD and autistic-like behaviors are associated with abnormalities of the cerebellum. Both the finding of a thicker corpus callosum and cerebellar deviations point to brain overgrowth in the deletion carrier; increase in head size and brain volume has long been associated with ASD (31).

Table 3

Subgroup Analysis of the Duplication and Deletion Carriers by Using the Presence of One or More Radiologic Features to Subdivide the Group and Test for Differences in Cognition and Behavior between Subgroups

	IQ				Vineland						SRS	
	FSIQ		VIQ		Composite		Communication		Socialization			
Parameter	Mean Score	*P* Value	Mean Score	*P* Value	Mean Score	*P* Value	Mean Score	*P* Value	Mean Score	*P* Value	Mean Score	*P* Value
Duplication carriers												
Decreased CC/WM and/or enlarged ventricles (group A)		.007*		.004*		.161		.230		.185		.768
Yes ($n = 20$)	78		80		77		79		80		72	
No ($n = 46$)	95		97		88		90		91		50	
Decreased cerebellar volumes (group B)		.406		.407		.017		.155		.006*		.088
Yes ($n = 9$)	84		86		73		79		77		73	
No ($n = 55$)	91		93		86		87		89		54	
Brain findings (groups A, B, D, E, F)		.046		.014		.022		.044		.029		.568
≥1 ($n = 27$)	83		84		78		80		82		65	
0 ($n = 40$)	95		97		89		91		92		51	
Deletion carriers												
Skull base abnormalities (group C)		.426		.468		.853		.641		.977		.327
Yes ($n = 19$)	83		80		80		78		83		65	
No ($n = 46$)	86		84		81		80		83		73	
Enlarged corpus callosum (group D)		.601		.717		.049		.067		.011		.016
Yes ($n = 12$)	89		87		75		75		74		88	
No ($n = 57$)	84		82		82		80		85		67	
Cerebellar tonsillar ectopia and/or Chiari I (group E)		.201		.168		.097		.008*		.062		.296
Yes ($n = 21$)	82		79		77		74		79		77	
No ($n = 48$)	86		84		82		82		86		68	
Brain findings (groups A, B, D, E, F)		.513		.990		.005*		.002*		.004*		.028
≥1 ($n = 36$)	84		83		77		76		79		79	
0 ($n = 33$)	86		82		85		84		89		63	

Note.—An analysis of covariance model was used to assess significance adjusting for age and sex. For all the comparisons that are statistically significant before Bonferroni correction ($P < .05$), the mean of the cognitive and behavioral score for the carriers with the radiologic finding(s) indicated greater impairment. The groups (A–F) are defined in the dendrogram in Figure 2. CC = corpus callosum, FSIQ = full-scale IQ, SRS = Social Responsiveness Scale, VIQ = verbal IQ, WM = white matter.

* Survived a Bonferroni correction over all six cognitive and/or behavioral scores ($\rho < 0.05$)

In the deletion carriers, a thick corpus callosum was associated with impaired social function. Deviations in volume and structure in the corpus callosum are a prevalent finding in ASD, including an increased risk of ASD in agenesis of the corpus callosum (32). Unlike the duplication carriers, the most striking link between the radiologic findings and the cognitive and behavioral scores was found by comparing the deletions in a subgroup with no radiologic findings to a subgroup with one or more radiologic abnormalities. Thus, for the deletion carriers, it may be clinically relevant in the future to screen for the brain-based radiologic abnormalities as a general indicator of risk for behavioral deficits reflected in the Vineland scales and Social Responsiveness Scale.

Preliminary radiologic results from 34 deletion carriers and seven familial noncarriers from an early Simons VIP publication (25) revealed an increase in frequency of craniocervical abnormalities, although no statistical analyses were performed. In our current study, a full checklist of neurodevelopmental radiologic features was used in 71 duplication carriers and the full cohort of

75 deletion carriers (more than double the previous cohort) were compared with both 109 population control participants and 58 familial noncarriers. It was previously shown that 25 16p11.2 child deletion carriers and 17 adult duplication carriers from a subset of the Simons VIP cohort had opposing differences in gray and white matter and cerebellar volumes by using automatic segmentation (FreeSurfer) of structural MR imaging (20). However, FreeSurfer was only able to assess a limited subset of the neuroradiologic features assessed in our study, and there were no statistically significant correlations between the brain volumes and measures of behavior or cognition. Qureshi et al (20) also included a summary of the radiologic interpretations by using a developmental checklist similar to that used in our study, but no formal statistical analyses were performed and no conclusions were drawn about the frequency of these findings in the carrier population. The volumetric findings from Qureshi et al (20) and our findings mirror the tendency toward macrocephaly and higher body mass index reported in 16p11.2 deletion carriers and the accompanying microcephaly and lower body mass index found in 16p11.2 duplications (33), which supports the hypothesis that genetic copy numbers have correlated phenotypic quantitative changes (34).

There are limitations to this study. Although our sample size is relatively large, our results could be heuristic to the cross-section of individuals enrolled in the Simons VIP. We excluded individuals from our analyses for excessive motion at MR imaging, which has the indirect effect of excluding the lowest functioning individuals (the average full-scale IQ for the excluded individuals was 13 points lower than in those who passed quality control; $P < .005$). Despite this, we were able to demonstrate associations with brain anatomy and cognition and behavior even after excluding individuals from what is likely one tail of the distribution. The average ages of the deletion and duplication carriers were different (Table 1), which reflects ascertainment bias in our sample, further discussed in the Appendix E1 (online). Clinical radiologic interpretations are not influenced by age discrepancies because judgments on the part of a radiologist are made in light of the age of the participant, and we addressed the age discrepancies between groups in a post hoc analysis. We were not able to provide interrater reliability estimates because the radiologists were forced to come to a consensus, but we did provide information regarding the number of cases in which there were discrepancies. We plan to interrogate the prevalence of these abnormalities across the lifespan in a longitudinal study; however, we are unable to address such questions with our cross-sectional sample.

The findings in this study exhibit a link between genetic cause, brain structure, and behavior. By using unsupervised machine learning, we established a set of radiologic features that are emblematic of the deletion and duplication carriers. Identification of one feature in an individual should prompt a search for the others because observing these features together in an individual could lead to the suggestion of genetic screening and could enable radiologists to potentially distinguish 16p11.2 deletion from other genetic changes or neurodevelopmental disorders. Additionally, our results linking the radiology findings to the cognitive and behavioral scores could assist clinicians in assessing prognosis, although further study is warranted to fully establish these links.

Acknowledgments: 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 Consortium. We appreciate obtaining access to phenotypic data on SFARI Base. Approved researchers can obtain the Simons VIP population dataset described in this study by applying at https://base.sfari.org.

Disclosures of Conflicts of Interest: J.P.O. disclosed no relevant relationships. **P.B.** disclosed no relevant relationships. **N.P.** disclosed no relevant relationships. **T.T.** disclosed no relevant relationships. **Q.C.** disclosed no relevant relationships. **J.L.** disclosed no relevant relationships. **D.D.** disclosed no relevant relationships. **O.A.G.** disclosed no relevant relationships. **J.V.H.** disclosed no relevant relationships. **J.I.B.** disclosed no relevant relationships. **T.P.R.** Activities related to the present article: disclosed no relevant relationships. Activities not related to the present article: disclosed personal fees from Elekta Oy, personal fees from Prism Clinical Imaging, personal fees from Siemens, personal fees from Johnson and Johnson, and personal fees from Guerbet. Other relationships: disclosed no relevant relationships. **R.B.** disclosed no relevant relationships. **S.S.N.** disclosed no relevant relationships. **P.M.** disclosed no relevant relationships. **E.H.S.** disclosed no relevant relationships.

References

1. Christensen DL, Baio J, Van Naarden Braun K, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years--autism and developmental disabilities monitoring network, 11 sites, United States, 2012. MMWR Surveill Summ 2016;65(3):1–23.

2. Dubovsky EC, Booth TN, Vezina G, Samango-Sprouse CA, Palmer KM, Brasseux CO. MR imaging of the corpus callosum in pediatric patients with neurofibromatosis type 1. AJNR Am J Neuroradiol 2001;22(1):190–195.

3. Peters JM, Sahin M, Vogel-Farley VK, et al. Loss of white matter microstructural integrity is associated with adverse neurological outcome in tuberous sclerosis complex. Acad Radiol 2012;19(1):17–25.

4. Wegiel J, Schanen NC, Cook EH, et al. Differences between the pattern of developmental abnormalities in autism associated with duplications 15q11.2-q13 and idiopathic autism. J Neuropathol Exp Neurol 2012;71(5):382–397.

5. Violante IR, Ribeiro MJ, Silva ED, Castelo-Branco M. Gyrification, cortical and subcortical morphometry in neurofibromatosis type 1: an uneven profile of developmental abnormalities. J Neurodev Disord 2013;5(1):3.

6. Duarte JV, Ribeiro MJ, Violante IR, Cunha G, Silva E, Castelo-Branco M. Multivariate pattern analysis reveals subtle brain anomalies relevant to the cognitive phenotype in neurofibromatosis type 1. Hum Brain Mapp 2014;35(1):89–106.

7. Pride NA, Korgaonkar MS, Barton B, Payne JM, Vucic S, North KN. The genetic and neuroanatomical basis of social dysfunction: lessons from neurofibromatosis type 1. Hum Brain Mapp 2014;35(5):2372–2382.

8. Boronat S, Mehan WA, Shaaya EA, Thibert RL, Caruso P. Hippocampal abnormalities in magnetic resonance imaging (MRI) of 15q duplication syndromes. J Child Neurol 2015;30(3):333–338.

9. Frazier TW, Embacher R, Tilot AK, Koenig K, Mester J, Eng C. Molecular and phe-

notypic abnormalities in individuals with germline heterozygous PTEN mutations and autism. Mol Psychiatry 2015;20(9):1132–1138.

10. Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH, et al. Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. Eur J Med Genet 2009;52(2-3):77–87.
11. Kumar RA, Marshall CR, Badner JA, et al. Association and mutation analyses of 16p11.2 autism candidate genes. PLoS One 2009;4(2):e4582.
12. Szatkiewicz JP, O'Dushlaine C, Chen G, et al. Copy number variation in schizophrenia in Sweden. Mol Psychiatry 2014;19(7):762–773.
13. Hanson E, Bernier R, Porche K, et al. The cognitive and behavioral phenotype of the 16p11.2 deletion in a clinically ascertained population. Biol Psychiatry 2015;77(9):785–793.
14. McCarthy SE, Makarov V, Kirov G, et al. Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet 2009;41(11):1223–1227.
15. Steinberg S, de Jong S, Mattheisen M, et al. Common variant at 16p11.2 conferring risk of psychosis. Mol Psychiatry 2014;19(1):108–114.
16. Stefansson H, Meyer-Lindenberg A, Steinberg S, et al. CNVs conferring risk of autism or schizophrenia affect cognition in controls. Nature 2014;505(7483):361–366.
17. Al-Kateb H, Khanna G, Filges I, et al. Scoliosis and vertebral anomalies: additional abnormal phenotypes associated with chromosome 16p11.2 rearrangement. Am J Med Genet A 2014;164A(5):1118–1126.
18. Schaaf CP, Goin-Kochel RP, Nowell KP, et al. Expanding the clinical spectrum of the 16p11.2 chromosomal rearrangements: three patients with syringomyelia. Eur J Hum Genet 2011;19(2):152–156.
19. Simons Vip Consortium. Simons Variation in Individuals Project (Simons VIP): a genetics-first approach to studying autism spectrum and related neurodevelopmental disorders. Neuron 2012;73(6):1063–1067.
20. Qureshi AY, Mueller S, Snyder AZ, et al. Opposing brain differences in 16p11.2 deletion and duplication carriers. J Neurosci 2014;34(34):11199–11211.
21. Owen JP, Chang YS, Pojman NJ, et al. Aberrant white matter microstructure in children with 16p11.2 deletions. J Neurosci 2014;34(18):6214–6223.
22. Chang YS, Owen JP, Pojman NJ, et al. Reciprocal white matter alterations due to 16p11.2 chromosomal deletions versus duplications. Hum Brain Mapp 2016;37(8):2833–2848.
23. Berman JI, Chudnovskaya D, Blaskey L, et al. Abnormal auditory and language pathways in children with 16p11.2 deletion. Neuroimage Clin 2015;9:50–57.
24. Jenkins J 3rd, Chow V, Blaskey L, et al. Auditory evoked M100 response latency is delayed in children with 16p11.2 deletion but not 16p11.2 duplication. Cereb Cortex 2016;26(5):1957–1964.
25. Zufferey F, Sherr EH, Beckmann ND, et al. A 600 kb deletion syndrome at 16p11.2 leads to energy imbalance and neuropsychiatric disorders. J Med Genet 2012;49(10):660–668. [Published correction appears in J Med Genet 2014;51(7):478.]
26. Beaton EA, Qin Y, Nguyen V, Johnson J, Pinter JD, Simon TJ. Increased incidence and size of cavum septum pellucidum in children with chromosome 22q11.2 deletion syndrome. Psychiatry Res 2010;181(2):108–113.
27. Schmitt JE, Yi JJ, Roalf DR, et al. Incidental radiologic findings in the 22q11.2 deletion syndrome. AJNR Am J Neuroradiol 2014;35(11):2186–2191.
28. Bauman ML, Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. Int J Dev Neurosci 2005;23(2-3):183–187.
29. Limperopoulos C, Bassan H, Gauvreau K, et al. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? Pediatrics 2007;120(3):584–593.
30. Tsai PT, Hull C, Chu Y, et al. Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. Nature 2012;488(7413):647–651.
31. Sacco R, Gabriele S, Persico AM. Head circumference and brain size in autism spectrum disorder: A systematic review and meta-analysis. Psychiatry Res 2015;234(2):239–251.
32. Paul LK. Developmental malformation of the corpus callosum: a review of typical callosal development and examples of developmental disorders with callosal involvement. J Neurodev Disord 2011;3(1):3–27.
33. Jacquemont S, Reymond A, Zufferey F, et al. Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. Nature 2011;478(7367):97–102.
34. Subramanian M, Timmerman CK, Schwartz JL, Pham DL, Meffert MK. Characterizing autism spectrum disorders by key biochemical pathways. Front Neurosci 2015;9:313.