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► **To cite this version:**

Sandra Martin, Borja Rodríguez-Herreros, Jared Nielsen, Clara Moreau, Claudia Modenato, et al.. Quantifying the Effects of 16p11.2 Copy Number Variants on Brain Structure: A Multisite Genetic-First Study. *Biological Psychiatry*, 2018, 84 (4), pp.253 - 264. 10.1016/j.biopsych.2018.02.1176 . hal-01870357

HAL Id: hal-01870357

<https://hal.umontpellier.fr/hal-01870357v1>

Submitted on 13 Nov 2018

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Quantifying the Effects of 16p11.2 Copy Number Variants on Brain Structure: A Multisite Genetic-First Study

Sandra Martin-Brevet, Borja Rodríguez-Herreros, Jared A. Nielsen, Clara Moreau, Claudia Modenato, Anne M. Maillard, Aurélie Pain, Sonia Richetin, Aia E. Jønych, Abid Y. Qureshi, Nicole R. Zürcher, Philippe Conus, 16p11.2 European Consortium, Simons Variation in Individuals Project (VIP) Consortium, Wendy K. Chung, Elliott H. Sherr, John E. Spiro, Ferath Kherif, Jacques S. Beckmann, Nouchine Hadjikhani, Alexandre Reymond, Randy L. Buckner, Bogdan Draganski, and Sébastien Jacquemont

ABSTRACT

BACKGROUND: 16p11.2 breakpoint 4 to 5 copy number variants (CNVs) increase the risk for developing autism spectrum disorder, schizophrenia, and language and cognitive impairment. In this multisite study, we aimed to quantify the effect of 16p11.2 CNVs on brain structure.

METHODS: Using voxel- and surface-based brain morphometric methods, we analyzed structural magnetic resonance imaging collected at seven sites from 78 individuals with a deletion, 71 individuals with a duplication, and 212 individuals without a CNV.

RESULTS: Beyond the 16p11.2-related mirror effect on global brain morphometry, we observe regional mirror differences in the insula (deletion > control > duplication). Other regions are preferentially affected by either the deletion or the duplication: the calcarine cortex and transverse temporal gyrus (deletion > control; Cohen's $d > 1$), the superior and middle temporal gyri (deletion < control; Cohen's $d < -1$), and the caudate and hippocampus (control > duplication; $-0.5 > \text{Cohen's } d > -1$). Measures of cognition, language, and social responsiveness and the presence of psychiatric diagnoses do not influence these results.

CONCLUSIONS: The global and regional effects on brain morphometry due to 16p11.2 CNVs generalize across site, computational method, age, and sex. Effect sizes on neuroimaging and cognitive traits are comparable. Findings partially overlap with results of meta-analyses performed across psychiatric disorders. However, the lack of correlation between morphometric and clinical measures suggests that CNV-associated brain changes contribute to clinical manifestations but require additional factors for the development of the disorder. These findings highlight the power of genetic risk factors as a complement to studying groups defined by behavioral criteria.

Keywords: 16p11.2, Autism spectrum disorder, Copy number variant, Genetics, Imaging, Neurodevelopmental disorders

<https://doi.org/10.1016/j.biopsych.2018.02.1176>

Autism spectrum disorder (ASD) and related neurodevelopmental disorders are defined behaviorally and characterized by a significant clinical and etiologic heterogeneity. As a consequence, investigating ASD under the assumption of an underlying homogeneous condition has resulted in controversial findings in the field of neuroimaging (1). Increased brain growth early in development (2–4) and alterations of many regional brain volumes (5) have been implicated in ASD, but results have proven difficult to replicate (1,6–8).

To mitigate some of these issues, cohorts of individuals with shared genetic risk factors have been assembled to minimize the noise introduced by etiologic and biological heterogeneity (9). Such a “genetic-first” study design provides the opportunity to

investigate a given neurodevelopmental risk (and associated mechanism) shared by individuals who carry the same genetic etiology irrespective of the psychiatric diagnosis.

Copy number variants (CNVs) at the 16p11.2 (breakpoints 4–5, 29.6–30.2 Mb-hg19) (10) are among the most frequent risk factors for neurodevelopmental and psychiatric conditions. There is a similar 10-fold enrichment of deletions and duplications in ASD cohorts (11,12), and both CNVs have large effects on IQ (Z scores of 1.5 and 0.8, respectively) and Social Responsiveness Scale (SRS) (Z scores of 1 and 2, respectively) (10,13–15). However, there are phenotypic differences between both CNVs: the 10-fold enrichment in schizophrenia cohorts (16,17) is only observed for duplications, and only

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deletions affect measures of language by 1.5 Z scores (18). Previous studies demonstrated “mirror” effects of both CNVs on head circumference and body mass index (13,19). Neuroimaging studies reported gene-dosage effects on global brain metrics (20,21). However, large global effects and sample size limited the interpretation of the regional analyses, any estimate of effect size, and the generalizability of study results across different ascertainment.

In the current study, we aimed at quantifying the effects of 16p11.2 deletions and duplications on brain structure. We also examined the generalizability of our results across cohorts, scanning sites, sex, and a broad age range. Finally, we aimed at understanding the influence of clinical ascertainment. In particular, we asked whether language, social responsiveness, IQ, or the presence of psychiatric disorders may impact any of the findings. To this end, we analyzed structural magnetic resonance imaging (MRI) performed at seven sites from two international cohorts of 16p11.2 CNV carriers, familial control subjects, and unrelated control subjects. Voxel- and surface-based methods were performed in parallel on 361 participants, including 307 individuals not previously analyzed at the regional level, using whole-brain statistical methods.

METHODS AND MATERIALS

Participants

Data were acquired in two different cohorts in North America and Europe. Enrollment in the Simons Variation in Individuals Project (22) included referral by clinical genetic centers or web-based networks, or active online registration of families, while in the European 16p11.2 consortium the families were directly recruited by the referring physician.

Carriers were ascertained regardless of clinical diagnoses or age. The CNV carriers were either probands ($n = 76$) referred to the genetic clinic for the investigation of neurodevelopmental and psychiatric disorders, or their relatives (parents [$n = 49$], siblings [$n = 14$], and other relatives [$n = 10$]). Familial control subjects were relatives who do not carry a 16p11.2 CNV.

All families participated in a larger phenotyping project, as previously reported (10,13,20–22). Trained neuropsychologists performed all cognitive and behavioral assessments, including tests of overall cognitive functioning (nonverbal IQ [NVIQ]) (23–27) and phonological skills (standard score of the nonword repetition) (28,29). Participants also completed a broad screening measure of social impairment, the SRS (30). Experienced, licensed clinicians provided clinical DSM-5 diagnoses (31), using all information obtained during the research evaluation. NVIQ scores and psychiatric diagnoses were available for all participants. SRS total score was available for 77% of the participants (72 of 78 deletion carriers, 57 of 71 duplication carriers, and 149 of 212 control subjects), and phonological measures for 43% of the participants (56 of 78 deletion carriers, 19 of 71 duplication carriers, and 81 of 212 control subjects). Full description of cognitive and psychiatric assessment is available in the [Supplemental Methods and Materials](#).

We analyzed data from 78 16p11.2 (breakpoints 4–5) deletion carriers, 71 duplication carriers, 72 familial control subjects, and 140 unrelated control subjects, including data not previously analyzed at the regional level on 64 deletion carriers,

54 duplication carriers, 51 familial control subjects, and 138 unrelated control subjects. The latter were selected among volunteers from the general population who had neither a major DSM-5 diagnosis nor a relative with a neurodevelopmental disorder.

The study was approved by the institutional review boards of each consortium. Signed informed consent was obtained from the participants or legal representatives. Full description of participants is available in [Table 1](#), [Supplemental Table S1](#), and the [Supplemental Methods and Materials](#).

MRI Data Acquisition and Processing

The MRI data included T1-weighted (T1w) anatomical images acquired at seven sites using different 3T whole-body scanners: Philips Achieva (Philips Healthcare, Andover, MA) and Siemens Prisma Syngo and TIM Trio (Siemens Corp., Erlangen, Germany). Four sites used multiecho sequences for 264 participants (52 deletion carriers, 51 duplication carriers, 21 familial control subjects, and 140 unrelated control subjects), and three sites used single-echo sequences for 97 participants (26 deletion carriers, 20 duplication carriers, and 51 familial control subjects). Thirty-four scans were excluded from the analysis based on standardized visual inspection, which identified significant artifacts potentially compromising the accurate tissue classification and boundary detection (details in [Supplemental Methods and Materials](#)).

Surface-Based Morphometry. In FreeSurfer 4.5.0 (<http://surfer.nmr.mgh.harvard.edu>), each participant's T1w image was registered to a custom hybrid template consisting of 48 subjects (12 deletion children, 12 noncarrier children, 12 duplication adults, and 12 noncarrier adults) (21). Then, we used FreeSurfer's volumetric (32) and surface-based (33) algorithms with default settings. We estimated the total intracranial volume (eTIV) (34), global brain measures, cortical thickness, and surface area. The cortical thickness and surface area maps were resampled in fsaverage5 space and spatially smoothed with a Gaussian kernel of 8-mm full width at half maximum.

Voxel-Based Morphometry. In parallel we processed subjects' T1w data within the computational anatomy framework of SPM12 (<http://www.fil.ion.ac.uk/spm>). T1w images were classified in different brain tissue classes using the “unified segmentation” (35) and an enhanced set of brain tissue priors (36). Aiming at optimal spatial registration, we applied the diffeomorphic registration algorithm DARTEL (37) followed by a Gaussian spatial smoothing with 8-mm full width at half maximum. Of note, total intracranial volume computed by SPM is referred to as TIV.

Regions of interest were extracted using maximum probability tissue labels (<http://www.neuromorphometrics.com>) within SPM12 using data from the OASIS project (<http://www.oasis-brains.org>).

All MRI scanning parameters and processing are detailed in the [Supplemental Methods and Materials](#).

Data Analysis

Our whole-brain voxel-based morphometry (VBM) (38) analysis used a factorial design to test for gene dosage-related local

Table 1. Population Characteristics

	Deletion		Control		Duplication	
	EU (n = 25)	SVIP (n = 53)	EU (n = 83)	SVIP (n = 129)	EU (n = 23)	SVIP (n = 48)
Age, Years, Mean (SD), Range [Minimum–Maximum]	21.2 (14) [6.3–53.6 ^{a,b}]	13.8 (9.5) [6.7–48 ^c]	28.5 (12.6) [7.8–62.5 ^d]	24 (14.5) [6.1–63.4]	32.5 (13.3) [9.8–58.1]	30.1 (15.4) [6.3–63.1]
Male/Female, n	14/11	30/23	56/27	71/58	13/10	25/23
HC Z Score	n = 23	n = 52	n = 46	n = 27	n = 22	n = 48
Mean (SD)	0.28 (1.23) ^{a,b}	1.34 (1.2)	-0.21 (1.3) ^c	0.32 (1) ^c	-0.64 (1.9)	-1.08 (1.4)
NVIQ, Mean (SD)	81 (13) ^b	89 (14)	107 (16) ^c	103 (12) ^c	78 (18) ^b	89 (20)
SRS Raw Total Score	n = 20	n = 52	n = 25	n = 124	n = 11	n = 46
Mean (SD)	62 (32)	70 (37)	33 (17) ^c	19 (13) ^c	84 (40) ^b	57 (38)
Phonological Skills	n = 9	n = 47	n = 3	n = 78	n = 3	n = 16
Mean (SD)	4.8 (1.5)	5.5 (2.4)	12.3 (2.1)	8.4 (2.2) ^c	10.7 (1.5)	6.25 (2.2)

Phonology skills were evaluated in SVIP with the comprehensive test of phonological processing, nonword repetition subtest (29); and in Europe with the developmental neuropsychological assessment, nonword repetition task (28). Mean standard scores are shown. Low sample size did not allow statistics on phonological skills of the European (EU) cohort. HC, head circumference; NVIQ, nonverbal IQ; SRS, Social Responsiveness Scale; SVIP, Simons Variation in Individuals Project cohort.

^aSignificantly different between copy number variant carrier groups in the same cohort; analysis of covariance, post hoc comparison, $p < .05$ Bonferroni corrected.

^bSignificantly different from the same genetic group in the other cohort; analysis of covariance, post hoc comparison, $p < .05$ Bonferroni corrected.

^cSignificantly different from all the genetic groups in the same cohort; analysis of covariance, post hoc comparison, $p < .05$ Bonferroni corrected.

gray matter (GM) volume differences within the general linear model framework of SPM12 (39). SPM t maps were generated with a voxel-level threshold of $p < .05$ after familywise error correction for multiple comparisons over the whole GM volume using Gaussian random field theory (40). We generated Cohen's d maps from familywise error-corrected t scores to show the unbiased magnitude of the effects (Supplemental Methods and Materials).

Surface-based analyses tested regional differences in cortical thickness and surface area using linear models. For each vertex in the cerebral cortex surface mesh, we ran a multiple regression analysis. The vertexwise results were corrected for multiple comparisons at a false discovery rate of $q < .05$ (41,42) (Supplemental Methods and Materials).

The main effects of linear and quadratic expansions of age, sex, MRI site, and NVIQ were included as additional variables. The cubic expansion of age did not show any significant effect and was subsequently removed from all analyses. In an attempt to increase the power of our analyses we controlled for the effect of the seven scanning sites by introducing them as a random factor in a linear mixed model. This approach did not change the obtained results.

Z scores for global brain metrics were obtained in CNV carriers and familial control subjects using the adjusted measures from unrelated control subjects as the reference population.

Regional analyses were also corrected for the SPM estimate of TIV, the mean cortical thickness, or the total cortical surface area. In addition to the linear effect of gene dosage, we investigated the quadratic term to identify nonreciprocal effects of both CNVs. Post hoc analyses comparing deletion carriers and control subjects as well as duplication carriers and control subjects identified regions predominantly altered by each CNV.

We analyzed the interaction of the genetic groups with the regressors (age, sex, MRI site, NVIQ) as well as the MRI parameters (single-echo vs. multiecho) and three other clinical variables: SRS, phonological processing (nonword repetition), and psychiatric diagnoses. NVIQ did not show any significant effect and was removed from the analyses on the whole dataset. Dice index was computed to estimate the overlap between 16p11.2-related alterations and statistical maps obtained from a large cross-disorder neuroimaging meta-analysis (<http://anima.fz-juelich.de>) (43). We computed the rate of overlap between both maps. Finally, to motivate future hypotheses we relied on the Neurosynth database (<http://neurosynth.org>) to meta-analytically decode the functional association of the structural alterations observed in the gene dosage analyses of the 16p11.2 CNV carriers. All these analyses are detailed in the Supplemental Methods and Materials.

Linear models on global metrics and regions of interest were performed in R, version 3.2.5 (<http://www.r-project.org>; R Project for Statistical Computing, Vienna, Austria), and voxel- and surface-based analyses in MATLAB 2016b (The MathWorks, Inc., Natick, MA).

RESULTS

Demographics

We analyzed 78 deletion carriers, 71 duplication carriers, and 212 familial and unrelated control subjects (Table 1,

Supplemental Table S1), including new data on 138 individuals and data on 307 individuals not previously analyzed with whole-brain statistical methods. Age ranges from 6 to 63 years. Deletion carriers and control subjects from the Simons Variation in Individuals Project cohort are younger than the same groups in the European cohort, and deletion carriers overall are younger than the other groups. There is no significant difference in sex ratio across genetic groups and cohorts. Mean NVIQ is 81 and 89 in deletion carriers, and 78 and 89 in duplication carriers for the European and Simons Variation in Individuals Project cohorts, respectively. Ninety percent of the deletion carriers, 69% of the duplication carriers, and 25% of familial control subjects meet criteria for at least one psychiatric diagnosis. Twelve categories of diagnoses are recorded across the CNV carrier groups, including ASD in 13% of deletion carriers and 11% of duplication carriers (Table 2).

Global Brain Metrics

Head circumference Z scores (Table 1) and eTIV (Figure 1A) correlate negatively with the number of genomic copies of the 16p11.2 locus in both cohorts. Both GM and white matter total volumes contribute to this effect on eTIV (Figure 1B, C). The effect sizes on global brain metrics are up to 1 Z score for the deletion and approximately -0.4 Z score for the duplication (Supplemental Table S2). FreeSurfer and SPM estimates of

TIV, GM, and white matter are comparable across groups, cohorts, and MRI parameters (Supplemental Figure S1). Gene dosage preferentially affects cortical surface area and not thickness (Figure 1E, F). Of note, age-related thinning of cortical thickness is not significantly different between genetic groups (Supplemental Figure S2).

Regional Brain Differences Related to the 16p11.2 CNVs

In both cohorts, the whole-brain VBM analysis shows a negative relationship between the number of genomic copies at the 16p11.2 locus and the volume of several brain regions. Alterations with an effect size >1 Cohen's *d* (detected with a conservative power of 74.4% for family-wise error-corrected $p < .05$) include the bilateral anterior and posterior insula, transverse temporal gyrus, and calcarine cortex (Figure 2A, Supplemental Table S3). Regions with smaller volumes in deletion carriers compared with control subjects and duplication carriers include the bilateral precentral gyrus and middle and superior temporal gyri. Altered regions with smaller effect sizes are detailed in Supplemental Table S3.

There is a high degree of overlap between VBM findings with large effects and regional cortical surface area alterations, namely the insula, transverse temporal gyrus, and calcarine cortex (negative gene dosage), as well as the precentral gyrus

Table 2. DSM-5 Diagnoses

	Deletion		Familial Control Subjects		Duplication	
	EU (<i>n</i> = 25)	SVIP (<i>n</i> = 53)	EU (<i>n</i> = 45)	SVIP (<i>n</i> = 27)	EU (<i>n</i> = 23)	SVIP (<i>n</i> = 48)
Neurodevelopmental Disorders	3	5	–	–	4	6
Intellectual Disability						
Communication disorder	16	53	–	2	–	3
Autism spectrum disorder	–	10	–	1	2	6
Attention-deficit/hyperactivity disorder	2	12	2	4	1	7
Specific learning disorder	1	14	1	–	4	4
Motor disorder, tic disorder	–	26	–	1	1	9
Schizophrenia Spectrum and Other Psychotic Disorders	–	–	–	–	1	–
Bipolar and Related Disorders	–	–	–	–	1	–
Depressive Disorders	2	3	7	1	5	9
Anxiety Disorders	2	7	–	3	9	13
Obsessive-Compulsive and Related Disorders	1	1	–	–	1	2
Trauma and Stressor-Related Disorders	1	–	–	–	–	2
Elimination Disorders	5	14	–	2	1	2
Disruptive, Impulse-Control, and Conduct Disorders	1	5	–	–	–	–
Substance-Related and Addictive Disorders	3	–	–	–	1	–
Feeding and Eating Disorders	–	–	2	–	–	–
Other Conditions That May Be a Focus of Clinical Attention	–	9	–	–	–	9

From the DSM-5 (31).

A total of 20 of 25 European (EU) cohort deletion carriers (80%) had at least one psychiatric diagnosis: 11 had one diagnosis and 9 had several diagnoses (between two and five); 17 of 23 EU cohort duplication carriers (74%) had at least one psychiatric diagnosis: 7 had one diagnosis and 10 had several diagnoses (two or three); 9 of 45 familial control subjects (20%) had at least one psychiatric diagnosis: 6 had one diagnosis and 3 had two diagnoses. In the Simons Variation in Individuals Project (SVIP) dataset, 50 of 53 deletion carriers (94.3%) had at least one psychiatric diagnosis: 6 had one diagnosis and 44 had several diagnoses (between two and eight); 32 of 48 SVIP duplication carriers (66.6%) had at least one psychiatric diagnosis: 10 had one diagnosis and 22 had several diagnoses (between two and five); 9 of 27 familial control subjects (33%) had at least one psychiatric diagnosis: 4 had one diagnosis and 5 had two diagnoses. In both cohorts, unrelated control subjects without psychiatric diagnosis were recruited.

Effects of 16p11.2 Copy Number Variants on Neuroanatomy

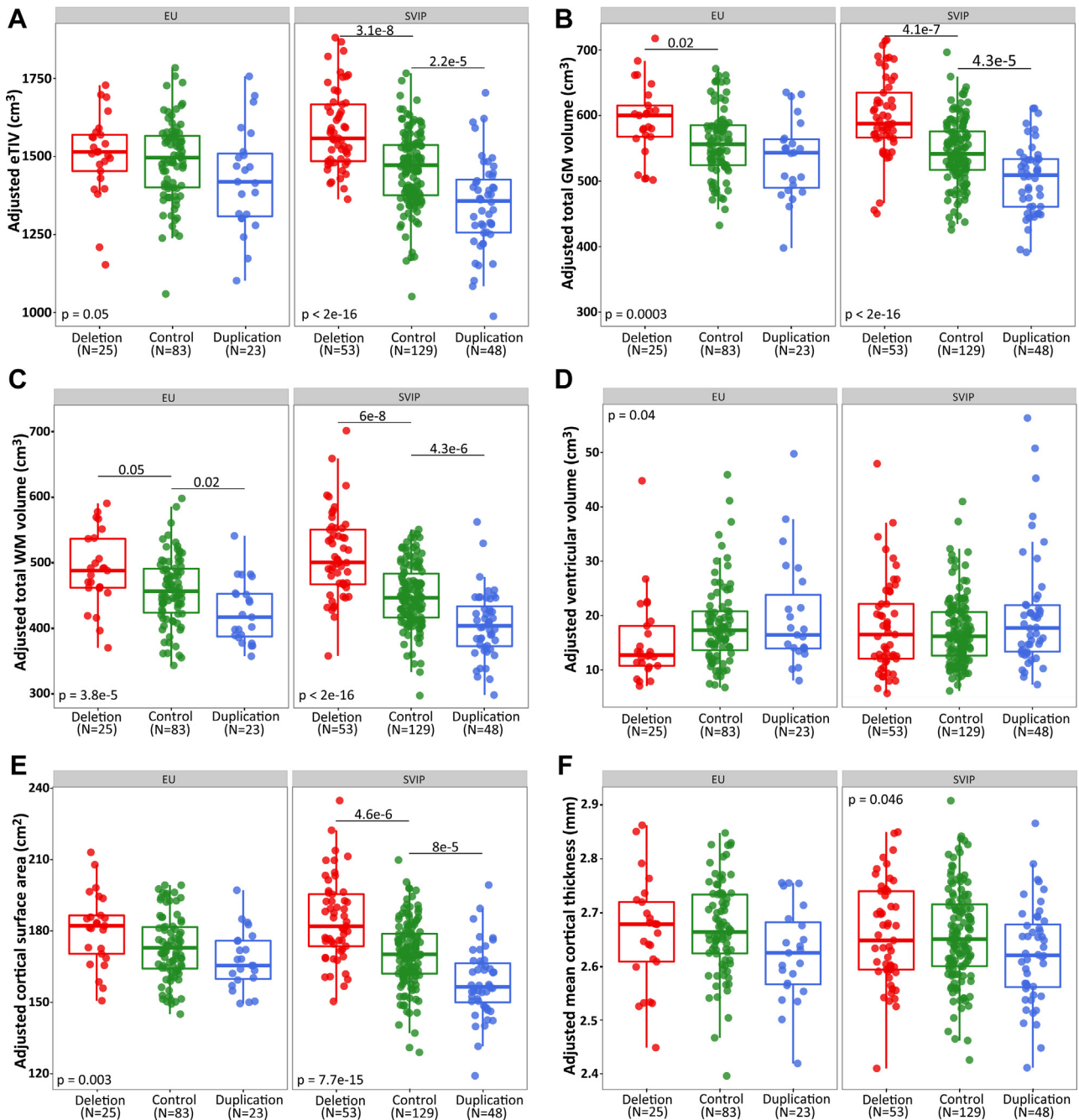


Figure 1. Effects of gene dosage on global brain measures in the European (EU) and Simons Variation in Individuals Project (SVIP) cohorts. Boxplots of (A) estimated total intracranial volume (eTIV), (B) gray matter (GM) volume, (C) white matter (WM) volume, (D) ventricular volume, (E) cortical surface area, and (F) mean cortical thickness in each genetic group separately for the EU and SVIP cohorts. Gene dosage effect is estimated with a linear model using the number of 16p11.2 genomic copies (1, 2, or 3), and including linear and quadratic expansions of age, sex, nonverbal IQ, and magnetic resonance imaging site as fixed covariates. In each box, the bold line corresponds to the median. The bottom and top of the box show the 25th (quartile 1 [Q1]) and 75th (quartile 3 [Q3]) percentiles, respectively. The upper whisker ends at the largest observed data value within the span from Q3 to $Q3 + 1.5 \times \text{interquartile range}$ ($Q3 - Q1$), and lower whisker ends at the smallest observed data value within the span for $Q1 - 1.5 \times \text{interquartile range}$. Circles that exceed whiskers are outliers. Post hoc comparisons show Bonferroni-corrected p values.

and superior and middle temporal gyri (positive gene dosage). Regions with smaller effect size and no overlap are shown in Figure 3; Supplemental Figure S3A, C; and Supplemental

Table S4. Cortical thickness, on the other hand, shows little overlap with the VBM results (Figure 3; Supplemental Figure S3B, D; and Supplemental Table S5).

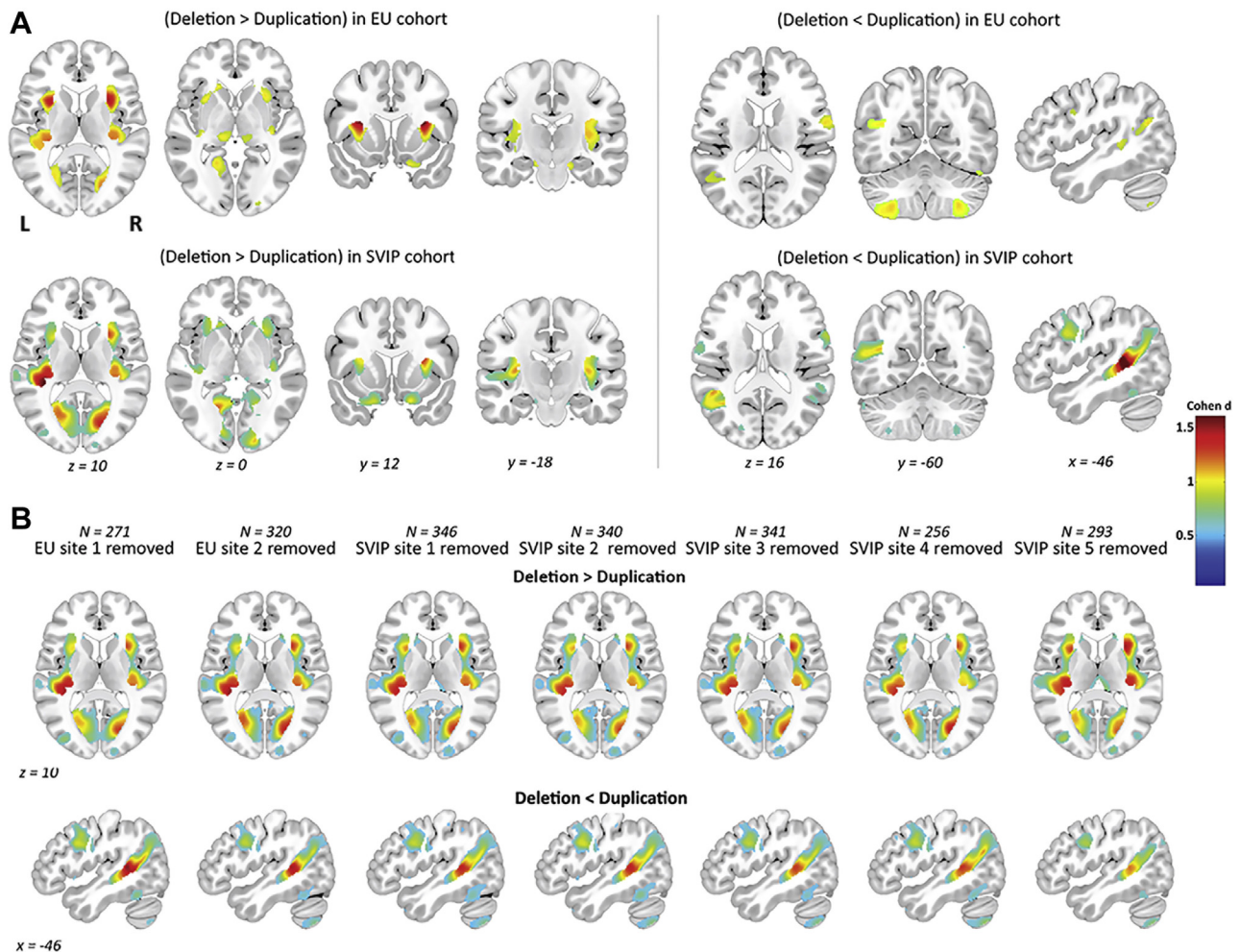


Figure 2. Effects of gene dosage on regional gray matter volume in the Europe (EU) and Simons Variation in Individuals Project (SVIP) cohorts. **(A)** Left panels (deletion > duplication) show voxel-based whole-brain maps, with the volumes of regions showing a negative relationship with the number of 16p11.2 genomic copies. Right panels (deletion < duplication) present the volumes of regions showing a positive relationship with the number of 16p11.2 genomic copies. **(B)** Negative and positive gene dosage effects on gray matter volume following a leave-one-out approach by systematically removing one of the magnetic resonance imaging sites. All the analyses are controlled for linear and quadratic expansions of age, sex, magnetic resonance imaging site, total intracranial volume, and nonverbal IQ. Results significant at a threshold of $p < .05$ familywise error corrected for multiple comparisons are displayed in standard Montreal Neurological Institute space. Color bars represent Cohen's d . L, left; R, right.

These regional results are not influenced by subjects' age, sex, cohort, MRI site, or MRI protocol (multiecho vs. single-echo): None of the variables shows an interaction with genetic groups (Figure 2B, Supplemental Figures S4 and S5A). In particular, a subgroup of participants who underwent both multi- and single-echo protocols presents the same alterations (Supplemental Figure S5B). NVIQ does not show any main effect on regional brain structure and was removed as a covariate for the subsequent analyses. Given the above observations, we pooled all data.

Relationship Between Total Brain Volume and Regional Differences

We examined the contribution of global differences to regional alterations. There was no relationship between global metrics and any of the aforementioned large effect regional findings,

even after adding GM volume as a covariate in the VBM analyses. We then tested for correlations between eTIV and the raw or adjusted volumes of some significant regions (Supplemental Figure S6). This demonstrates that small, average, or large brains contribute equally to the regional effects of 16p11.2 CNVs.

Mirror Effects Versus Differential Contribution of CNVs to Regional Differences

To differentiate reciprocal from nonreciprocal effects driven by either the deletion or the duplication carriers, we compared the linear and quadratic effects of gene dosage. The nonreciprocal effects of the 16p11.2 deletion and duplication identified by the quadratic term are detailed in Supplemental Figure S7. Post hoc analyses show that the deletion preferentially impacts the volume and surface area of the calcarine cortex and the

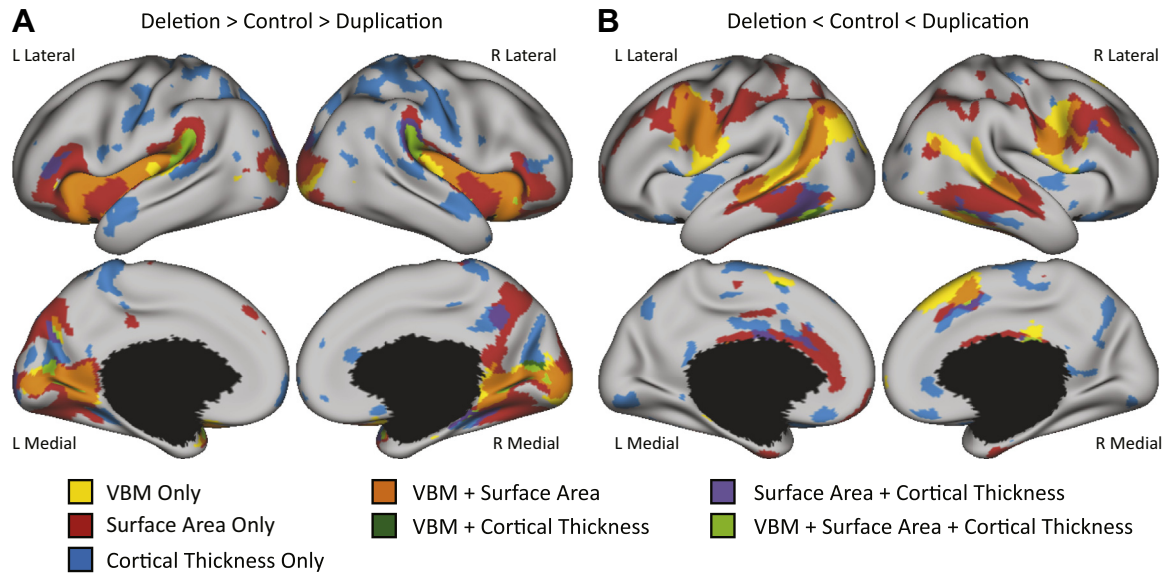


Figure 3. Overlap between voxel-based and surface-based results for cortical alterations associated with gene dosage. The relationship between gene dosage and the morphometric features was compared in the pooled sample ($n = 361$). The voxel-based and surface-based statistical maps are thresholded at the multiple comparisons–corrected p value and then projected on the cortical surface mesh. Regions with effect size ≥ 1 Cohen’s d and overlapping between voxel-based and surface-based analyses are (A) the bilateral insula, transverse temporal gyrus, calcarine cortex and (B) the precentral, superior and middle temporal gyri. L, left; R, right; VBM, voxel-based morphometry.

transverse temporal gyrus (deletion > control) and the superior and middle temporal gyri (deletion < control), with absolute effect size $>|1|$ Cohen’s d . The duplication carriers do not show any neuroanatomical differences with effect size $>|1|$ Cohen’s d . We observe GM volume changes in the caudate

and hippocampus with Cohen’s d between $|0.5|$ and $|1|$ (duplication < control) (Figure 4B, Supplemental Table S6). Differences with smaller effect sizes or identified only by one of the analytical methods such as alterations in the cerebellum, precentral gyrus, and cingulate are detailed in the

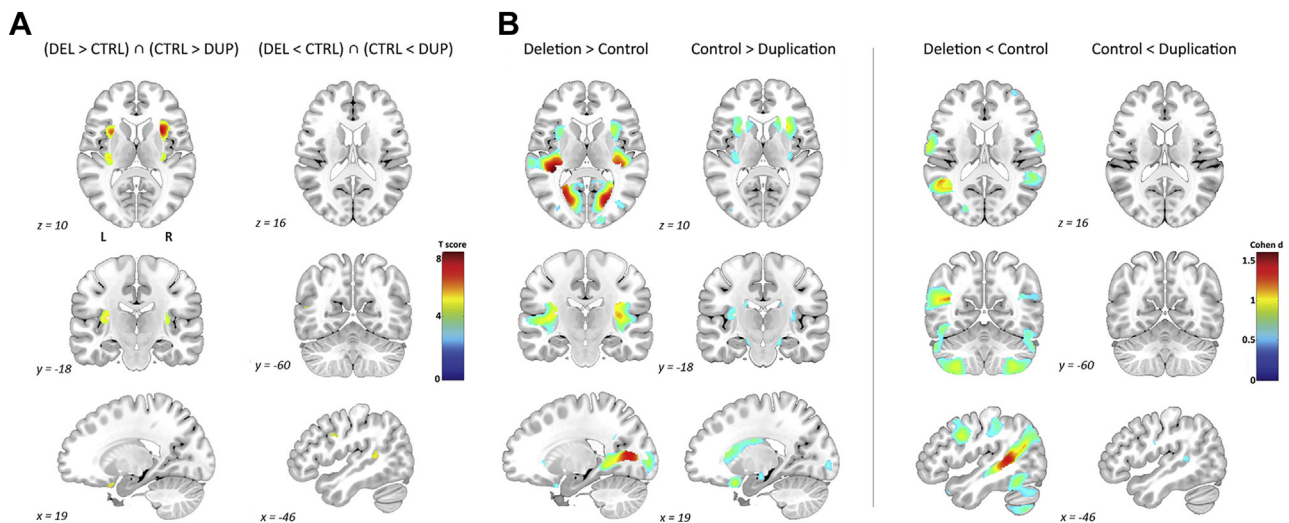


Figure 4. Differential and overlapping contribution of deletion and duplication to the regional gray matter volume differences. (A) Results of voxel-based whole-brain maps from the conjunction analysis of both negative (deletion > control AND control > duplication) and positive (deletion < control AND control < duplication) gene dosage. The main mirror pattern is the insula. (B) Results of voxel-based whole-brain maps showing the effect size in regions with larger volume in deletion carriers compared with control subjects (deletion > control), in control subjects compared with duplication carriers (control > duplication), in regions with smaller volume in deletion carriers compared with control subjects (deletion < control), and in control subjects compared with duplication carriers (control < duplication). Results significant at a voxel-level threshold of $p < .05$ familywise error corrected for multiple comparisons are displayed in standard Montreal Neurological Institute space. Color bars represent t scores for panel (A) and Cohen’s d for panel (B). CTRL, control individuals; DEL, deletion carriers; DUP, duplication carriers; L, left; R, right.

Supplemental Table S6 and Supplemental Figures S8A–D and S9C–F.

The reciprocal mirror effects of the 16p11.2 deletion and duplication are restricted to the bilateral insula. The post hoc conjunction analysis shows that the deletion is associated with an increase of the volume and surface area of the insula, and the duplication is associated with a decrease of this region (Figure 4A, Supplemental Table S6). We do not observe reciprocal effects of gene dosage for cortical thickness measurements (Supplemental Figure S9A, B).

Relationship With Psychiatric Diagnosis and Cognitive Traits

Because the 16p11.2 locus is associated with more than one psychiatric diagnosis, we quantified the overlap of our findings with a large, cross-disorder neuroimaging meta-analysis [<http://anima.fz-juelich.de> (43)]. We observe that the 16p11.2-related VBM map overlaps 33% of the meta-analytic map (Dice index): 46% for the cluster including the left insula, 28% for the right insula, and none for the dorsal anterior cingulate cortex.

We used Neurosynth to meta-analytically decode the psychological terms most closely associated with the main anatomical clusters identified in the VBM analysis. Supplemental Table S7 illustrates the domains most associated with each cluster. The transverse, superior, and middle temporal gyri (regions predominantly affected in deletion carriers) show top associations with language, phonology, and auditory terms. The anterior insula and caudate (alterations found in duplication carriers) are associated with terms such as reward, pain, and executive function (Supplemental Figure S10). Recognizing such inverse inferences can provide hypotheses for future studies but are unable to support strong conclusions.

However, the measures of NVIQ, SRS, and phonological processing measured in participants do not show main effects or interact with the gene dosage effects. The presence of low general intelligence (NVIQ), language impairment (measured by phonological processing), or poor social skills (SRS), or the presence and number of comorbid psychiatric diagnoses, does not change any of the neuroanatomical findings associated with the 16p11.2 deletion or duplication.

Ascertainment and Additional Factors Contributing to Changes in Brain Structure

We tested whether ascertaining carriers for neurodevelopmental symptoms could bias our results. Because clinical ascertainment may enrich as well for additional neurodevelopmental factors present in CNV carriers and their families, we investigated potential brain alterations in the family members who do not carry a 16p11.2 CNV. Comparing control subjects from deletion families ($n = 51$) and unrelated control subjects shows changes in volume and thickness with medium effect size (>0.5 Cohen's d) of the left posterior insula and right lingual gyrus; changes of volume also include the putamen and hippocampus (Figure 5, Supplemental Figure S14E). No effect was found for the cortical surface area (Supplemental Figure S13E).

However, comparing deletion or duplication carriers with familial or unrelated control subjects does not change any of the global (Supplemental Table S2 and Supplemental Figure S11) or regional findings reported above (Supplemental Figures S12, S13A–D, and S14A–D).

DISCUSSION

This large, multisite dataset combines new and previously published data to expand our understanding of the neuroanatomical differences associated with 16p11.2 deletions and

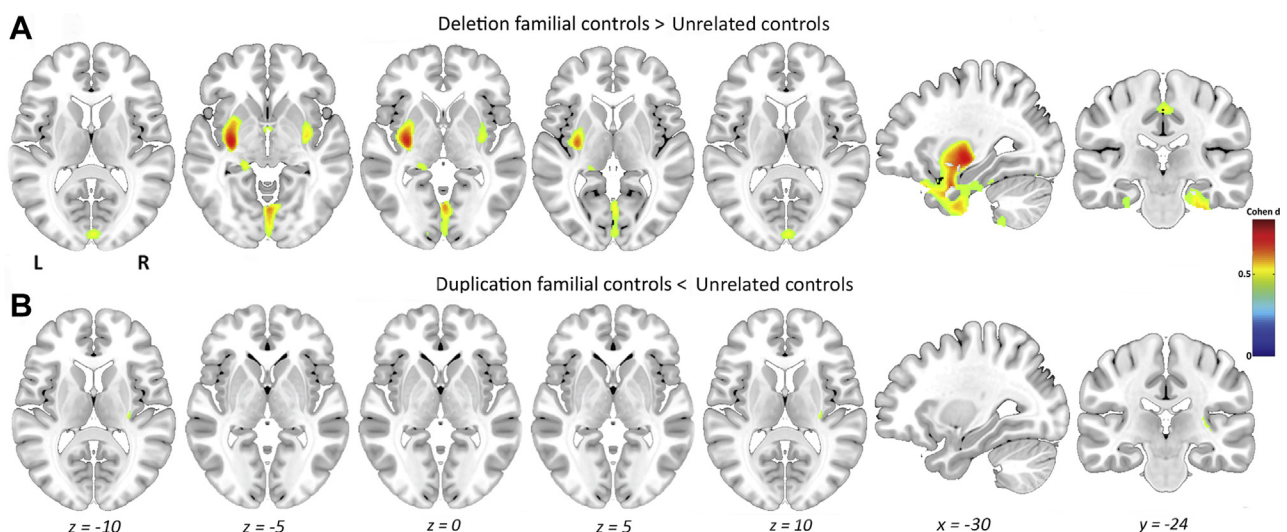


Figure 5. Contribution of familial control subjects to the regional gene dosage-dependent gray matter volume differences. Results of voxel-based whole-brain maps showing (A) regions with larger volume in control subjects from deletion families ($n = 51$) compared with unrelated control subjects ($n = 140$); and (B) regions with smaller volume in control subjects from duplication families ($n = 21$) compared with unrelated control subjects ($n = 140$). Results significant at a voxel-level threshold of $p < .05$ familywise error corrected for multiple comparisons are displayed in standard Montreal Neurological Institute space. Color bars represent effect size (Cohen's d). L, left; R, right.

duplications. The effect of the reciprocal CNVs on brain structure is generalizable across heterogeneously ascertained cohorts and remains significant beyond differences in MRI scanners, imaging protocols, analysis with two complementary computational methods, sex, age, and presence and number of comorbid psychiatric diagnoses. We extend previous neuroimaging studies by characterizing the reciprocal and differential effect of deletions and duplications on brain structure. While 16p11.2 deletions and duplications impact reciprocally bilateral insula [a gateway for sensory interoception, self-recognition, and emotional awareness (44)], differences in other brain areas are predominantly associated with either CNV.

Recent publications have questioned the reliability of neuroimaging studies that are prone to both type I and II errors (45). Our results provide robust estimates for CNV effect sizes on brain structure. Our sample size is adequate to detect the large effects associated with both CNVs, greatly reducing the probability of spurious findings. In imaging genetics, it has often been assumed that genetic variants may have larger effects on imaging phenotypes than on clinical traits or psychiatric risk (45). Our study shows, however, that the effect size of CNVs on brain structure is similar to their effect previously published for cognitive and behavioral traits (13,18). The effect of the deletion is approximately twice that of the duplication for global and regional brain volumes as well as clinical traits (such as IQ loss) (13).

The brain regions showing gene dosage effects are implicated in phonology, language, reward, and executive function networks. These are diverse functions that are each complex and heterogeneous. Nonetheless, the associations raise hypotheses for future studies. Similarly, the spatial overlap between our findings and the meta-analytical results performed across all Axis I psychiatric diagnoses from the DSM-IV-TR (43) may provide clues to pathological patterns underlying the risk for psychiatric diagnoses conferred by 16p11.2 CNVs.

The effects of CNVs on brain structure are not changed by ascertainment for either neurodevelopmental or psychiatric symptoms. Differences in IQ, language ability, or social responsiveness or the presence and number of psychiatric diagnoses do not influence any of the findings. We have previously reported a similar observation for cognition showing that the 16p11.2 deletion is associated with a decrease in IQ of 25 points regardless of whether carriers have intellectual disabilities or intelligence in the normal range (13).

This observation is consistent with an additive model underlying psychiatric disorders (46). Under this assumption, brain alterations associated with CNVs contribute to, but do not necessarily correlate with, a psychiatric diagnosis because additional brain alterations or other factors are required for the onset of the disorder. This is in agreement with studies demonstrating that GM changes in the superior temporal gyrus, insula, and cingulate are observed in individuals both diagnosed with psychosis and at high risk for developing psychosis (47).

Contrasting familial and unrelated control subjects reveals regional differences partially overlapping with the 16p11.2 gene dosage alterations. Of note, these alterations involve cortical thickness as opposed to CNV-related cortical surface

changes. This may suggest the presence of additional factors in these families ascertained in the neurodevelopmental clinic. Assortative mating in families (in particular when the CNV is inherited) may also contribute to an increase of risk factors (48).

We are not implying that our findings are specific to the 16p11.2 locus. Differences in global and local GM volumes as well as surface and thickness have been observed in similar regions in 22q11.2 deletion carriers, another large-effect-size genetic risk factor for psychiatric conditions (49–51). They are also reminiscent of decreased regional volumes in brain areas associated with emotion and face processing demonstrated in individuals with a 7q11.23 deletion (52,53). It is still unclear whether these shared alterations in brain structure relate to similar changes in tissue properties and underlying molecular mechanisms, but they may suggest neuroanatomical convergence across different genetic risk factors. This is illustrated by a study of several genetically modified mouse models of ASD and intellectual disability showing that their regional neuroanatomical alterations can be grouped in three different clusters (6).

Limitations

The broad age range of our dataset (6–63 years of age) is a potential limitation. However, we did not find any interaction between age and effects of gene dosage. The global and regional alterations remain unchanged in age-specific subgroups, with the caveat of a significant decrease in power (Supplemental Figures S2 and S4A). The developmental onset of global and regional differences in 16p11.2 CNV carriers remains unknown, but the insula, striatum, and thalamus are also altered in a 7-day-old 16p11.2 deletion mouse model (54,55), suggesting an early developmental effect. However, specific anatomical effects are difficult to interpret between humans and mouse models. The multisite data represents another limitation and can introduce false-positive findings. However, investigating the impact of sites using main as well as random effects did not identify any biases introduced by the different scanners: this means that the effect of the CNV may be more important than the noise introduced by the multiple MRI sites. Finally, the missing clinical data may limit our power to detect correlations between the brain morphometric measurements and the cognitive and clinical data.

The strong results of this multisite genetic-first neuroimaging study provide a robust characterization of 16p11.2 deletion and duplication effects on neuroanatomy. The deletion and duplication of the same genetic interval may affect brain regions in opposing ways, but other structures are preferentially altered by one of the two CNVs. The morphometric effect sizes are comparable to those previously recorded on cognitive traits. Results are generalizable across sites, computational methods, age, sex, and ascertainment for psychiatric or neurodevelopmental disorders. This suggests that these brain alterations are related to the risk conferred by the CNVs rather than the clinical manifestations observed in carriers. This highlights the relevance of studying genetic risk factors as a complement to groups

defined on the basis of behavioral criteria. Future longitudinal studies are required to establish the onset of these alterations.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by Simons Foundation Grant Nos. SFARI219193 (to RLB) and SFARI274424 (to AR); a Bursary Professor fellowship of the Swiss National Science Foundation (SNSF) (to SJ); SNSF Grant No. 31003A160203 (to AR); SNSF Sinergia Grant No. CRSII33-133044 (to AR); SNSF National Centre of Competence in Research Synapsy and project Grant No. 32003B_159780 (to BD); Foundation Parkinson Switzerland and Foundation Synapsis (to BD); European Union Seventh Framework Programme (FP7/2015-2018) Grant No. 604102 (Human Brain Project) (to BD); the Roger De Spoelberch and Partridge Foundations; a Canada Research Chair in neurodevelopmental disorders (to SJ), and a chair from the Jeanne et Jean Louis Levesque Foundation (to SJ). This research was enabled by support provided by Calcul Quebec (<http://www.calculquebec.ca>) and Compute Canada (<http://www.computeCanada.ca>).

We thank all of the families at the participating Simons Variation in Individuals Project (VIP) sites, as well as the Simons VIP Consortium. We appreciate obtaining access to imaging and 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>. Statistical support was provided by data scientist Steven Worthington, at the Institute for Quantitative Social Science, Harvard University. We are grateful to all families who participated in the 16p11.2 European Consortium.

The authors report no biomedical financial interests or potential conflicts of interest.

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Received Oct 18, 2017; revised Feb 1, 2018; accepted Feb 24, 2018.

Supplementary material cited in this article is available online at <https://doi.org/10.1016/j.biopsych.2018.02.1176>.

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