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Appendix E1

Inclusion and Exclusion Criteria

Individuals with 16p11.2 CNVs were recruited in various ways, including referral by clinical genetic centers or testing laboratories, web-based networks, or by self-referral of families who learned about the Simons VIP project (*http://SimonsVIPconnect.org*) with the initial goal of recruiting 100 deletion and 100 duplication carriers. Unaffected family members were invited to participate in the study and 109 population controls were recruited from the community. Informed consent was obtained from all individuals participating in the study according to the Committee on Human Research requirements at each individual institution, which included permission to perform cross-site evaluation of the data. Included in the consent was permission to make all the MR images and cognitive and behavioral testing data available for download upon approval from the Simons Foundation (*https://sfari.org/resources/sfari-base*).

Initial screening and review of medical records occurred at Emory University, a member institution of the larger Simons VIP consortium. Only participants with recurrent breakpoints at BP4–BP5 of 16p11.2 without other pathogenic CNVs or other known genetic diagnoses or syndromes were invited to enroll into the imaging portion of the Simons VIP. In the deletion carriers, we found 44 de novo mutations, 15 inherited mutations, two germline mutations, and 18 individuals with unknown status. In the duplication carriers, we found 11 de novo mutations, 45 inherited mutations, and 23 individuals with unknown status. We found ascertainment bias in the age distributions of the deletion and duplication carriers. The proband in each family was a child because children with developmental delays are referred to neurologists and identified as a 16p11.2 deletion or duplication carrier as a result of genetic testing. Cascade testing in the family of a duplication proband was much more likely to reveal other family members (eg, siblings and parents) with the duplication, while cascade testing rarely reveals a family member with a deletion, which reflects different patterns of inherited versus de novo mutations. As such, our cohort of deletion carriers predominantly consists of children, while the duplication cohort is composed of children and their affected family members, including adults.

Individuals or their family members were excluded if they were not fluent in English, or had a history of environmental insults that could affect neurocognitive status, such as fetal alcohol syndrome, birth asphyxia, or prematurity. Population controls with any major DSM-IV diagnosis (on the basis of clinical psychologist review) or with a history of ASD, other developmental disorders, dysmorphic features, or genetic abnormalities in a first-degree relative were excluded. Population control participants were also excluded for evidence of psychiatric or developmental disorder including the following: a Symptom Checklist 90 (known as SCL-90) score greater than 62, an axis 1 psychiatric diagnosis from the the Diagnostic Interview for Children, severe substance abuse, elevations on behavioral indexes (the Adult Behavior Checklist or the Child Behavior Checklist) in the presence of clinician suspicion of psychiatric disorder, or if cutoffs on the Broader Autism Phenotype Questionnaire were met and clinician suspected ASD. Population control subjects also had a chromosome microarray, neurologic examination, a photograph evaluation for dysmorphology of anatomic features, and a clinical psychologist review.

Image Acquisition

No individuals were sedated at MR imaging. The youngest children, younger than 3 years (10 participants), were imaged at times that coincided with their afternoon sleep schedule, which permitted the acquisition of high quality images with limited motion. MR imagers across the five sites were matched for resolution and regularly calibrated to ensure consistent data acquisition by using the Alzheimer's Disease Neuroimaging Initiative and Biomedical Informatics Research Network phantoms and Owl Image monitoring system. Temporal changes were limited: system distortions and nonlinearity and signal-to-noise ratios were tracked and were stable across the study duration. Baseline imaging in a single traveling volunteer was performed at all sites to assure protocol consistency (interpreted by a study radiologist) before enrollment was initiated. ME–magnetization-prepared rapid gradient-echo T1-weighted, T2-weighted SPACE and T2-weighted fluid-attenuated inversion recovery images were acquired with a manufacturer-supplied 16-channel coil by using a 3-T MR imager (Tim Trio; Siemens) at Waltham's Hospital in Boston, and a 32-channel coil system by using a 3-T MR imager (Tim Trio; Siemens) at the University of California, Berkeley (affiliated with the University of California, San Francisco clinical site) and the Children's Hospital of Philadelphia. A three-dimensional inversion-recovery-turbo-field echo T1-weighted image (essentially a ME–magnetization-prepared rapid gradient-echo), three-dimensional T2-weighted image, and three-dimensional fluid-attenuated inversion recovery image was obtained by using a manufacturer-supplied 16-channel coil by using a 3-T MR imager (Achieva; Philips Healthcare, Amsterdam, the Netherlands) at University of Washington and Baylor. The imaging parameters were matched across sites on the basis of the Alzheimer's Disease Neuroimaging Initiative standard. The parameters for the sequences for the Siemens and Phillips sites are presented in Table E1.

Radiological Review

At least two neuroradiologists reviewed the images for each participant. If there was a discrepancy between the two interpretations, a third neuroradiologist performed a read, then all three neuroradiologists and a pediatric neurologist came to a consensus on the results. The images in population control participants were interpreted by two of the neuroradiologists identified in the Materials and Methods sections, and the images in familial noncarriers were interpreted by all three of the neuroradiologists to ensure that the radiologic features were not part of the background familial genetics of the carrier groups. Fifty-three images in the 75 deletion carriers were read twice, and the remaining 22 images were read three times to resolve disagreement between the initial two reads. Likewise, 51 images in the 71 duplication carriers were read twice and the remaining 20 images in duplication carriers were read three times.

Checklist for Radiological Reads

The checklist of development-related features included the following. Unless otherwise noted, these were subjective assessments based on the experience of the pediatric neuroradiologist:

1. Thick corpus callosum (dysmorphic, ie, lacking the morphologic structure of the typical corpus callosum and thicker in appearance)

2. Thin corpus callosum volume (dysmorphic, ie, lacking the morphologic structure of the typical corpus callosum and thinner in appearance)

3. Pituitary abnormality (abnormally large pituitary, small pituitary, or ectopic posterior pituitary)

4. Brainstem abnormality (abnormal volume or morphologic structure)

5. Cerebellar tonsillar ectopia (defined as ≤5 mm below foramen magnum, measured by drawing a line from the inner margins foramen magnum (basion to opisthion) and measuring the inferior-most part of the tonsils)

6. Chiari 1 malformation (defined as >5 mm below foramen magnum measured by drawing a line from the inner margins foramen magnum [basion to opisthion] and measuring the inferior-most part of the tonsils and with or without syrinx in visualized upper cervical spinal cord)

7. Decreased cerebellar hemisphere volume

8. Decreased cerebellar vermis volume

9. Craniocervical junction abnormality (too narrow or crowded, or abnormal morphologic structure)

10. Platybasia

11. Dens abnormality (abnormal volume or morphologic structure)

12. Cortical malformation (such as gray matter heterotopia, polymicrogyria, or focal cortical dysplasia)

13. Enlarged ventricles

14. Basal ganglia or thalamus abnormality (abnormal volume or morphologic structure)

15. Hippocampal abnormality (abnormal volume or morphologic structure)

16. Decreased white matter volume

Table E1. Image Acquisition Parameters for Various Sequences

Parameter	3-T Siemens Tim Trio			3-T Phillips Achieva		
	T1	T2	TFLR	T1	T2	TFLR
Sequence name	MP-RAGE	T2 VISTA	3D TFLR	IR-TFE	T2	3D TFLR
Sequence type	3D IR-GRE	3D TSE	3D IR-TSE	3D IR-GRE	2D TSE	3D IR-TSE
Orientation	Sagittal	Sagittal	Sagittal	Sagittal	Transverse	Sagittal
FOV-frequency	256	256	256	256	256	256
FOV-phase	240	240	240	93.8*	93.8*	100*
Acquisition matrix	256 × 256	256 × 256	256 × 256	256 × 256	256 × 256	256 × 256
Pixel size-Frequency (mm)	1	1	1	1	1	1
Pixel size-phase (mm)	1	1	1	1	1	1
Section thickness (mm)	1	1	1	1	1	1
Section gap (mm)	0	0	0	0	0	0
No. of sections	160	160	160	160	160	160
TR (ms)	2300	2500	6000	2300	3200	6000
TE (ms)	3	249	323	2.98	402	388
TE2/TEeff	NA	100	151	NA	NA	NA
Flip angle (deg)	9	90	90	9	90	90

TI	900	NA	2100	900	NA	2100
Turbo/EPI factor	192	78	141	NA	7	133
Profile order	Linear	Linear	Linear	Linear	Special	Linear
Halfscan	No	No	0.8 × 0.8	NA	NA	0.875
Bandwidth	240	400	500	240	751	781
No. of averages	1	1	1	1	1	1
Concatenations	1	1	1	1	1	1

Note.—3D = three dimensional, FOV = field of view, EPI = echo planar imaging, GRE = gradient-recalled echo, IR = inversion recovery, MP-RAGE = magnetization-prepared rapid acquisition gradient echo, TE = echo time, TEeff = effective echo time, TE2 second echo time, TFE = turbo field echo, TFLR = turbo fluid attenuated inversion recovery, TI = inversion time, TR = repetition time, TSE = turbo spin echo, VISTA = volume isotropic turbo spin-echo acquisition.

*Data are percentages.

Table E2. Deletion Carriers and Population Controls Removed from Larger Cohort

Parameter	Deletion Carriers	Population Control Participants	*P* Value, Deletion vs Population Control Participants	Duplication Carriers	Familial Non-Carriers	*P* Value, Duplication vs Familial Non-Carrier
Thick Corpus Callosum	10/56	0/50	<.0014*	1/48	0/47	>.999
Thin Corpus Callosum	1/56	0/50	>.999	11/48	1/47	.0037*
Cerebellar Tonsillar Ectopia	18/56	3/50	<.011	2/48	5/49	.44
Chiari I Malformation	5/56	0/50	.059	0/48	2/49	.50
Decreased Cerebellar Hemisphere Volume	1/56	5/50	>.999	5/48	2/49	.27
Decreased Cerebellar Vermis Volume	4/55	0/50	.12	7/46	1/48	.029
Craniocervical Junction Abnormality	17/55	1/50	<.001*	2/46	2/47	>.999
Platybasia	6/51	1/50	.112	2/48	0/49	.25
Dens Abnormality	12/52	0/50	<.002*	3/47	4/45	.71
Enlarged Ventricles	1/56	0/50	>.999	15/48	1/49	<.001*
Decreased White Matter Volume	1/55	0/50	>.999	14/48	0/49	<.001*

Note.—Data are numerator and denominator unless otherwise mentioned. Duplication carriers and familial noncarriers were removed from the larger cohort until age ($P < .093$) and sex ($P < .84$) were matched, resulting in 49 duplication carriers and 49 familial noncarriers. Frequencies of the 11 salient radiologic findings from Table 2 were reassessed for difference in frequency by using $\rho < 0.05$ ($P < .0045$) in the subgroups generated.

*$Q < 0.05$.

Table E3. Volumetric Results Obtained from FreeSurfer that Corraborate the Qualitative Radiologic Assessments of White Matter and Cerebellar Volume Decreases

Parameter	Mean No. of Deletion Carriers ($10^4 \times mm^3$) ($n = 49$)	Mean No. of Duplication Carriers ($10^4 \times mm^3$) ($n = 55$)	Mean No. of Familial Non-Carriers ($10^4 \times mm^3$) ($n = 36$)	Mean No. of Population Control Participants ($10^4 \times mm^3$) ($n = 75$)	*P* Value, Deletion vs Familial Non-Carrier	*P* Value, Deletion vs Population Control	*P* Value, Duplication vs Familial Non-Carrier	*P* Value, Duplication vs Population Control
White Matter Volume	47.0 ± 8.5	38.3 ± 8.4	40.9 ± 8.0	46.7 ± 6.1	.0007*	.03	2.09e-6*	5.11e-13*

Cerebellar Volume	15.2 ± 1.6	12.8 ± 1.7	14.5 ± 1.5	14.2 ± 1.3	.056	.008*	7.80e-3*	1.42e-7*

Note.—Data are ± standard deviation unless otherwise mentioned. FreeSurfer was performed on the T1-weighted structural images with sufficient image quality; the quality control procedure is detailed in Qureshi et al (20). An analysis of covariance adjusted for age and sex covariates was performed.

* $Q < 0.05$.