Topographic Shifts in Functional Connectivity and Reduced Lateralization in 16p11.2 Deletion Carriers: A Genetics-First Approach to Understanding Autism

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Keywords: autism spectrum disorder, functional connectivity, brain networks, brain lateralization

Posted Date: May 13th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-28618/v1>

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Abstract

Background: The study of autism has been confounded by genetic and etiologic heterogeneity. The current study utilized a genetics-first approach to investigate the underlying neurobiology of autism by studying individuals with copy number variation at 16p11.2. Our aim was to investigate the prevailing theories of brain connectivity in autism – specifically, in regard to (1) distributed brain networks, (2) local and distant connectivity, and (3) functional lateralization.

Methods: We analyzed resting-state functional connectivity MRI acquired in 26 carriers of a 16p11.2 deletion and 42 age-matched control participants. We also compared the functional connectivity metrics with measures of language ability, IQ, and social behavior.

Results: We do not find widespread disruption of canonical large-scale networks in the deletion carriers. Nor do we find quantitative differences in the degree of local and distant connections. Instead, we discover unique connections in 16p11.2 deletion carriers that are not present in the control participants. Specifically, functional coupling between auditory cortex and regions of the default network is present only in the deletion carriers. In addition to the topographic shifts in functional connectivity, we observe reduced right hemispheric lateralization in the deletion carriers and less left hemispheric lateralization in individuals with poorer language ability.

Conclusions: Links or connections between primary sensory areas and higher-order association areas violate fundamental large-scale circuit properties by functionally connecting brain regions specialized in local (hierarchical) processing with those that specialize in distant (parallel) processing. Aberrant hemispheric lateralization and connections between auditory and default networks may underlie difficulties with language and social interactions.

Background

The last decade began with the field of autism brain research coming under scrutiny for divergent results and lack of reproducibility across studies. This sentiment was captured by a prominent review article at the time, advocating to reconceptualize the disorder as “the autisms” in order to account for this plurality [1]. In response, recent efforts have focused on identifying sources of variance, to again, reach a unified neurobiological model for autism spectrum disorder (ASD). An impressive grassroots consortium, the autism brain imaging data exchange or ABIDE, addressed the high variability that can arise from low power, and aggregated data across many prior studies [2]. Müller and colleagues identified methodological choices as a major source of heterogeneous results [3]. Others have reconciled conflicting data through changes that occur through development [4, 5]. Another source of variance in ASD is the extensive genetic and etiologic heterogeneity. At least 71 ASD risk loci and 65 risk genes have been reported [6], but none of the known causes of ASD account for more than 1-2% of cases [7].

To address this source of heterogeneity the Simons foundation pioneered a genetics-first approach to ASD [8]. *De novo* copy number variations (CNVs) may account for up to 15% of sporadic cases of autism

[9]. 16p11.2 is among the most frequently occurring CNV associated with ASD, at 0.76% (95% CI, 0.51-1.12) [10]. The locus contains MAPK3, a key regulator downstream from other ASD targets, but like other large *de novo* CNVs 16p11.2 likely contains multiple modest-effect ASD-risk genes [6, 11]. Carrying a 16p11.2 deletion increases the odds of developing autism or developmental delay by 38.7-fold (95% CI, 13.4-111.8), and the duplication increases the odds by 20.7-fold (95% CI, 6.9-61.7)[12]. Accordingly, Simons VIP has first focused its efforts on the effects of deletion and duplication carriers of the recurrent ~600 kb BP4-BP5 16p11.2 CNV. While variable expressivity and incomplete penetrance are not rare, but are actually characteristic of ASD-risk loci, including 16p11.2 [13], the advantage of a genetics-first approach is that it is better able to isolate the contributions of a particular ASD-risk locus to a resulting phenotype. The present study investigates the resting-state functional connectivity of individuals with this copy number variation.

Focus has shifted from explaining autism as a localized brain disorder of particular structures to one involving higher-order association areas as a large-scale neural systems disorder [1, 14]. This paradigm shift is concordant with neuropsychological testing. Basic information processing is intact (or even enhanced), whereas tasks that place high demand on information integration are impaired [14]. Frith first synthesized the core problem under a unified cognitive model of “weak central coherence” [15], which was reframed in terms of functional connectivity as the theory of “underconnectivity” [16] and more recently the “dysconnectivity” model [2, 5]. In other words, ASD may spare brain regions that involve local specialization (segregation) while targeting highly distributed large-scale networks involved in global coherency (integration). Nonetheless, there have been conflicting results across studies, which have variously shown hyper- and hypoconnectivity [17] (see full reviews [3, 18]).

In this study, our aim was to investigate these prevailing theories of connectivity in the neurobiology of ASD from the benefit of studying a clinically ascertained cohort that carries only a single known ASD-risk CNV. The present study analyzed both intra-hemispheric and interhemispheric functional connectivity in deletion carriers of the 16p11.2 CNV. Our results converge on large-scale brain networks involved in social cognition and communication.

Methods

Participants

Details on the large-scale recruitment and screening of the carriers of the 16p11.2 CNV carriers and control participants have been described previously [8, 19]. Carriers of the 16p11.2 CNV were enrolled only if recurrent breakpoints occurred at BP4-BP5, and no other pathogenic CNVs or other known genetic diagnoses or syndromes were found. The initial level of the study focused on phenotypic assessment. This included the Autism Diagnostic Observation Schedule (ADOS), Autism Diagnostic Interview (ADI), broad screening measures of social impairment such as the Social Responsiveness Scale (SRS), Vineland-II, and Social Communication Questionnaire, as well as general measures of cognitive abilities

with the Differential Ability Scales-II (DAS-II) and Wechsler Individual Achievement Test (WIAT-III) among others.

A subset of these participants who were eight years and older and able to tolerate an MRI scan were recruited to the second level of assessment focused on neuroimaging. Data acquisition occurred at two imaging core sites: University of California sites (UC) and Children Hospital of Philadelphia (CHOP). All aspects of the study at UC were performed at University of California – San Francisco, except for scanning, which was performed at University of California – Berkeley to maintain conformity with the CHOP scanner. Participants had structural MRI, resting-state functional MRI, task-based functional MRI, diffusion-weighted imaging and magnetoencephalography collected on matched scanner platforms. Control participants were recruited directly by the two imaging core sites (CHOP and UC), screened for ASD, neuropsychiatric disease [19], and matched for age, sex, handedness, and non-verbal IQ. Additional neuropsychiatric assessments were completed during the neuroimaging visits. The battery included the DIAS-II, WIAT-III, Wechsler Abbreviated Scale of Intelligence (WASI), Clinical Evaluation of Language Fundamentals (CELF-4), Clinical Test of Phonological Processing (CTOPP), Delis-Kaplan Executive Function System (D-KEFS), Purdue Pegboard, and Ishihara plates. Carriers completed the CELF and D-KEFS during the neuroimaging visit, as this had not been done previously.

Twenty-six child deletion carriers and 42 age-matched controls (ages 8-17) served as the comparison group (**Table 1**). A total of 38 child deletion carriers and 52 control participants were screened, and only those with high-quality imaging were included (detailed in the sections below). Of the 68 children included, 44 were imaged at UC and 24 at CHOP. Gender and handedness did not significantly differ between groups. On behavioral measures, a significant difference ($p < 0.05$) in Verbal Intelligence Quotient (VIQ), Non-Verbal Intelligence Quotient (NVIQ), and the CELF were observed in deletion carriers. Additionally, 7/25 and 10/18 child deletion carriers met criteria for ASD on the ADOS and ADI respectively. Finally, a more fine-grain motion parameter, micro-movements > 0.1 mm, showed a significant difference in children (**Table 1**). All participants were evaluated for mental disorders using the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (**Table 2**). A final clinical diagnosis of ASD was given to 5 child deletion carriers. There was no overlap in individuals given a diagnosis of intellectual disability with those diagnosed with ASD.

Table 1

	Controls	Deletion Carriers
n	42	26
Mean Age $\pm$ SD	12.5 $\pm$ 3	11.6 $\pm$ 2
Age Range	8–17	8–16
Male : Female	28:14	14:12
Left : Right	8:34	6:20
FSIQ	108.6 $\pm$ 13	88.1 $\pm$ 15*
VIQ	109.6 $\pm$ 15	84.3 $\pm$ 18*
NVIQ	107.2 $\pm$ 11	92.0 $\pm$ 14*
CELF, $\leq$ 85	109 $\pm$ 12, 1/39	76 $\pm$ 20*, 16/23
SRS, $\geq$ 60	16.1 $\pm$ 11, 0/39	77.7 $\pm$ 40*, 18/25
Vineland-II, $\leq$ 85	107.2 $\pm$ 10, 0/38	82.2 $\pm$ 14*, 17/25
SCQ, $\geq$ 15	2.0 $\pm$ 2, 0/39	10.6 $\pm$ 8*, 6/25
ADOS	-	7/25
ADI	-	10/18
sSNR	225.1 $\pm$ 73	201.0 $\pm$ 49
vSNR	69.3 $\pm$ 11	65.6 $\pm$ 9
Micro-mov>0.5mm	1.1 $\pm$ 2	1.0 $\pm$ 2
Micro-mov>0.1mm	23.0 $\pm$ 17	38.3 $\pm$ 27*

Table 2

	DEL n=25 (%)
Phonological Processing Disorder (315.39)	15 (60)
Language Disorders (315.31, 315.32, 307.9)	10 (40)
Autism Spectrum Disorder (299.00, 299.80)	5 (20)
Intellectual Disability (317, 318, 319)	5 (20)
ADHD Diagnosis (314.00, 314.01, 314.9)	6 (24)
Learning Disorders (315.0, 315.1, 315.2, 315.9)	2 (8)
Coordination Disorder, Developmental (315.4)	8 (32)
Behavior Disorder (312.9, 313.82)	3 (12)
Anxiety Disorder (300.0, 300.02)	3 (12)
Tic Disorder (307.2, 307.22, 307.3)	2 (8)
Mood Disorders (300.4, 300.9)	0 (0)

Duplication carriers of the 16p11.2 CNV were also recruited. This consisted of nineteen adult duplication carriers and 56 age-matched controls (ages 18-62). However, only 3/19 met broad impairment on SRS, and none of the duplication carriers met criteria for ASD on ADOS (0/19). Again, this is in contrast to deletion carriers with 72% meeting broad social impairment on SRS, and 52% meeting ASD criteria with ADOS or ADI. With a cognitive-behavioral phenotype of adult duplication carriers similar to control participants we focused the analysis on the child deletion carrier group.

The institutional review boards at UC, CHOP, University of Washington Medical Center, Baylor University Medical Center, Boston Children's Hospital, Emory University, Geisinger Medical Center, Columbia University, and Harvard University approved the study procedures.

MRI Data Acquisition and Quality Assessment

High-quality resting-state functional MRI scans obtained from the UC and CHOP neuroimaging sessions were the primary focus of this study. All images were acquired at the UC or CHOP sites with matched 3T TIM Trio MRI scanners (Siemens) and 32-channel phased-array radio-frequency head coils supplied by the vendor. The functional images were acquired during a six-minute eyes-open resting state session with a gradient-echo echo planar imaging sequence that was sensitive to blood oxygenation level-dependent (BOLD) contrast (parameters: repetition time = 3,000 msec, echo time = 30 msec, 3.0 x 3.0 x 3.0-mm voxels, flip angle = 85°, 47 axial slices acquired with interleaved acquisition and no gap). All anatomical and functional images were inspected manually for cerebral cortex clipping, signal inhomogeneity, ghosting, and radio-frequency noise or spiking using a standard methodology (Harvard Center for Brain

Image Processing

The functional images underwent the following preprocessing steps common to functional connectivity analyses: 1) first four volumes discarded to allow for signal stabilization; 2) AFNI's 3dDespike dampens large fluctuations in the resting-state BOLD time series that are likely due to head motion; 3) slice-timing correction (SPM2; Wellcome Department of Cognitive Neurology, London, UK); 4) rigid body correction for head motion with MCFLIRT in the FSL suite [20]; 5) registration to MNI space by transforming the functional images to an EPI template (SPM2; Wellcome Department of Cognitive Neurology, London, UK); 6) smoothed with 6-mm full-width half-maximum Gaussian kernel; 7) band-pass temporal filtering (0.01–0.08 Hz) and regression of six motion parameters and signal from the white matter, ventricles, and whole brain.

MRI Motion

Special attention was placed on reducing bias from motion. Despite spatial registration and regression of motion estimates head motion is associated with decreased functional coupling in the default and frontoparietal control networks, and increased local functional coupling – including left and right motor regions [21, 22]. In conjunction with manual inspection for macro-movements in the form of MRI artifacts, automated measures were calculated to assess for micro-movements. Slice-based temporal signal-to-noise ratio (sSNR), and micro-movements were calculated by determining the number of movements from one volume to the next that exceeded 0.5 mm of root mean squared displacement in 3D space (micro-movements >0.5 mm). Participants were excluded based on automated quality control measures of sSNR < 100 and/or more than 15 micro-movements >0.5 mm. One hundred eighty-eight participants were screened, from which 143 were included. No significant difference was found in sSNR deletion carriers between controls and 16p11.2 carriers (225.1 ± 73 vs. 201.0 ± 49 respectively; $t = 1.49$, $p = 0.14$; Cohen's $d = 0.38$), or micro-movements >0.5 mm (1.1 ± 2 vs. 1.0 ± 2 respectively; $t = 0.26$, $p = 0.80$; Cohen's $d = 0.05$). Duplication carriers also had no significant difference from control participants in sSNR (212.5 ± 76 vs. 216.8 ± 53 respectively; $t = 0.28$, $p = 0.02$, Cohen's $d = 0.06$), and micro-movements >0.5mm (2.2 ± 2 vs 1.8 ± 3 respectively; $t = -0.59$, $p = 0.55$, Cohen's $d = 0.16$). Additionally, in pre-processing an additional measure was taken to perform AFNI's 3dDespike, which dampens large fluctuations in the resting-state BOLD time series that are likely due to head motion. The analysis was performed on these subjects.

On a more fine-scale measure, the two groups did show a significant difference in the number of micro-movements > 0.1 mm: child controls (23.0 ± 17) vs. child deletion carriers (38.3 ± 27) ($t = -2.85$, $p < 0.01$,

Cohen's $d=0.67$). The data was re-analyzed with stricter motion criteria. This excluded any participant, independent of group, with 5 or more micro-movements >0.5 mm, 70 or more micro-movements >0.1 mm, and "minor" manual quality control issues. This reduced control participants from 42 to 38, and deletion carriers from 26 to 20. There continued to be no significant difference in age, sex, handedness, sSNR, or micro-movements > 0.5 mm, and now micro-movements > 0.1 mm were no longer significant (child controls (20.5 ± 16) vs child deletion (29.8 ± 19); $t = -1.25$, $p = 0.22$, Cohen's $d=0.53$). The analysis was re-run with these patient groups without a major change in results (**Supplemental Figure 1**).

Regional Parcellation Approach

The cerebral cortex was parcellated into 61 regions per hemisphere based on the 17-network solution [23]. The matrix elements reflect the z-transformed Pearson correlation coefficient between each pair of 61 intra-hemispheric regions' BOLD time series for both the child control and deletion groups. The matrix labels and matrix divisions are based on the seven networks described by Yeo and colleagues. The matrices allow for visualization of within-network and between-network correlations. A difference matrix is then computed using an ANOVA after controlling for age, sex, handedness, site of imaging, and micro-movements > 0.1 mm.

Degree Connectivity, Voxel-Level Approach

In graph theory, degree centrality, or simply degree, refers to the total number of direct connections to a given node. The degree centrality of local and distant functional connectivity was estimated using methods similar to prior work [24, 25]. The data was first subsampled to 4-mm isotropic voxels for computational efficiency. A matrix of Pearson's r correlation coefficient values was computed across the whole-brain mask (see methods of [24]). For each voxel, the degree of connectivity was computed by counting the number of voxels it was correlated to above a threshold of $r > 0.25$. Moreover, correlated voxels within a 16-mm radius sphere were counted towards local connectivity, i.e., within a 3-voxel length from the target, as this spatial distance is optimal for a local and distant segregation of functional connectivity data (Sepulcre et al., 2010). Voxels outside this radius sphere were counted towards distant connectivity. Group mean cortical maps were generated separately for the degree of local and distant connectivity. An independent data set was used to calculate the test-retest reliability of using local and distant connectivity as a measure. Sixty-six subjects (mean age 21.3 ± 3 , range 19-29; 34 female) from a larger dataset [26] were scanned, and then scanned again, with a median delay of 65 days (range 2-175) for the second scan. Slice SNR and motion were similar between the two visits. Both local connectivity ($r=0.97$) and distant connectivity ($r=0.87$) are highly reliable measures. A global measure of local and distant connectivity was estimated by summing the total number of links for each participant's degree centrality map across the cortex. The global group mean was then compared using a t-test. The aim was to assess whether the sum total links, local and distant, significantly differed between 16p11.2 carriers and controls.

Regional differences in the degree of connectivity were assessed between controls and 16p11.2 carriers. Local and distant connectivity maps were standardized for each subject by converting to Z scores. This allows for comparison of data across subjects. A t-test was then run on each 4-mm node using *Matlab* (v7.11), and false discovery rate (FDR) correction was calculated with a MNI cortical gray-matter mask to limit the number of comparisons. These results were replicated using an independent methodology from FSL's *randomise* function (v5.0.4). Statistical results were nearly identical between the two methods (unpublished data). As an alternative to FDR correction, *randomise* performs a threshold-free cluster enhancement of the data, akin to a bootstrap. Again, there was no discrepancy between alternative statistical methods, providing some internal validity.

Degree of Differential link Approach

Individual whole brain correlation matrices can discern group differences when analyzed link-by-link (or connection-by-connection), as described elsewhere [27]. Briefly, a mask of 4968 voxels covering the whole brain (cortex, subcortex, brainstem and cerebellum) was used to extract the time course of low-frequency BOLD fluctuations. This resulted in a 4968 x 4968 matrix of Pearson's correlation coefficients for each individual. The connectivity maps were then standardized, by converting to Z scores, so that maps could be compared across participants. Thus, each node (i) has a z -transformed- r value associated with every other node across the cortex ($i-j$, $i-k$, $i\ldots n$). A two-tailed t-test is run on each link ($i-j$), comparing link ($i-j$) in all participants in the first group to the second group to assess whether the null hypothesis that the given link is similar between the two groups can be rejected. For instance, if only links $i-a$, $i-f$, $i-p$ are significantly different among the possible 4967 possible connections to node i , then a value of 3 is displayed on the cortical map at node i , which represents three differential links. Similar to the prior analysis, nodes within a 16-mm radius sphere were counted towards local connectivity (~32 possible links), and outside the radius sphere were counted towards distant connectivity (~4935 links). Of note, no values were removed from any participant on the t-test (in particular, z -transformed $r < 0.25$ were included) as to avoid applying nonparametric constraints to a parametric dataset. Inclusion of these correlations is supported by evolving conceptions of functional connectivity. Dynamic functional connectivity, from seconds to minutes, has shown time-varying profiles for canonical resting-state network; a time-compressed average correlation value (used in our analysis), may be negative or positive depending on the time point sampled [28-30]. An FDR correction at 0.05 was performed to correct for multiple comparisons. The advantage of this kind of analysis is that it is a hypothesis-free, data-driven, method of identifying regions with a differential topographical pattern of connectivity between groups – simultaneously across the cortex. To visually demonstrate this, as a proof-of-principle, a seed was placed at regions with the greatest number of differential links (specifically >50 at $p < 0.001$). The group mean resting-state functional connectivity map shows topographically distinct patterns of functional connectivity for controls and 16p11.2 carriers. A t-test was intentionally not performed between the two seed-based maps to avoid circularity in analysis.

As a final step all resulting maps were projected onto cerebral hemisphere of PALS surface (PALS-B12) provided by Caret Software [31, 32] using the interpolated voxel algorithm and average fiducial mapping.

Intrinsic Laterality Index

An intrinsic laterality index (iLI) was calculated using the same methodology described previously [33]. Initially, each cerebral hemisphere was divided into 199 equally space 7-mm spherical regions. The BOLD time series was then extracted for each of the 398 cortical regions and correlated with the other regions' BOLD time series. In an iterative fashion for each of the regions, the BOLD time series of a seed region in the left hemisphere was correlated with the BOLD time series of a target region also in the left hemisphere. The correlation between left seed and left target will be referred to as LL. Correlations were also calculated for the left seed and right homologous target (LR), right homologous seed and left target (RL), and right homologous seed and right homologous target (RR). The iLI was then calculated with the following equation: $iLI = [(LL - RL) - (RR - LR)] / (|LL| + |LR| + |RR| + |RL|)$. An iLI was calculated for each pairwise seed/target correlation in the respective left and right hemispheres.

An independent sample was used to create a mask of the most lateralized functional connections in the cerebral cortex. The sample of 100 healthy participants was selected from a larger sample of 1570 participants [26] based on the number of micromovements >0.1 mm during the resting session; however the independent sample moved less than the CNV carriers or adult control participants (27.4 +/- 18.5 movements > 0.1 mm; see Table 1 for comparison). The independent sample's functional images were acquired with identical imaging parameters and MRI scanners matched to the UC and CHOP scanners. However, a 12-channel head coil was used with the independent sample rather than a 32-channel head coil. A threshold of $|iLI| > 0.3$ [as done previously [33]] was used to determine the most lateralized connections. In total, there were 21 left-lateralized connections comprised of 18 unique regions and 15 right-lateralized connections comprised of 22 unique regions in the iLI mask. The left- and right-lateralized connections from the mask were used to calculate mean left and right iLIs for each CNV carrier and control participant.

The test-retest reliability of the iLI was also calculated in another independent sample. The sample included 69 participants who were scanned twice over multiple days (interscan intervals varied between 2 and 175 days) and was derived from the same sample of 1,570 participants described above [26]. The iLIs have good reliability (Left: $r = 0.63$, $p < 0.001$; Right: $r = 0.65$, $p < 0.001$).

Multiple linear regression models were used to test for group differences in iLI. The following nuisance variables were controlled for: sex, age, handedness, site of imaging, and number of movements greater than 0.1 mm during the resting-state scan. The same models were used to test for the relationship between iLI and measures of language ability and visuospatial ability with the addition of group membership added to the list of aforementioned nuisance variables. We limited our tests to the following *a priori* hypothesized relationships: (1) left lateralization and language ability, as measured by VIQ and the CELF score; and (2) right lateralization and visuospatial ability, as measured by NVIQ. All statistical analyses were conducted in SPSS (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY). As a precautionary measure, the analyses excluded three individuals (one CNV deletion carrier and two adult

control participants) with iLIs classified as outliers. An outlier was defined as an iLI that was more than 1.5 interquartile ranges greater than the third quartile or smaller than first quartile.

Results

Preserved Global Resting-state Architecture

The whole-brain mean correlation matrices capture the entirety of intra-hemispheric resting-state data for the 42 age-matched controls and 26 child deletion carriers (**Figure 1**). The deletion carrier matrix has a similar overall network architecture compared to control participants. Both groups have positive within-network correlations (at the diagonals), and largely a similar pattern of positive and negative between-network correlations. Differences at $p < 0.05$ uncorrected are shown in the smaller bottom right matrix, although no single connection survived correction for multiple comparisons. Overall, this broad-scale view of intra-hemispheric functional connectivity demonstrates that 16p11.2 deletion carriers preserve the general organization of large-scale networks. In other words, the carrier group does not have a disorganized, unrecognizable connectivity matrix; nor is there any single large-scale network, such as the default network, that is wholly disrupted.

Degree Centrality for Local and Distant Connectivity

We then estimated the degree centrality of local and distant functional connectivity across the cortex for 16p11.2 deletion carriers. Typically, the human cortex shows a high degree of local connectivity at primary sensory cortices, medial prefrontal cortex and precuneus; whereas a high degree of distant connectivity is seen at association cortices, particularly at cortical hubs [24, 25]. The child control subjects replicated well the local and distant connectivity maps of degree centrality seen in a prior study using healthy adults [25], upon which our current methodology is based. Deletion carriers have a high degree of local links at primary cortices and the medial surface, and a high degree of distant links at heteromodal association areas similar to controls share the same general architecture as controls in terms of regions with a low or high degree centrality across the cortex (**Figure 2A, D**). Although regional differences do occur (shown later), at a broad level the maps are very similar. Deletion carriers have a high degree of local links at the medial surface and primary cortices, and high degree of distant links at heteromodal association areas similar to controls. Furthermore, these degree centrality maps do not demonstrate a uniform global decrease for all distant connections, or a uniform global increase in local connectivity across the cortex for deletion carriers. This assertion can be quantified by summing the total number of links across the cortex for each subject's local map, and independently, their distant map. The group mean for the global sum of degree links is shown for local and distant connectivity (**2B, E**). No significant group differences are observed in local connectivity ($t = -1.39$, $p = 0.18$, Cohen's $d = 0.34$) or distant connectivity ($t = 1.21$, $p = 0.23$, Cohen's $d = 0.30$). To investigate further whether differences occurred at particular brain regions, the difference in degree centrality for controls minus deletion carriers is shown (**2C, F**) for local connectivity and distant connectivity as a t-statistic map (**2C, F**). A significantly greater degree of connections in controls is seen in red ($p < 0.05$), and a greater degree of connectivity in deletion

carriers is seen in blue ($p < 0.05$). A decrease in the degree of local connectivity is seen in deletion carriers at the temporoparietal junction (TPJ), anterior medial prefrontal cortex (aMPFC), paracentral gyrus, and visual cortex. An increase in degree of local connectivity in deletion carriers is observed at lateral temporal cortex (LTC), the insula, posterior cingulate (PCC), anterior cingulate and the ventromedial prefrontal cortex. In regards to the distant map a decrease in the degree of distant connectivity in deletion carriers is observed at the inferior temporal gyrus, precentral gyrus, aMPFC, and cingulate sulcus; whereas an increase in the degree of connectivity is observed in deletion carriers at the superior temporal gyrus (STG), parietal operculum (OP), inferior and middle frontal gyrus (IFG, MFG), and the pre-Supplemental motor area (pre-SMA). Notice how these particular regions in blue in deletion carriers had a greater number higher degree of distant connections in deletion carriers despite an overall trend towards fewer long-range connections. In other words, both directions of change were observed (regions with increased degree in carriers and increased degree in controls). Regardless, no regions survived correction for multiple comparisons using FDR correction. These results were replicated using an independent methodology, FSL's *randomise* function (v5.0.4). Statistical results were nearly identical between the two methods. Additionally, *randomise* can perform a threshold-free cluster enhancement of the data akin to a bootstrap as an alternative to FDR. No regions survived correction for multiple comparisons using either method. The left hemisphere is representative of the pattern seen in the right hemisphere, which similarly did not have any regions that survived FDR correction. Overall, quantitative differences in degree centrality were not a salient feature in differentiating the functional connectivity of control participants from 16p11.2 deletion carriers.

Degree of Differential Links Reveal Systems with Topographically Distinct Connectivity

Whole-brain correlation matrices of individuals can also discern unique connections in one group but not the other when analyzed link-by-link. The advantage of this kind of analysis is that it is a hypothesis-free, data-driven method for identifying distinct patterns of functional connectivity simultaneously across the cortex. As detailed in the methods section, if node i is connected to nodes a, f, p in one group but not the other, such that z-transformed- r is significantly different at those particular links, then a value of 3 will be displayed on the cortical map at node i (i.e., the 3 differential links $i-a, i-f, i-p$ are counted while all of the shared connections are not ($i-x, i-...n$)). Only differential links (DL) significant at $p < 0.001$ (two-tailed) are displayed (**Figure 3**). The results show that differential links are non-uniformly distributed across the cortex. These regions with a high concentration of DL are 'hot spots' of distinct functional connectivity. The auditory cortex, insula, middle-posterior cingulate and the paracentral gyrus had the greatest number of differential links for local connectivity (**3A**). For distant connectivity, the auditory cortex and insula were again present, but additionally the inferior parietal lobule (IPL), left MFG, right IFG, posterior mid-cingulate and posterior cingulate sulcus, and dorsal and anterior MPFC were regions with the greatest differential links (**3B**). Areas circumscribed in black denote DL that survived multiple comparisons from FDR correction.

Knowing that quantitative differences did not significantly differ between our groups in the number of connections at a given node (from the degree centrality analysis), a differential link should then represent a topographical displacement of a node to its connection (from $i \rightarrow j$ in controls to $i \rightarrow k$ in carriers); akin to a qualitative rather than a quantitative change. To visually demonstrate differences in topography, as a proof-of-principle, a seed was placed at these aforementioned hot spots (specifically regions with >50 DL at $p < 0.001$). The precise seed locations are displayed on the left hemisphere distant connectivity DL map (**Figure 4, upper box**), which was overlaid with the borders of the 7-network solution to better characterize network-level changes [23]. The mean group maps for seed-based functional connectivity are displayed with control participants on the left, and 16p11.2 deletion carriers on the right. Statistical testing was intentionally not performed on this seed-based analysis to avoid circularity. The first three seed locations share an interwoven story (**Figure 4A-C**). First, the IPL shows correlation with the canonical default network (DN) in control participants (7-network, salmon) (**4A**). In deletion carriers the IPL does not appear to be functionally connected to the dorsal medial prefrontal cortex (dmPFC). Nor are there the expected negative correlations to the superior parietal lobule (SPL) of the dorsal attention network (dATN), or OP of the ventral attention/salience network (SN) visualized in controls (7-network, green and magenta respectively). Next, the auditory cortex, specifically at the secondary auditory cortex (or A2) is seeded (**4B**). The control group demonstrates the canonical pattern of connectivity restricted to nearby primary sensorimotor cortices (7-network, blue) with scant connectivity to the medial surface. The auditory seed in deletion carriers, however, shows aberrant connectivity to the dmPFC as well as robust connectivity to the other nodes of the default network (7-network, salmon). This is not an artifact of anatomical displacement of the seed location between groups, or from projection volume-space to surface-space (**Supplemental Figure 2**). Remarkably, these are the same regions that appeared to be missing from seeding the left IPL. Thus, two independent, large-scale resting-state networks are functionally correlated in deletion carriers, and these links between networks are not present in controls. Specifically, the secondary auditory cortex is functionally connected to the TPJ, lateral temporal cortex, temporal pole, IFG and MFG – which constitute the Dorsal Medial subsystem of the Default Network [34, 35] (**Figure 4 lower box**, in salmon). Finally, if that particular node at dmPFC is seeded (**4C**) deletion carriers do indeed show connectivity to the other regions of the DN, proving that the region is not functionally disconnected (or “underconnected”) from the rest of the cortex. It is not the case that the cortex itself at the dmPFC is problematic - as one would surmise if a study only performed a region of interest analysis in isolation at the IPL. It is also noteworthy that if this dataset had been approached with a traditional region-of-interest seed-based approach, one may incorrectly conclude that the dmPFC node is disconnected from the rest of the DN, and point to problematic cortex at that location. Alternatively, one could also conclude that the DN is “underconnected” between the IPL and the dmPFC. Both of these inferences are incorrect because we demonstrated that a seed placed at the dmPFC in deletion carriers continued to connect robustly to the other nodes of the dorsal medial subsystem of the DN as well as to the auditory cortex. It is conceivable that the very early functional magnetic resonance imaging (fMRI) studies of ASD fell into this methodological trap. Here the dmPFC is differentially connected to the auditory node. Additionally, this node of the dorsal medial subsystem has diminished connectivity to the posterior IPL and PCC of the DN core (**Figure 4 lower box**, in yellow). This suggests that the dorsal medial

subsystem is no longer tightly integrated to the core DN. More recent higher-resolution, intra-individual analyses describe no DN Core subsystem at all, but two parallel interdigitated system that give the appearance of a DN Core when group averaged [36]. In this re-interpretation the explanation becomes more simple - Network B of the DN is affected in 16p11.2 deletion carriers, while Network A of the DN is not. Collectively these three seed-based locations reveal a shift in topographical connectivity.

The second major finding in the left hemisphere involves the posterior insula (**4D**). Control participants show strong connectivity ($z(r) \geq 0.5$) constrained locally to area circumscribed around the seed, which explains its preferential clustering in a winner-take-all parcellation to the somatomotor network (7-network, blue). Mild negative correlations are seen at parts of the dorsolateral prefrontal cortex of the frontoparietal control network (FPCN) (7-network, orange) and the SPL of the dATN (7-network, green). This pattern of light blue is remarkably similar to the negative correlation pattern seen from seeding the auditory cortex in control participants in Figure 4A. Deletion carriers, in contrast, display strong connectivity extending into the middle insula and the OP – both nodes of the SN (7-network, magenta). One may recollect that enhanced functional connectivity between the Somatomotor and Salience networks was suggested by the correlation matrix in Figure 1. Negative correlations have also shifted to the default network, as one would expect with the salience network [37]. The posterior insula is the primary sensory cortex for interoception (heat, pain, sensual, visceral and homeostatic sensations) [38-40]. The middle insula re-represents this construct with emotionally salient and environmental stimuli [41-44], and finally the anterior insula then integrates this percept with motivational, social and cognitive conditions [43] as a site of multimodal integration [27]. Craig proposes a forward gradient starting with the interoceptive sensation x (posterior insula), to the subjective feeling of x (middle insula), and then the meta-cognitive self-awareness of x (anterior insula) [43]. This model is also in line with the 7-network parcellation used in our analysis: posterior insula (somatomotor network, blue), middle insula (SN, magenta), and anterior insula (FPCN, orange).

Aberrant links in both cases, auditory-DN and posterior insula-SN, violate fundamental large-scale circuit properties by functionally connecting regions that specialize in local and hierarchical processing of sensation (somatomotor network) with regions that specialize in parallel-processing across widely-distributed networks (default and salience networks.).

Differential links also reveal shifts in topographic connectivity in the right hemisphere (**Supplemental Figures 3 and 4**).

Characterization of Group-Level Behavior

Hahamy and colleagues [45] reported that high inter-subject variability in connectivity is the idiosyncratic defining feature of ASD, and the magnitude of change correlated with behavior. They used 5 sites from the ABIDE dataset, but 6 of the 20 sites in ABIDE collapsed data from ASD subtypes (autistic disorder,

Asperger syndrome, pervasive development disorder-not otherwise specified) [2]. Could this high inter-subject variance simply reflect the high degree of genetic heterogeneity? In contrast, 16p11.2 deletion carriers show stereotyped group-level behavior. A representative aberrant link connecting the auditory cortex with the TPJ of the default network can demonstrate this correlation with behavior at the group-level. The differential functional connectivity between carriers and controls in the left auditory node and left TPJ node is shown (**Figure 5**). The mean z-transformed Pearson's r is displayed after regression of nuisance variables (age, sex, handedness, scanner site, and micromovements greater than 0.1 mm), which is referred to as the 'adjusted' value. In controls the adjusted mean $z(r) = 0.06 \pm 0.24$ and median $z(r) = 0.03$, while deletion carriers have an adjusted mean $z(r) = 0.40 \pm 0.28$, and median $z(r) = 0.45$ (**5A**). Statistical significance was not assessed between the two groups as to avoid circularity. The result is similar for the raw, uncorrected mean value in controls $z(r) = 0.05 \pm 0.24$ and deletion carriers $z(r) = 0.43 \pm 0.29$. The Shapiro-Wilk test confirms normality for the adj. left auditory-TPJ link ($W = 0.941$, $p = 0.19$), and Levene's test confirms equal variance between groups ($F = 0.80$, $p = 0.37$). These results demonstrate that the difference at this link is not simply from high variance in deletion group, but rather a shift in the curve that represents a gain-of-function connection not present in controls. Next, a scatterplot of the left auditory-TPJ link $z(r)$ is plotted against Vineland-II scores, a measure of adaptive social behavior (abnormal ≤ 85) (**5B**). The black line represents the best line of fit for the entire cohort ($r = -0.46$). Similarly, the correlation with SRS (a measure of broad social functioning, abnormal ≥ 60) is $r = 0.42$. The differential link is also plotted against CELF scores, a comprehensive battery of language ability (abnormal ≤ 85) (**5C**). The black line represents the best line of fit for the entire cohort ($r = -0.39$). This is a moderate group-level correlation between the connection strength of the left auditory cortex and the left TPJ with behavior – both to social impairment (Vineland-II) and language (CELF). The bolded triangles on the Vineland-II plot represent individuals with a DSM diagnosis of phonological processing disorder, and conversely on the CELF plot the bold triangles represents those who met diagnostic criteria for ASD (on either ADI or ADOS). Interestingly, at the level of greatest aberrant link strength ($\sim 0.8-1$) only the subgroup of carriers with both language and social impairment (bolded triangles) are present. Of note, regression of group from the results removes the factor being studied. The aim of this study is to characterize group-level effects of the 16p11.2 CNV, induced from many other environmental, genetic and epigenetic factors that affect a particular individual.

The other main hubs of the default network showed connectivity similar to the auditory-TPJ node: left auditory-PCC (mean $z(r)$: 0.07 ± 0.3 controls and 0.41 ± 0.3 in deletions, Vineland-II $r = -0.46$ and CELF $r = -0.41$), and left auditory-dMPFC (mean $z(r)$ 0.12 ± 0.2 controls and 0.44 ± 0.2 in deletions, Vineland-II $r = -0.37$ and CELF $r = -0.35$). Interestingly, with the topographical shift of the auditory node away from the somatomotor network the auditory node is no longer connected to the posterior insula (mean $z(r)$ 0.29 ± 0.2 controls and -0.02 ± 0.2 in deletions) with reversal in direction in correlation with group behavior (Vineland-II $r = 0.39$ and CELF $r = 0.40$). Finally, to reiterate that the core default network is intact, and not directly involved, the left dMPFC-PCC node is functionally connected in both groups (mean $z(r)$: 0.44 ± 0.3 controls and 0.36 ± 0.3 in deletions) without correlation with group behavior (Vineland-II $r = 0.13$ and CELF $r = 0.14$).

Reduced Right-hemispheric Functional Lateralization

Functional lateralization was assessed by measuring the functional connectivity in the most lateralized connections of the cerebral cortex, which were determined in an independent sample of 100 participants without any history of psychiatric illness. The regions involved in the most left-lateralized connections were found almost exclusively in the default network (**Figure 6A, left**), whereas the regions in the most right-lateralized connections were found in the ventral attention, dorsal attention, frontoparietal control, and visual networks (**Figure 6A, right**). After determining the most lateralized connections in the independent sample, we then calculated the mean iLI for each subject in the 16p11.2 CNV sample. Group differences in mean left and right lateralization are shown (**Figure 6B**). The deletion carriers were less lateralized in the right hemisphere than age-matched controls even after controlling for age, sex, handedness, imaging site, and micromovements ($t = -2.44$, $p = 0.02$, Cohen's $d = 0.69$); however, the deletion carriers' left iLI did not differ significantly from age-matched controls' left iLI ($t = 1.75$, $p = 0.09$, Cohen's $d = 0.52$).

Reduced Left-hemispheric Laterality in Individuals Relates to IQ and Language

As follow up to the group difference in the deletion carriers, we tested whether any relationship existed between (1) left functional lateralization and language ability and (2) right functional lateralization and visuospatial ability (**Figure 7**). As hypothesized, language ability was negatively correlated with left iLI when controlling for sex, age, handedness, imaging site, and micro-movements (VIQ: $t = -3.01$, $p = 0.004$, $R^2 = 0.14$; CELF: $t = -1.96$, $p = 0.05$, $R^2 = 0.07$). In other words, as left lateralization increased (i.e., became more negative), language ability likewise increased. The relationship between left iLI and VIQ remained, even after controlling for group ($t = -2.21$, $p = 0.03$, $R^2 = 0.08$); however, the relationship between left iLI and CELF went away after controlling for group ($t = -0.97$, $p = 0.34$, $R^2 = 0.02$). No relationship was found between right iLI and visuospatial ability (NVIQ: $t = 0.59$, $p = 0.56$, $R^2 = 0.01$).

Discussion

While much of the global architecture of large-scale functional networks was preserved, differences in regional intra-hemispheric and interhemispheric functional connectivity were observed in the 16p11.2 deletion carriers. Our data did not support either the longstanding theory of global *underconnectivity* or a modernized quantitative model of *dysconnectivity*. Instead, the most robust finding in 16p11.2 deletion carriers is the discovery of hot spots of unique connections not present in control participants. These brain regions, with a high degree of differential links, demonstrate topographic shifts in the pattern of functional connectivity.

Global architecture is preserved in 16p11.2 deletion carriers

Deletion carriers retained the organization of canonical large-scale networks. On the surface this may not seem remarkable, but this finding comes in face of the longstanding theory of “underconnectivity” in autism¹⁶. Just and colleagues make a subtle distinction in contrast to the earlier cognitive model of “weak central coherence”¹⁵, which implicated a top-down problem (“a conductor problem at a symphony”). *Underconnectivity*, on the other hand, asserts an emergent property problem (“a jam session in which the musicians cannot hear each other”). The authors implicated reduced synchronization as the core problem in autism, i.e., the correlation strength (Pearson’s r) between distant regions¹⁶. We did not find diminished connectivity (r) between and among the TPJ, the medial PFC, and the precuneus/PCC to suggest disruption of the default network. Intrahemispheric correlation matrices of deletion carriers showed robust within-network correlations, and retained organization of large-scale networks in general. Similarly, in a meta-analysis by Müller and colleagues [3], only 2 of 22 autism studies that showed support for global *underconnectivity* actually used a whole-brain field of view – the rest presumed that the effects were global by extrapolating from specific ROI analyzed.

The propensity for a given region to harbor local versus distant connections is non-uniformly distributed across the cortex^{19,24}. Deletion carriers also maintained the global pattern for a given brain region to preferentially harbor local (hierarchical) versus distant (parallel) processing. A model of ASD should not only discern differences from controls, but also account for the preservation of the vast majority of brain functions – as ASD is not synonymous with global intellectual disability.

A quantitative change in the number of connections is not the salient feature of the data

With the growing popularity of graph theory [46] *underconnectivity* slowly morphed into a quantitative model of *dysconnectivity* [2, 5]. In deletion carriers, the mean degree centrality did not show a significant decrease in the global number of distant connections or a global increase in the number of local connections - although a trend was observed. Perhaps global degree is not a robust property of autism in general, and in part explains the multitude of conflicting reports showing both increased and decreased local and distant connectivity across the literature [3, 18]. Our intra-hemispheric data does lend some support to regional differences in degree connectivity. We observed non-uniform or regional changes in the number of connections, which were indifferent to the modality of the cortex involved (primary cortices, unimodal or heteromodal association areas). Both directions of change (increased degree in carriers and increased degree in controls) were seen at different parts of the cortex for each difference map. Similar findings have been reported in recent works with advanced methodology [2, 17, 47-49] among others). Nonetheless, the disparity at any given region did not meet a threshold to surpass correction for multiple

comparisons. Thus, pure quantitative differences in the degree of connectivity were not the salient feature of 16p11.2 deletion carriers.

Differential link analysis revealed hot spots of distinct topographical patterns of connectivity.

Qualitatively different topographical patterns of connections are thought to underlie individual differences in thought and behavior, and are associated with variability in intrinsic functional connectivity [50, 51], particularly at heteromodal association areas [52]. On a group level, autistic individuals report very different internal thoughts compared to control participants [53, 54]. A search for unique connections, or significantly different links between the two groups was conducted using a hypothesis-free, data-driven method. Differential links were not uniformly distributed across the cortex, but instead clustered to particular regions, which we termed ‘hot spots’. Knowing that number of connections did not significantly differ between our groups for any given brain region, a differential link should then represent a topographical displacement of a node to its connection. We found that differential links were not uniformly distributed across the cortex, but instead clustered to particular regions, which we termed ‘hot spots’. It then follows that a seed placed at a hot spot should reveal group-level, distinct topographical differences in the pattern of functional connectivity. This is what was found, and is described below.

Distant Differential Links: The auditory cortex is aberrantly connected to the default network

The purpose of the seed-based analysis was to visualize the hypothesized differences in topography. We intentionally avoided statistical tests here to avoid circularity. In 16p11.2 deletion carriers the auditory cortex and default network are functionally correlated in the left hemisphere. This is extremely peculiar as the transformation of sensation-to-perception in the auditory system proceeds in a hierarchal manner (ex/ A1, A2 or V1, V2, V3, etc.) [55], whereas default network functions as a cortical hub, and does not directly encounter low-level sensations [24, 27]. In other words, a primary sensory area and higher-order association area share functional connectivity. Specialization requires segregation [55], and in deletion carriers a single process, namely aberrant connection between large-scale networks, could preclude the fidelity necessary for proper functionality of both networks.

The aforementioned cluster of differential links is at the superior temporal gyrus (secondary auditory cortex) and abuts the transverse temporal gyrus (primary auditory cortex). From this location one would expect abnormalities in translation of sound frequencies into phonemes, which is exactly what is seen phenotypically. The most frequent DSM diagnosis given to this cohort was phonological processing disorder (15/26 or 58% of participants). Previous reports also describe a delay of auditory evoked cortical response (M100) in 16p11.2 carriers [56, 57]. The auditory node is functionally connected, in particular, to each of the previously described nodes of the Dorsal Medial subsystem of the DN (dmPFC, TPJ, LTC,

Temporal pole) [34, 35]. The default network engages in a variety of self-generated thoughts related to social cognition, but the Dorsal Medial subsystem has been more specifically linked to ‘mentalizing’, or ‘theory of mind’ (see review[34]). Deficits in social cognition are a key phenotypic characteristic of this cohort – as demonstrated on the SRS, and Vineland-II [19, 58, 59]. This is not a reverse inference as the differential link analysis was a hypothesis-free, data-driven method.

The relationship between functional connectivity and group-level behavior was explored further. Hahamy and colleagues reported high inter-subject variability in topographic connectivity to be the idiosyncratic defining feature of ASD, and the magnitude of change correlated with behavior⁴¹. They used 5 sites from the ABIDE dataset, but 6 of the 20 sites in ABIDE collapsed data from ASD subtypes (autistic disorder, Asperger syndrome, pervasive development disorder-not otherwise specified)². Could this high inter-subject variance simply be a reflection of the high degree of genetic heterogeneity? In 16p11.2 deletion carriers topographic connectivity demonstrated well-behaved group behavior, not violating normality or having variance drastically different from controls. A representative differential link (left auditory-TPJ) demonstrated a shift in the curve of functional connectivity, from a median $z(r)$ of 0.03 to 0.45, and represents a ‘gain-of-function’ connection from the deletion of the 16p11.2 CNV. In relation to behavior, the left auditory–TPJ showed group-level behavioral association with both social impairment (Vineland-II and SRS) and language dysfunction (CELF). Interestingly, at the level of greatest aberrant link strength (~ 0.8 -1) only the subgroup of carriers with both language and social impairment are present.

The regions of the left-lateralized dorsal medial subsystem of the DN are also the same regions that are attributed to the language network (Mesulam et al., 2014). Interestingly, the meta-analysis of 526 studies performed by Andrews-Hanna using Neurosynth software found meta-analytic maps of the dorsomedial subnetwork pertaining to not only ‘mentalizing’ and ‘social cognition’, but also to ‘story comprehension’ and ‘semantic/conceptual processing’ (Andrews-Hanna et al., 2014); This is in contrast to the DN-Core, which was associated most with ‘self-related processes’, and the medial temporal subsystem which is linked to ‘past’ and ‘future autobiographical thought’. Structures associated with language during a task may integrate into the default network at rest.

It is also noteworthy that if this dataset had been approached with a traditional region-of-interest seed-based approach, one may incorrectly conclude that the dmPFC node is disconnected from the rest of the DN, and point to problematic cortex at that location. Alternatively, one could also conclude that the DN is “underconnected” between the IPL and the dmPFC. Both of these inferences are incorrect because we demonstrated that a seed placed at the dmPFC in deletion carriers continued to connect robustly to the other nodes of the dorsal medial subsystem of the DN as well as to the auditory cortex. It is conceivable that the very early fMRI studies of ASD fell into this methodological trap.

Additionally, deletion carriers did not demonstrate the robust anticorrelation to the dATN when seeding the IPL, particularly at the superior parietal lobule and parietal operculum that is typically present in healthy controls (Fox et al., 2005). The magnitude of anticorrelation has been correlated to cognitive performance (Kelly et al., 2008). Here, that very same strong anti-correlation is seen instead shifted to the

auditory cortex seed in deletion carriers, further supporting a topographical shift to that of default network connectivity. At the same time, the dmPFC seed is, at the same time, less robustly connected the nodes of the Core DN subsystem - namely the posterior IPL and the precuneus/PCC. In typically developing individuals the PCC fractionates to ventral and dorsal components - with the ventral portion functionally connected to the medial temporal lobe, and the dorsal component functionally connected to the medial PFC (Leech et al., 2011). We observed minimal functional connectivity in deletion carriers from the dorsomedial PFC to the dorsal PCC or posterior IPL (of the Core-DN) as is seen in typically developing individuals (Andrews-Hanna 2011). Thus, the shift in topographic connectivity to include the auditory cortex may have secondarily affected the dorsal subsystem's tight integration to the Core-DN. More recent higher-resolution, intra-individual analyses describe no DN Core subsystem at all, but two parallel systems Network A (overlapping with the Medial Temporal subsystem) and Network B (overlapping with Dorsal Medial subsystem) that give the appearance of a DN Core when group-averaged⁴². In this re-interpretation the explanation becomes more simple - Network B of the DN is affected in 16p11.2 deletion carriers, while Network A of the DN is not. It also must be kept in mind that in real-time these 2-3 subsystems work together in parallel to achieve normal social cognition, and form the canonical default network.

Distant Differential Links: The posterior insula is aberrantly connected to the salience network

The second major finding is aberrant connectivity between the posterior insula (somatomotor network) with the middle insula and parietal operculum (salience network, SN). A forward gradient adjacent but functionally disparate insular cortex starts with the interoceptive sensation x (posterior insula, blue somatomotor 7-network), to the subjective feeling of x (middle insula, magenta salience 7-network), and then the meta-cognitive self-awareness of x and as a site of multimodal integration (anterior insula, orange FPCN 7-network)^{43,44-47}. Concordant with this theory is the fact that human insular cortex is disproportionately (~30%) larger than in macaques, and the anterior insula has no direct homologue⁴⁸. Analogous to the aberrant auditory-DN connection, here the aberrant posterior insula-SN link violates the fundamental organizing principle of primary sensorimotor cortex. Local processing is required to maintain the fidelity of interoceptive sensations, but again deletion carriers have functional connections to regions that specialize in distant connectivity and parallel processing. Individuals with ASD do not lack emotions, but report distorted emotional experiences, and an impaired ability to recognize the emotional states of others correctly [60-62]. Interestingly, the left posterior insula was among the most robust cluster identified in the left hemisphere across methodologies in the largest aggregated dataset of ASD patients [2].

Local connectivity and potential underlying neurobiological mechanism

The differential links analysis also revealed hot spots of distinct local connectivity. Unique connections clustered around the auditory cortex, insula, middle cingulate/cingulate sulcus and the paracentral gyrus. The actual topographic change cannot be visualized well using seed-based maps (similar to the distant DL), since the scope of this difference is < 16 mm. Regardless, if seeded at hot spots local DL maps show nearby spread of functional connectivity, analogous to a bleeding ink blot (not shown).

We have previously shown that the increase in gray matter volume in 16p11.2 deletion carriers was primarily due an effect of the CNV on surface area rather than cortical thickness (Qureshi et al., 2014). This would implicate an increase in the number of neuroprogenitor cells rather than say, a lack of pruning (which would primarily affect cortical thickness). This was corroborated in a zebrafish model of the 16p11.2 CNV (Golzio and Katsanis, 2013). An increase in neuroprogenitor cells translates to an increase in the number of minicolumns. Minicolumns are thought to be the fundamental unit of information processing (Buxhoeveden and Casanova, 2002; Casanova et al., 2002), consisting of radially oriented arrays of pyramidal neurons (layers II-VI), interneurons (layers I-VI), axons and dendrites. In autism minicolumns have been reported to be increased in number, narrower in width, with reduced neuropil space and smaller neuron cell bodies and nucleoli (Casanova et al., 2006; Minshew and Williams, 2007). Moreover, reduced inhibitory control over excitatory activity (Rubenstein and Merzenich, 2003) within and between minicolumns could undermine the development of functionally discrete minicolumns, such that boundaries become blurred and under-selective (Courchesne and Pierce, 2005). This would allow for local excitation to last too long, and spread too far across local patches of cortex. This leads to retaining an abnormal number and pattern of local connections, and reduces the temporal and spatial resolution of input from distant sources (Courchesne and Pierce, 2005). In other words, minicolumn pathology may simultaneously disrupt local and distant circuit organization (Just et al., 2004). Our own data showed regions with a high degree of overlap in the local and distant hotspots of differential links. This is particularly evident at the superior temporal gyrus, posterior insula, middle cingulate and cingulate sulcus. Aberrant links on the seed-based analysis (Figures 5 and 6) were always between adjacent large-scale matrices. For instance, the DN at the middle temporal gyrus is immediately inferior to the auditory cortex, and the cingulate sulcus borders the FPCN and DN. Thus, it may be possible that reduced lateral inhibition of minicolumns in 16p11.2 carriers leads to aberrant local connectivity, and then aberrant distant connectivity.

Since this CNV is associated with the expansion of cortical surface area, it is not completely surprising that the 16p chromosome segment is among the most extreme cases of positive selection among humanoid species. Positive selection of 16p was seen after humans and great apes separated from orangutans, and positive selection continued after the divergence of human and chimpanzee lineages (Johnson et al., 2001). A multitude of genes contribute to the expansion of the cortex, but 16p11.2

deletion carriers demonstrate the result of the malfunctioning of just one of the CNVs positively selected for in the course of evolution.

Deletion carriers show reduced lateralization and correlation with behavioral measures

Contrary to our hypothesis, functional lateralization was significantly reduced in the right hemisphere between deletion carriers and control participants at the group-level. Most reports of atypical functional lateralization in 16p11.2-related deficits or disorders point toward a dampening of functional lateralization in the left hemisphere, or a shift from left-hemisphere lateralization to the right [63-66]. Despite the lack of group difference found in functional lateralization of the left hemisphere, individuals with lower language scores had lower functional lateralization.

Limitations

The current study includes several limitations. The current study lacks a replication sample and includes fewer participants than is desired due to the rare nature of the CNV and our insistence on including only high-quality neuroimaging data. Additionally, we aimed to remove the confound of genetic heterogeneity in the study of autism. The limitation of this approach is that variable expressivity and incomplete penetrance characteristic of ASD-loci. Nevertheless, the results were not driven by outliers, but rather group-level shifts that represents a gain-of-function. As with most fMRI studies of ASD low-functioning individuals may have been excluded from high motion. Additionally, 0/19 adult duplication carriers meet ASD criteria on ADOS perhaps from ascertainment bias. As such, the current study did not attempt to make any inferences in regard as to the reciprocal nature of this CNV. Also, no inference can be made about the longitudinal course of deletion carriers through adulthood. Group differences in resting-state functional connectivity can arise artificially from differences in motion between groups, misregistration of anatomical differences, and heterogeneity of functional connectivity within individuals of a group. Concerted effort was made to address these issues, but can always remain as alternate explanations to group differences in functional connectivity.

Conclusions

In conclusion, differential links in 16p11.2 deletion carriers violate fundamental large-scale circuit properties by functionally connecting large-scale networks that 1) segregate data locally and hierarchically (auditory, visceral sensory) with those that 2) integrate data using parallel-processing across widely-distributed networks (default, salience). Shared resting-state architecture between auditory and default networks from aberrant connections may preclude the fidelity necessary for proper language and social processing, and serve as a new model for ASD.

List Of Abbreviations:

anterior medial prefrontal cortex (aMPFC), autism brain imaging data exchange (ABIDE), Autism Diagnostic Interview (ADI), Autism Diagnostic Observation Schedule (ADOS), autism spectrum disorder (ASD), blood oxygenation level-dependent (BOLD), copy-number variant (CNV), Children Hospital of Philadelphia (CHOP), Clinical Evaluation of Language Fundamentals, fourth edition (CELF), Delis-Kaplan Executive Function System (D-KEFS), default network (DN), Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV), Differential Ability Scales-II (DAS-II), differential links (DL), dorsal attention network (dATN), dorsal medial prefrontal cortex (dmPFC), false discovery rate (FDR), frontoparietal control network (FPCN), functional magnetic resonance imaging (fMRI), inferior frontal gyrus (IFG), inferior parietal lobule (IPL), intrinsic laterality index (iLI), lateral temporal cortex (LTC), left seed and left target (LL), left seed and right homologous target (LR), middle frontal gyrus (MFG), Non-Verbal Intelligence Quotient (NVIQ), parietal operculum (OP), posterior cingulate (PCC), pre-Supplemental motor area (pre-SMA), right homologous seed and left target (RL), right homologous seed and right homologous target (RR), slice-based temporal signal-to-noise ratio (sSNR), Social Responsiveness Scale (SRS), superior parietal lobule (SPL), superior temporal gyrus (STG), temporoparietal junction (TPJ), University of California (UC), ventral attention/salience network (SN), Verbal Intelligence Quotient (VIQ), Wechsler Individual Achievement Test (WIAT-III)

Declarations

Ethics approval: Consent was obtained, and study procedures were approved by the relevant institutional review boards at Children Hospital of Philadelphia and University of California Berkeley.

Availability of data and materials: We appreciate obtaining access to phenotypic data on SFARI Base. Data were also provided in part by the Brain Genomics Superstruct Project of Harvard University and the Massachusetts General Hospital, (Principal Investigators: Randy Buckner, Joshua Roffman, and Jordan Smoller). Approved researchers can obtain the Simons VIP population dataset described in this study by contacting the Simons Foundation Autism Research Initiative. They can also obtain the Brain Genomics Superstruct data by going to <https://dataverse.harvard.edu/dataverse/GSP>.

Competing Interests: The authors declare no competing financial interest.

Funding: A.Q. was supported by NIH/NINDS 5R25NS065743; and J.S. by the National Institutes of Health (NIH) grant K23EB019023.

Author contributions: All the authors contributed to design, analysis, and interpretation of the data. A.Y.Q. and J.A.N. wrote the manuscript. All the authors read, revised, approved the final manuscript.

Acknowledgements: We thank Randy Buckner for insights and guidance on the analysis. 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.

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Figures

Supplemental Figure 1

Figure 1

Comparing intrinsic functional connectivity within and between cortical networks in the children groups. The mean correlation matrices are displayed for the 42 age-matched control participants (top), 26 child deletion carriers (middle), and the difference between the two groups (bottom). The cerebral cortex was parcellated into 61 regions per hemisphere based on the 17-network solution from Yeo and colleagues (see parcellation scheme depicted between the correlation matrices). The matrix elements reflect the z-

transformed Pearson correlation coefficient between each pair of the 61 intrahemispheric regions' BOLD time series for the two group matrices (top and middle). The matrix labels and matrix divisions (i.e., the gray lines on the correlation matrix) are based on the seven networks described by Yeo and colleagues. The between network correlation fall along the diagonal and are positive (warm colors), whereas the within-network correlations are off the diagonal and are both positive (warm colors) and negative (cool colors). The difference matrix (bottom) is calculated with an ANOVA after controlling for the following variables with a linear regression: sex, age, handedness, imaging site, and the number of micromovements greater than 0.1 mm. The small matrix shows only the connections (i.e., non-black elements) with group difference $p < 0.05$. No connections survive an FDR correction for multiple comparisons, $q < 0.05$. FPCN, frontoparietal control network; dATN, dorsal attention network; SN, salience or ventral attention network; SomMot, somatomotor network

Figure 2

Figure 2

Local and distant functional connectivity in control participants and 16p11.2 CNV deletion carriers across the cortex. The degree centrality for local connectivity is estimated as the number of links correlated ($r > 0.25$) to each 4-mm node within a 16-mm spherical radius (A), and degree centrality for distant connectivity is estimated as the number of links correlated ($r > 0.25$) to each 4-mm node outside of its 16-mm spherical radius (D). The resulting value of each node is projected on an inflated brain surface map

for each hemisphere. For visualization purposes, the entire range of results is best captured by displaying the 2nd – 98th percentile of the absolute number of degree links for the mean control maps; specifically, 110 local links and 3080 distant links represent the 2nd percentile of the mean control map (dark gray is $\leq 2\%$), and 500 local links and 5490 distant link are the 98th percentile for controls (dark red is $\geq 98\%$). Next, the total number of links were summated across the cortex for each subject's local, and separately, distant maps as global estimates. The group mean for the global sum of degree links is shown for local connectivity (B) and distant connectivity (E). Error bars reflect the standard error of the mean. Although a trend is observed for increased local and decreased distant connectivity in deletion carriers, no significant group difference is found in either local connectivity ($t=-1.39$, $p=0.18$, Cohen's $d=0.35$) or distant connectivity ($t=1.21$, $p=0.23$, Cohen's $d=0.03$). Finally, the difference of degree centrality for controls minus deletion carriers (from A,D) is displayed for local connectivity (C) and distant connectivity (F) as a t-statistic map. Significantly more connections in controls are in red ($p<0.05$), and significantly more connections in deletion carriers are in blue ($p<0.05$). Both directions of change are observed at focal brain regions in local and distant connectivity maps, and it is not the case that only deletion > control is seen on the local map, and only control > deletion is seen on the distant map. Notably, no regions survived FDR correction for multiple comparisons.

Figure 3

Figure 3

Local and distant functional connectivity in control participants and 16p11.2 CNV deletion carriers across the cortex. The degree centrality for local connectivity is estimated as the number of links correlated ($r > 0.25$) to each 4-mm node within a 16-mm spherical radius (A), and degree centrality for distant connectivity is estimated as the number of links correlated ($r > 0.25$) to each 4-mm node outside of its 16-mm spherical radius (D). The resulting value of each node is projected on an inflated brain surface map

for each hemisphere. For visualization purposes, the entire range of results is best captured by displaying the 2nd – 98th percentile of the absolute number of degree links for the mean control maps; specifically, 110 local links and 3080 distant links represent the 2nd percentile of the mean control map (dark gray is $\leq 2\%$), and 500 local links and 5490 distant link are the 98th percentile for controls (dark red is $\geq 98\%$). Next, the total number of links were summated across the cortex for each subject's local, and separately, distant maps as global estimates. The group mean for the global sum of degree links is shown for local connectivity (B) and distant connectivity (E). Error bars reflect the standard error of the mean. Although a trend is observed for increased local and decreased distant connectivity in deletion carriers, no significant group difference is found in either local connectivity ($t=-1.39$, $p=0.18$, Cohen's $d=0.35$) or distant connectivity ($t=1.21$, $p=0.23$, Cohen's $d=0.03$). Finally, the difference of degree centrality for controls minus deletion carriers (from A,D) is displayed for local connectivity (C) and distant connectivity (F) as a t-statistic map. Significantly more connections in controls are in red ($p<0.05$), and significantly more connections in deletion carriers are in blue ($p<0.05$). Both directions of change are observed at focal brain regions in local and distant connectivity maps, and it is not the case that only deletion > control is seen on the local map, and only control > deletion is seen on the distant map. Notably, no regions survived FDR correction for multiple comparisons.

Left Hemisphere Distant Connectivity

Figure 4

Figure 4

Seeds placed at hot spots of differential links act as proof-of-principle for distinct topographical patterns of connectivity. Locations investigated are displayed on the left hemisphere distant connectivity DL map (upper box), and overlaid with the borders of the 7-network cortical parcellation (Yeo et al., 2011). Mean group maps for seed-based functional connectivity are displayed (A-D). The z-transformed-r correlation strength to the particular seed location is displayed (red ≥ 0.5 to deep blue ≤ -0.4) (A) In controls the

inferior parietal lobule (IPL) (-46 -62 24) shows correlation with the canonical default network (DN) (7-network, red). In deletion carriers the IPL does not appear to be functionally connected to the dmPFC. Nor is there the expected negative correlation to the superior parietal lobule or parietal operculum (7-network, green- dorsal attention, magenta - salience networks respectively). (B) The auditory cortex (-62 -16 0) in controls shows canonical connectivity restricted to nearby primary sensorimotor cortices (7-network, blue) and scant connectivity to the medial surface. Surprisingly, deletion carriers show connectivity to the dmPFC that appeared missing from the IPL seed, as well as other nodes of the DN (7-network, red). The expected negative correlations of the DN are visualized here. (C) If that particular node at dmPFC (-6 50 24) is seeded deletion carriers do indeed show connectivity to other regions of the DN, proving that the region is not functionally disconnected from the rest of the cortex. Notable differences include differential involvement of the auditory cortex node, diminished connectivity to the posterior IPL and PCC. Collectively these three seed-based locations reveal a shift in topographic connectivity. Specifically, the auditory cortex is functionally correlated to the TPJ, lateral temporal cortex, temporal pole, IFG and MFG of the Dorsal Medial subsystem of the DN (lower box, red). (D) The posterior insula (-38 -22 8) in controls show strong connectivity ($z(r) \geq 0.5$) restricted to the somatomotor network (7-network, blue). In deletion carriers strong connectivity extends to the middle insula and OP of the salience network (7-network, magenta). Mild anti-correlation with the PCC and medial PFC is also observed DEL, deletion carriers; CON, control participants

Figure 5

Figure 5

A representative aberrant link demonstrates group-level correlation with behavior. The differential link between the left auditory cortex and left tempoparietal junction (TPJ) node of the default network is shown. (A) The mean adjusted z-transformed Pearson's r is after regression of nuisance variables (age, sex, handedness, scanner site, and micromovements greater than 0.1 mm). Child controls (CON) mean $z(r) = 0.06 \pm 0.24$ and median $z(r) = 0.03$. For deletion carriers (DEL) the adjusted mean $z(r) = 0.40 \pm 0.28$,

and median $z(r) = 0.45$. Statistics were not conducted here to avoid circularity. (B) A scatterplot of the left auditory – TPJ link $z(r)$ is plotted against Vineland-II scores, a measure of adaptive social behavior (abnormal ≤ 85). The bolded triangles here represent individuals that met DSM for phonological processing disorder. The black line represents the best line of fit for the entire cohort at $r = -0.46$. (C) A scatterplot of the aberrant link plotted against CELF scores, a comprehensive battery of language ability (abnormal ≤ 85). Here, the bolded triangles represent individuals that met diagnostic criteria for ASD on either ADI or ADOS. The black line represents the best line of fit for the entire cohort at $r = -0.39$.

Figure 6

Figure 6

Lateralized brain regions that make up the functional connections included in the mean left and right intrinsic laterality indices (A). The most lateralized brain regions were defined based on the functional connectivity patterns from an independent sample of 100 individuals. The 18 left-lateralized regions (yellow regions, left) that make up the 21 most left-lateralized connections are primarily part of the default network as defined by the seven-network parcellation in (Yeo et al, 2011). The 22 right-lateralized regions (blue regions, right) that make up the 15 right-lateralized connections are part of the ventral and dorsal attention networks, frontoparietal control network, and visual network as defined by the seven-network parcellation in (Yeo et al., 2011). Deletion carriers have reduced functional lateralization (B). The 26 child deletion carriers have reduced mean laterality in the right hemisphere compared to the 42 age-matched control participants. The mean laterality indices were calculated for each individual based on the most lateralized regions and connections previously described. Then, the mean laterality indices were adjusted to remove the effects of age, sex, handedness, imaging site, and number of micromovements greater than 0.1 mm during the resting-state run. *, $p=0.02$; DEL, deletion CNV carriers; CON, control participants.

Figure 7

Figure 7

Functional laterality in the left hemisphere is related to language ability. The scatter plots depict the relationship between mean left laterality index and two measures of language ability, VIQ (A) and the CELF score (B) across the entire sample of children. In other words, the mean left laterality index becomes more lateralized (i.e, more negative) as VIQ (A) and the CELF score (B) increase. The adjusted mean left laterality index in A is the left iLI after controlling for age, sex, handedness, imaging site, number of

micromovements greater than 0.1 mm, and group; whereas the adjusted mean left laterality index in B controls for all of the same variables except group. DEL, deletion CNV carriers; CON, control participants.

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