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Differences in Intrinsic Gray-Matter Connectivity and their genomic underpinnings in Autism Spectrum Disorder

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Abstract

Background

Autism is a heterogenous neurodevelopmental condition accompanied by differences in brain connectivity. Structural connectivity in autism has mainly been investigated within the white matter. However, many genetic variants associated with autism highlight genes related to synaptogenesis and axonal guidance, thus also implicating differences in 'intrinsic' (i.e. gray-matter) connections in autism. Intrinsic connections may be assessed *in vivo* via so-called intrinsic global and local wiring costs.

Methods

Here, we examined intrinsic global and local wiring costs in the brain of N=359 autistic individuals and N=279 controls, aged 7-31 years from the EU-AIMS Longitudinal European Autism Project (LEAP). FreeSurfer was used to derive surface mesh representations to compute the estimated length of connections required to wire the brain within the gray-matter. Vertex-wise between-group differences were assessed using a general linear model. A gene expression decoding analysis based on the Allan Human Brain Atlas was performed to link neuroanatomical differences to putative underpinnings.

Results

Group differences in global and local wiring costs were predominantly observed in medial and lateral prefrontal brain regions, in inferior temporal regions, and at the left temporoparietal

junction. The resulting neuroanatomical patterns were enriched for genes previously implicated in the etiology of autism at the genetic and transcriptomic level.

Conclusion

Based on intrinsic gray-matter connectivity, the study investigated the complex neuroanatomy of autism and linked between-group differences to putative genomic and/or molecular mechanisms to parse the heterogeneity of autism and provide targets for future subgrouping approaches.

Introduction

Autism spectrum disorder (ASD) is a highly heterogenous neurodevelopmental condition characterized by difficulties with social communication, as well as restricted and repetitive behavior (1). Studies suggest that autism is associated with differences in neuroanatomy and structural brain connectivity (2). Investigating these differences and their putative genetic mechanisms might aid to identify targeted support.

Magnetic resonance imaging (MRI) has extensively been used to examine differences in brain connectivity *in vivo*. For example, functional and structural studies suggest that autism is accompanied by differences in global (i.e. long-range) and local (i.e. short-range) connectivity compared to non-autistic individuals even though there is some dispute over the sign and scale of these difference. Some studies report decreased global and increased local connectivity in autism (3–6), while others report significant increases (7) or decreases in global and local functional connectivity, respectively (8). Such heterogeneity in findings might be due to various reasons including variability in participant demographics, sample sizes, or connectivity features (8–11). Evidence also suggest that differing brain connectivity is associated with clinical traits in autism, i.e. alterations in functional brain connectivity in limbic, frontoparietal and motor regions are associated with repetitive behavior (12), and with the severity of core autism features (13). Thus, examining brain connectivity may provide important novel insights into the neurobiological underpinnings of different clinical autistic traits.

Most previous neuroimaging studies investigating brain connectivity in autism focused on so-called 'extrinsic' connections that pass through the cortical white matter (e.g. 14). However,

genetic and transcriptomic studies suggest that many genetic variants associated with autism include genes related to synaptogenesis, maturation and axonal guidance, hence also implicating differences in 'intrinsic' (i.e. gray-matter) connectivity in the pathophysiology of the condition (15–17). Intrinsic cortico-cortical connections are confined to the cortical sheet and travel in parallel to the cortical surface (18). As described in histological studies, such intrinsic connections can range up to a few millimeters and are found in the prefrontal (19), visual, and somatosensory cortex (20). Intrinsic connections are related to various surface-based geometric features such as surface area (21), cortical separation distances (22), and Gaussian curvature (23) and are considered to be 'intrinsic' as they are independent from the two-dimensional folding of the cortex, and cannot be removed without deformation of the cortical surface (22). Therefore, measuring intrinsic connections provides insights that are not accessible by traditional volume-based approaches, and may provide *in vivo* proxy markers that are closely related to the aetiology of autism (i.e., genetic underpinnings).

To date, there is only one study that has examined differences in intrinsic connectivity *in vivo* in autistic adults (6). This study examined the intrinsic organization of the cortex using geodesic distances which represent the shortest path between two points (i.e. vertices) along the cortical surface, thus running in parallel to intrinsic horizontal connections (22). These distances were subsequently used to estimate intrinsic wiring costs. Global wiring costs were quantified by 'mean separation distances' (MSD) representing the average geodesic distance from a vertex to the rest of the surface. Local wiring costs were estimated using 'geodesic circles' projected onto the cortical surface. Here, the radius of a geodesic circle represents the 'intra-areal' wiring costs (i.e., costs required to wire the cortex within the circle), while the perimeter represents the 'inter-

areal' wiring costs (i.e., costs associated with wiring the cortex outside the circle) (see supplement and SF1 for schematic illustration). The study by Ecker et al. (6) reported that the brain in autism is characterized by (i) reduced global wiring costs, (ii) increased inter-areal wiring costs, and (iii) decreased intra-areal wiring costs compared to the non-autistic brain suggesting that intrinsic measures of brain morphometry are particularly well suited to describe the neuroanatomy of autism, and are conceptually linked to specific neurobiological mechanisms.

So far, direct evidence linking differing intrinsic brain connectivity to specific genes and neurobiological mechanisms remain missing. However, studies leverage the power of the spatial gene expression data provided by the Allan Human Brain Atlas (AHBA, 24) to link imaging phenotypes to putative genetic mechanisms (25–27). Utilizing the AHBA, it is possible to genetically 'decode' patterns of neuroanatomical variability, i.e., to identify genes with a spatial pattern of expression resembling the neuroimaging map. The resulting list of genes with a significant spatial correlation can be tested for an enrichment of specific gene sets previously associated with autism.

The objective of the current project was therefore to (i) replicate and extend earlier finding on intrinsic wiring costs in a large and clinically heterogeneous sample of autistic individuals and controls provided by the EU-AIMS Longitudinal European Autism Project (LEAP, 11), and (ii) to establish the putative genomic and/or molecular mechanisms mediating these differences in the brain to facilitate future subgrouping approaches.

Methods and Materials

Participants

The data used in this study was provided by the multicentered EU-AIMS LEAP project. A comprehensive description of the sample has been published elsewhere (11,25,28). In brief, N=359 (male=258,female=101) individuals with autism and N=279 (male=178,female=101) controls between the age of 6-30 years with structural MRI data were included in this study (Table 1). A detailed description of inclusion/exclusion criteria, clinical assessments, and medication status can be found elsewhere (Supplement and 25). Independent ethics committees approved the study, and written informed consent was obtained for all participants.

MRI data acquisition

All participants underwent MR-imaging in 3-T scanners located at six different sites using comparable acquisition paradigms: (i) University of Cambridge, U.K., (ii) King`s College London, U.K., (iii) Central Institute of Mental Health, Mannheim, Germany, (iv) Radboud University Medical Centre, Netherlands, (v) University Medical Centre Utrecht, Netherlands and (vi) Rome University, Italy. For all participants, a high-resolution structural T1-weighted image was acquired with full head coverage (slice thickness=1.2mm, in plane resolution=1.2x1.2mm^2, see supplement TS2). Subsequently, these T1-weighted images were used for surface reconstruction with FreeSurfer software.

Cortical surface reconstruction using FreeSurfer

FreeSurfer, version_6.0.0 was used to obtain cortical surface representations for each T1-weighted image of N=708 individuals within the LEAP sample. These fully automated processes have been described in detail elsewhere (29,30). All surface reconstructions underwent thorough quality assessments (see 25). In total, a sample of N=638 individuals was used, N=359 in the autistic group and N=279 in the non-autistic group. For reasons of computational efficiency (6), surface reconstructions were downsampled to 40,962 vertices per hemisphere (*fsaverage6* template), and pial surfaces were used for the computation of global and local wiring costs.

Estimation of global wiring costs: Mean Separation Distance (MSD)

We initially computed Mean Separation Distances (MSD) to estimate the degree of global intrinsic wiring costs based on the individual's pial surface reconstruction. MSDs represent the average geodesic distance between each vertex and all other vertices characterizing the cortical surface (see supplement and SF1 for details). Geodesic distance computations were performed using the "Fast-Marching" toolbox for MATLAB (R2021a, The Mathworks) that provides 'exact' geodesic distances (31–33). This resulted in a *n*-by-*n* matrix of distances *D* with zero values in the diagonal, with *n* indicating the number of vertices of the surface. The elements of *D* thus hold the geodesic distance from a vertex to all other vertices. The mean values of row/columns (1-by-*n*) of this matrix represent the MSD for each vertex.

Estimation of local wiring costs: Radius Function and Perimeter Function

Subsequently, we estimated local wiring costs by means of geodesic circles. Each circle had a radius *r* and a perimeter *p* and covered 5% of the total surface area. This percentage was chosen as (i) it has been shown to elicit a stable cortical pattern incorporating both the high frequency local variations at lower scales, and more global trends at higher scales (see 22), and (ii) to make our findings comparable to previous reports (6). The resulting radius of each circle determines the 'radius function', and was used to estimate the 'intra-areal' wiring costs at that vertex, i.e. the minimum length of connections required to connect a vertex with other vertices inside this area. In turn, the perimeter of the circle was used to estimate the 'inter-areal' wiring costs at a vertex that indicate the length of connections required to wire a vertex with neighboring vertices outside the given area (6,22). We examined MSD, radius function, and perimeter function at each vertex with a 2mm smoothing kernel (6), and examined the robustness of the results across different smoothing filters (see supplement).

Surface-based statistical analyses

For statistical analyses, the SurfStat toolbox (https://www.math.mcgill.ca/keith/surfstat/) for MATLAB (33) and R (34) was used. Vertex-wise between-group differences in MSD, perimeter function, and radius function were examined with a general linear model (GLM) incorporating group, sex, and acquisition site as fixed effect factors, and age, full-scale IQ (FSIQ), and pial surface area (SA) as continuous covariates and ε_i is the residual error at vertex i.

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197 $Y_i=\beta_0+\beta_1 Group+\beta_2 Sex+\beta_3 Age+\beta_4 FSIQ+\beta_5 Site+\beta_6 SA+\varepsilon_i$

198

199 Coefficient β_1 was used to estimate the between-group differences that were normalized by the
200 corresponding standard error. Corrections for multiple comparisons were performed using
201 random-field-theory based cluster analysis for non-isotropic images with a cluster-based
202 significance threshold (p_{clust}) of 0.05 (two-tailed) (35). Effect sizes associated with each model
203 term were assessed using Cohen's f, where values of 0.1, 0.25, and 0.4 indicate small, medium,
204 and large effects, respectively. In the autistic group, brain-behavior correlations between
205 differences in MSD, perimeter function, and radius function and the severity of autism traits were
206 examined using Pearson's r. Autism traits were assessed using ADOS (36), ADI-R (37), SRS-2 (38),
207 SSP(39), and RBS-R (40).
208 We also examined the effects of medication status and compared our results across distinct age-
209 stratified subgroups, i.e., adults (>18years), adolescents (12-18years), children (<12years), and a
210 group with mild learning disability, i.e., >12years and IQ<75 (see supplement for details). To
211 compare differences in intrinsic wiring costs with other morphometric features, we also
212 examined between-group differences in vertex-wise measures of surface area (SA) (41) and local
213 gyrification index (lGi, 42), with focus on the relationship between variability in MSDs and SA (see
214 supplement for details).

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216 **Gene Expression Decoding Analysis**

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To link the neuroanatomical findings of differences in intrinsic gray-matter wiring costs to putative genetic underpinnings, a gene expression decoding analysis (GEDA) was performed (25,27). The GEDA within Neurosynth/Neurovault (43,44) assessed the spatial correlation between gene expression data of N=20,787 genes from the Allen Human Brain Atlas (24) and the neuroanatomical findings from the vertex-wise between-group comparisons. A comprehensive description of the enrichment analyses is provided in the Supplement.

Results

Subject demographics

There were no differences between the autistic and non-autistic group in terms of age (t(638)=0.28,p=0.78), and total surface area (t(638)=0.15,p=0.88). However, autistic individuals had a significant lower full-scale IQ (mean=98.8,SD=9.73) than non-autistic controls (mean=104.75,SD=18.24) (

Table ***1***). We covaried for these measures in all subsequent analyses. Our sample included more autistic males than male controls, and more males than females (Table 1).

Differences in wiring costs

Global intrinsic wiring costs. Autistic individuals showed significant differences in MSDs compared to the control group. More specifically, the brain in autism showed significantly decreased MSDs in several clusters that included the (i) right lateral and medial prefrontal cortex (approximately Brodman area (BA) 10/11/46), (ii) left lateral orbitofrontal cortex and medial prefrontal cortex (BA 10/11), and (iii) left rostral middle frontal cortex and pars triangularis (BA 44/45/46) (Figure 1A,B). Effect sizes for the main effect of group (mean=0.04,SD=0.03) and all other model terms are displayed in Figure 1C. These differences are most pronounced during adulthood and adolescents (see Figure 2 and ST4) and are stable across smoothing filters (see SF6), and when covarying for medication status (see SF4).

Local intrinsic wiring costs. The brain in autism was also characterized by clusters with significantly increased perimeter function. Here, significant increases in autism were observed in the (i) right rostral middle frontal cortex (BA 46), (ii) right middle temporal cortex (BA 20/21), and in the (iii) left inferior parietal cortex (BA 39) (Figure 1A). Additionally, we observed clusters with significantly decreased radii. These decreases were predominantly observed in the (i) right rostral middle frontal cortex (BA 9/46), (ii) left inferior parietal cortex (BA 39), (iii) left supramarginal gyrus (BA 40), (iv) left superior frontal cortex (BA 9), (v) left lateral orbitofrontal cortex (BA 11),

and in the (vi) medial orbitofrontal cortex (BA 12/32) (Figure 1A). Effect sizes for the main effect of group (Perimeter function: mean=0.04,SD=0.03; radius function: mean=0.037,SD=0.03) and all other model terms are displayed in Figure 1C. For the perimeter function, these differences were most pronounced during adolescents, and across adolescence and adulthood for the radius function (Figure 2 and ST4).

Brain behavioral correlations. To assess the association between intrinsic global and local wiring costs and autistic traits, we correlated the average MSD, perimeter function, and radius function in significant clusters of the autistic group with the severity of autism traits. Within these clusters, increased perimeter function was significantly positive correlated ($0.14<r<0.2$) with severity of autism traits. There were no significant correlations between MSDs and radius function and measures of trait severity (Supplement SF2).

Gene Set Enrichment Analysis. To link imaging phenotypes to potential genetic underpinnings in autism, we performed a gene decoding analysis using the AHBA. This resulted in N=480, N=382 and N=322 significant genes for MSD, perimeter function, and radius function, respectively (nominal $p<0.01$). These gene sets showed an enrichment for (i) differentially expressed genes (DEG) in autism, (ii) for biological pathways, and (iii) for different cell types and gene coexpression modules underpinning typical brain development. For MSD, we found an enrichment in downregulated DEGs, namely CTX.down.M10, CTX.down.M16, CTX.down.M4 (45) and ASD.DEGs.down (46) (Figure 3A) which have been associated with the Gene Ontology (GO) terms representing synaptic functioning and neuronal genes (45,46). Perimeter function was enriched

for DEGs that are upregulated or downregulated, namely CTX.up.M9, CTX.down.M16 (45), ASD.DEGs.up (46), and DEG (47) (Figure3A) that have been linked to GO terms representing inflammatory response and neuronal firing rate (45,46). For radius function, we found an enrichment for DEGs that are upregulated in autism, namely CTX.up.M20 (45) (Figure 3A) which have been linked to GO terms representing development and regulation of cell differentiation (45). Furthermore, we found an enrichment for biological pathways of the KEGG Pathway database (48). The MSD gene list was enriched for the pathways of axonal guidance, calcium signaling pathway, and retrograde endocannabinoid signaling (Figure3B). Perimeter function was enriched for pathways of neurodegeneration for multiple diseases and retrograde endocannabinoid signaling. Radius function was enriched for the calcium signaling pathway (Figure 3B).

Additionally, we observed an enrichment for coexpression modules underpinning typical brain development (49). More specifically, gene sets of MSD and radius function were significantly enriched for modules representing “synaptic transmission” (modules 2 and 15) that have been associated with GO terms calcium signaling, synaptic transmission, and neuroactive ligand-receptor interaction (49) (Figure 3C). Enrichment analysis resulted in an enrichment for different cell types (50), e.g., for excitatory neurons in MSD, for pericytes, oligodendrocyte precursor cells, excitatory neurons, and radial glia cells in perimeter function, and for endothelial cells, interneurons, and excitatory neurons in radius function (Figure 4). The pathway and process enrichment analysis conducted with Metascape highlighted several significant GO terms for MSD, perimeter function and radius function, e.g., nervous system development, synaptic signaling, and neuron projection morphogenesis (Supplement FS2).

Discussion

Here, we examined between-group differences in intrinsic wiring costs of the brain in a large and clinically diverse sample of autistic individuals and non-autistic controls and established their link to putative genomic mechanisms. Using measures of intrinsic wiring costs, we established that the intrinsic organization of the brain in autism differs significantly from the non-autistic brain in both global and local wiring costs. Differences in local wirings costs were also significantly correlated with the severity of autism traits. Brain regions with differences in intrinsic wiring costs were enriched for genes known to be implicated in autism. Our study therefore highlights the importance of examining intrinsic features of brain anatomy as putative biomarkers for autism that might aid future subgrouping approaches.

Initially, we assessed global wiring costs using Mean Separation Distances (MSDs) which represent the average length of connections required to wire each vertex to the rest of the cortex. We established that autistic individuals had significantly reduced MSDs in several frontal lobe regions including the dorsolateral-prefrontal cortex (DLPFC), the orbitofrontal cortex, and the pars triangularis. A similar pattern of differences in global wiring costs has been reported in an earlier study by Ecker at al., conducted in a smaller sample of male autistic adults (6). Notably, differences in the intrinsic organization of the cortex have also been reported in other mental health conditions such as major depression disorder (51), schizophrenia (52,53), and ADHD (54). However, the neuroanatomical patterns associated with these differences vary widely between conditions and are distinct from the pattern we observe in ASD. Moreover, the pattern of differences in intrinsic wiring costs only partially overlapped with the patterns of regional

variations associated with measures of surface area and/or lGI (42,55,56), suggesting that each feature measures distinct aspects of the cortical architecture (Supplement SF7).Differences in local wiring costs, on the other hand, were estimated based on the radius and perimeter function representing the wiring potential within and between a given cortical area, respectively. We observed decreased radius function, and increased perimeter function in autism, with largest effects in frontal, temporal, and parietal brain regions. The radius function is often inversely correlated with the perimeter function of the cortex, i.e., a decreased radius of a geodesic circle is associated with an increased perimeter. Consequently, in regions with significant differences in local wiring costs, a reduced radius function implies easier wiring within the region, while an increased perimeter function indicates a facilitated development of connections between areas. As with MSDs, a similar regional pattern of differences in local wiring costs has been reported in autism previously (6). Our study thus replicates prior findings in an independent sample of males and females, and children and adolescents, generalizing our findings to the wider autism spectrum.

Differences in intrinsic brain connectivity have previously been linked to a differential (i.e. a more non-uniform) expansion of the cortical surface (57), and a divergent growth of the brain in autism, particularly in frontal and temporal lobes, that may be driven by an accelerated cortical expansion of the surface and might lead to a higher degree of folding (57–59). In turn, the differential cortical expansion in autism may be related to the increased density of minicolumns and therefore to a reduced neuropil space (60,61), which mainly contains axons of GABA-ergic inhibitory interneurons (62). Consequently, this reduction in neuropil space may contribute to an imbalance between excitation to inhibition (i.e. E/I imbalance) (63,64), and a perturbed

formation of the brain's neurocircuitry, which has also been implicated in other neuropsychiatric conditions such as schizophrenia (65). Moreover, our analyses within age-stratified subgroups showed that differences in intrinsic wiring costs are age-dependent and may therefore be sensitive to different neurodevelopmental stages. However, while intrinsic measures of brain connectivity have been related to specific aspects of the cortical architecture, it is important to note that the wiring costs examined in this study are a theoretical measure, i.e., do not represent the actual length of connections per se, but rather indicate the 'wiring potential' within the cortical sheet. Within this framework, reduced global wiring costs may imply an facilitated development of shorter cortico-cortical fibers at the cost of longer connections (23), supporting the notion of a preference for local over global information processing in autism (66). Regions with reduced global wiring costs partially overlapped with regions previously shown to be functional overconnected in ASD (67,68), thus suggesting that reduced intrinsic wiring might also enhance the degree of functional connectivity. Furthermore, studies employing diffusion tensor imaging have reported reduced white matter connectivity in autism (69), which may suggest that the formation of the grey- and white-matter neurocircuitry of the brain in autism are linked (see also (70)).

Next, we examined the relationship between differing wiring costs and the severity of autism traits within the autism group. Notably, there were no significant correlations between significant clusters of MSDs and the severity of autism traits. Global features of intrinsic brain connectivity that cut across functionally specialized brain areas, may therefore not be specific enough to clearly delineate specific symptom domains. Differences in local wiring costs, which we observed in frontal, temporal and parietal regions were significantly correlated with the severity of social

and repetitive traits as measured by ADOS (36), ADI-R (37), RBS-R (40), SSP (39), and SRS-2 (38). Many of these brain regions are part of the large-scale neurocognitive networks underpinning autism traits, e.g., the 'social brain' network (71) and the cortico-striatal-thalamo circuitry (72) that has previously been associated with repetitive behavior in autism (73–75). However, it is important to note that the effect sizes associated with these brain-behavioral correlations are small, as these regions represent isolated components of the wider neurocognitive networks underpinning autism. Thus, future research employing spatially-unbiased vertex-wise approaches is needed to link differences in wiring costs to specific symptom domains.

Last, we leveraged the spatial gene expression data provided by the AHBA (24) to establish whether the patterns of differences in wiring cost also map onto the putative etiological mechanisms underpinning autism. Overall, we found that the pattern of differences in MSDs, perimeter function, and radius function were enriched for genes previously implicated in the etiology of autism by genetic and transcriptomic studies. For example, we observed an enrichment of genes known to be downregulated in the post-mortem cortex in autism, e.g. CTX.down.M10, CTX.down.M4, and CTX.down.M16, that map onto GO terms 'synaptic functioning' and 'neuronal genes' (45,46). Additionally, we tested for an enrichment of gene co-expression modules underpinning typical brain development based on the spatio-temporal transcriptomic atlas provided by Kang et al. (49) observing an enrichment of modules 2 and 15 for MSD and radius function, which are linked to the GO term 'synaptic transmission'. Based on the E/I imbalance hypothesis, we tested for an enrichment of specific cell types (47). However, against our hypothesis of an involvement of inhibitory interneurons in particular, the pattern of differences we observed for MSDs, perimeter function and radius function were more closely

386 associated with the spatial gene expression patterns expressed in excitatory neurons. This implies
387 that differences in global and local wiring costs cannot be attributed to intraneuronal gene
388 expression signatures exclusively. Future histological studies are therefore needed to identify the
389 specific neuro-architectural underpinning of our *in vivo* findings. We also functionally annotated
390 the genes enriched within the MSD, perimeter function and radius function via pathway/process
391 enrichment analysis using Metascape (76), where we observed a functional enrichment of several
392 KEGG pathways and GO terms, i.e., 'Nervous system development', 'Neuronal system' (48), and
393 'synaptic signaling' (77). This is in line with previous genetic studies reporting a dysregulation of
394 axonal growth and guidance in autism, and an atypical formation and functioning of synaptic
395 connections (15–17). Thus, although it is difficult to determine whether imaging phenotypes are
396 the cause or the result of autism, our finding of an enrichment of autism-associated genes
397 suggests that intrinsic wiring costs are related to the genetic underpinnings of autism and might
398 therefore be etiologically relevant.
399 There are several limitations to our study. First, as noted above, our study did not access intrinsic
400 connections directly but estimated these using wiring costs. Wiring costs should therefore be
401 considered a proxy measure representing the estimated 'wiring potential' of brain regions rather
402 than the actual connection length (6). Second, we utilized the AHBA to link differences in brain
403 connectivity to putative mechanisms which is the most comprehensive gene expression data set
404 to date (24). However, the gene expression atlas contains data from adult donors exclusively
405 while our sample also includes children and adolescents. Moreover, the coverage of the gene
406 expression data within the AHBA is significantly lower than the spatial resolution of the
407 neuroimaging data. Third, so far, we looked at between-group differences exclusively, which

typically have small effects due to the large heterogeneity of autism. In the future, it will be important to determine how such markers may be used to parse heterogeneity in autism, and whether putative neurobiological subgroups converge onto distinct clinical phenotypes and/or neurodevelopmental outcomes that could aid clinical decisions and facilitate personalized support approaches.

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Table 1: Table of autistic participants and non-autistic control subjects. Age ranged from 6 to 30 in the autistic group and the non-autistic group. Full-scale IQ ranged from 40 to 148 in the autistic group and from 50 to 142 in the non-autistic group. The ADI scores from social interaction scale ranged from 0 to 29, from the communication scale from 0 to 26 and from the restricted and repetitive behaviors scale from 0 to 12. The ADOS scores ranged from 2 to 10 for the total score, from 3 to 10 for the social affect score and from 1 to 10 for the restricted and repetitive score. ADI-R = Autism Diagnostic Interview-Revised; ADOS= Autism Diagnostic Observation Schedule, SRS = Social Responsiveness Scale-2, RBS-R = Repetitive Behaviors Scale- Revised, SSP = Short Sensory Profiles.

Variable	Non-autistic Group (N=279)		Autistic Group (N=359)		Statistic		
	N	%	N	%	χ^2	df	p
Sex					4.72	1	0.03*
Male	178	63.8	258	71.9			
Female	101	36.2	101	28.1			
	Mean	SD	Mean	SD	t	df	p
Age (years)	17.35	5.9	17.49	5.51	0.3	577	0.76
Full-scale IQ	104.75	18.25	98.92	19.76	-3.86	617	0.0001*
ADI-R scores							
Social interaction			16.7	6.69			
Communication			13.24	5.63			
Restricted and repetitive behaviour			4.3	2.66			
ADOS scores							
Social affect			6.12	2.6			
Restricted and repetitive behaviour			4.63	2.7			
SRS-2			70.11	12.1			
RBS-R			16.34	13.94			
SSP			139.43	27.27			
Total surface area	228532	22854.8	228852	24246.4	0.17	613	0.86
Mean separation distance	122.94	5.61	122.91	5.71	-0.05	602	0.95
Perimeter Function	291.39	14.98	291.89	15.13	0.42	600	0.67
Radius Function	41.08	1.92	41.09	1.93	0.04	600	0.97

Figure 1: Neuroanatomical results. Panel A shows the t statistics for the unthresholded contrast autism against non-autistic controls for MSD, perimeter function and radius function, respectively. Panel B shows clusters with significantly increased (orange) and decreased (blue) MSD, perimeter function and radius function in autism relative to controls (random-field-theory-based cluster corrected $p<0.05$, two-tailed). Panel C shows the effect sizes associated with individual model terms. MSD = Mean separation distance; ASD =Autism Spectrum Disorder; SA = Total Surface Area; FSIQ = Full-scale IQ, L = left, R = right.

Figure 2: Neuroanatomical results within age groups.

Clusters are significantly increased (orange) and decreased (blue) for MSDs, perimeter function and radius function relative to controls (random field-theory-based cluster corrected $p<0.05$, two-tailed). Panel A highlights differences in the adult group, i.e. > 18 years. Panel B highlights differences in the adolescent group, i.e. 12 to 18 years. Panel C highlights differences within the children group, i.e. < 12 years. Panel D highlights differences in the adult and adolescent group, i.e. > 12 years with an IQ < 75. MSD = Mean separation distance.

Figure 3: Gene set enrichment analysis. Panel A shows significant odds ratios at a false discovery rate (FDR) corrected p threshold of 0.01 resulting from the gene set enrichment analyses for genes expressed in the different t-maps for MSD, perimeter function and radius function, respectively. Gene sets were subdivided into sets with differential gene expression in autism and sets representing ASD risk genes that contain either common variants (ASD.risk.common) or rare de novo variants (ASD.risk.DeNovo). Gene sets are annotated and labeled based on their original

publication. CTX = cortex; DEG = differentially expressed gene; down = down-regulated expression in autism; up = upregulated expression in autism. Panel B shows genes sets taken from different KEGG pathways. Axon_Gui = Axonal Guidance, Cal_Sig = Calcium Signaling pathway, Cell_Adh = Cell Adhesion, Endocan_Sig = Retrograde Endocannabinoid Signaling, Neurodeg = Pathways of Neurodegeneration. Panel C shows set enrichment of genes mediating typical brain development as reported in the spatiotemporal transcriptome data set provided by Kang et al. (47). Set names contain their respective coexpression module label (e.g., M1), followed by their functional description based on their Gene Ontology term enrichment. Panel D shows spatiotemporal expression profiles of brain gene modules significantly enriched in the MSD map for module 2 (left panel) enriched for genes implicated in synaptic transmission, and for module 15 (right panel) enriched for synaptic transmission. The x-axis shows the developmental time frame (pcw=postconception weeks) and the y-axis shows the different brain regions: OFC=orbital prefrontal cortex; DFC=dorsolateral prefrontal cortex; VFC=ventrolateral prefrontal cortex; MFC=medial prefrontal cortex; M1C=primary motor (M1) cortex; S1C=primary somatosensory (S1) cortex; IPC=posterior inferior parietal cortex; A1C=primary auditory (A1) cortex; STC=superior temporal cortex; ITC=inferior temporal cortex; V1C=primary visual (V1) cortex; HIP=hippocampus; AMY=amygdala; STR=striatum; MD=mediodorsal nucleus of the thalamus; CBC=cerebellar cortex.

Figure 4: Cell type enrichment analyses. Panel A is a schematic illustration of cell types in germinal zones of the developing cortex, adapted from Polioudakis et al. (50). CP=cortical plate; Cpi=inner cortical plate; Cpo=outer cortical plate; SP=subplate; IZ=intermediate zone;

SVZ=subventricular zone; iSVZ=inner subventricular zone; oSVZ=outer subventricular zone; VZ=ventricular zone; RG=radial glia; IP=intermediate progenitor; MN=newborn migrating excitatory neuron; EN=excitatory neuron; IN=interneuron; O=oligodendrocyte precursor; E=endothelial cell; P=pericyte; M=microglia. Panel B shows cell-type enrichment odds ratios and associated -log10(q) values for gene sets expressed in the t-map for MSD. Cell types are colored and labeled based on Polioudakis et al. (50) (see also Figure 4A). MP=mitotic progenitor; OPC=oligodendrocyte precursors; CGE/MGE=caudal and medial ganglionic eminence-derived interneurons; IP=intermediate progenitors; oRG/vRG=outer and ventricular radial glia. Panel C shows cell-type enrichment odds ratios and associated -log10(q) values for gene sets expressed in the t-map for perimeter function. Panel D shows cell-type enrichment odds ratios and associated -log10(q) values for gene sets expressed in the t-map for radius function.