Title: Inter-individual differences in cortical thickness and their genomic underpinnings in autism spectrum disorder

Authors: Christine Ecker, PhD [1,2,*]; Charlotte M Pretzsch, PhD [2]; Anke Bletsch, PhD [1]; Caroline Mann, PhD [1]; Tim Schaefer, PhD [1]; Sara Ambrosino, PhD [3]; Julian Tillmann, PhD [4]; Afsheen Yousaf, PhD[1]; Andreas Chiocchetti, PhD [1], Michael V Lombardo, PhD [5,6]; Varun Warrier, PhD [6]; Nico Bast, PhD [1]; Carolin Moessnang, PhD [7,8]; Sarah Baumeister, PhD [7,8]; Flavio Dell'Aqua, PhD [2]; Dorothea L. Floris, PhD [9]; Mariam Zabihi, PhD [9]; Andre Marquand, PhD [9]; Freddy Cliquet, PhD [10]; Claire Leblond, PhD [10]; Clara Moreau, PhD [10]; Nick Puts, PhD [2]; Tobias Banaschewski, MD, PhD [8]; Emily Jones, PhD [11]; Luke Mason, PhD [11]; Sven Bölte, PhD [12,13]; Andreas Meyer-Lindenberg, MD, PhD [7]; Antonio Persico, MD [14]; Sarah Durston, PhD [3]; Simon Baron-Cohen, PhD [6]; Will Spooren, PhD [15]; Eva Loth, PhD [2]; Christine M Freitag, MD, PhD [1]; Tony Charman, PhD [4]; Guillaume Dumas, PhD [10]; Thomas Bourgeron, PhD [10]; Christian F. Beckmann, PhD [9]; Jan K. Buitelaar, MD, PhD [9]; the EU-AIMS LEAP Group; Declan G. Murphy, MD [2]

Affiliations:

(1) Department of Child and Adolescent Psychiatry, University Hospital, Goethe University, Frankfurt am Main, Germany
(2) Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK
(3) Department of Psychiatry, University Medical Center Utrecht Brain Center, Utrecht University, Utrecht, The Netherlands
(4) Clinical Child Psychology, Department of Psychology, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK
(5) Laboratory for Autism and Neurodevelopmental Disorders, Center for Neuroscience and Cognitive Systems @UniTn, Istituto Italiano di Tecnologia, Rovereto, Italy
(6) Autism Research Centre, Department of Psychiatry, University of Cambridge, Cambridge

(7) Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany
(8) Department of Child and Adolescent Psychiatry, Central Institute of mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany
(9) Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands
(10) Human Genetics and Cognitive Functions Unit, Institut Pasteur, UMR3571 CNRS, University de Paris, F-75015, Paris, France
(11) Centre for Brain and Cognitive Development, Birkbeck, University of London
(12) Center for Neurodevelopmental Disorders (KIND), Center for Psychiatry Research, Department of Women's and Children's Health, Karolinska Institutet & Stockholm Health Care Services, Region Stockholm, Sweden
(13) Department of Child and Adolescent Psychiatry, Stockholm Health Care Services, Region Stockholm, Sweden
(14) Department of Child and Adolescent Neuropsychiatry, "Gaetano Martino' University Hospital, University of Messina, Messina, Italy
(15) Roche Pharmaceutical Research and Early Development, NORD Discovery and Translational Area, Roche Innovation Center Basel, Switzerland

* corresponding author: Dr. Christine Ecker, Department of Child and Adolescent Psychiatry, Deutschordenstrasse 50, University Hospital, Goethe University, 60528 Frankfurt am Main; Tel: + 44 (0)69 6301 84705; Email: christine.ecker@kgu.de

Number of words: ~4500 excluding references

Number of tables: 1

Number of figures: 4

Keywords: autism spectrum disorder, brain anatomy, cortical thickness, genetics

Abstract

Objective. Autism Spectrum Disorder (ASD) is accompanied by highly individualized neuroanatomical deviations that potentially map onto distinct genotypes and clinical phenotypes. However, the link between biological pathways and differences in brain anatomy, which may pave the way towards targeted therapeutic interventions, remains poorly understood.

Methods. Our study examined neurodevelopmental differences in cortical thickness (CT) and their genomic underpinnings in a large and clinically diverse sample of 360 individuals with ASD and 270 typically developing controls (aged 6–30 years) within the EU-AIMS Longitudinal European Autism Project (LEAP). We also examined neurodevelopmental differences and their potential pathophysiological mechanisms between clinical ASD subgroups, which differed in the severity and pattern of sensory features.

Results. In addition to significant between-group differences in 'core' ASD brain regions (i.e. fronto-temporal and cingulate regions), we found that ASD individuals manifested as neuroanatomical outliers within the neurotypical CT range in a wider neural system, which was enriched for genes known to be implicated in ASD on the genetic and/or transcriptomic level. Within these regions, the individuals' total (i.e. accumulated) degree of neuroanatomical atypicality was significantly correlated with the higher polygenic scores for ASD, and other psychiatric conditions, and scaled with measures of symptom severity. Differences in CT deviations were also associated with distinct sensory subgroups, especially in brain regions expressing genes involved in excitatory rather than inhibitory neurotransmission.

Conclusions. Our findings corroborate the link between macroscopic differences in brain anatomy and the molecular mechanisms underpinning heterogeneity in ASD, and provide future targets for stratification and subtyping.

Introduction

There is increasing recognition that major psychiatric conditions are highly heterogeneous at both the causative and phenotypic level (1). Assessing this heterogeneity is crucial in allowing more precise inferences about the aetiological mechanisms underpinning these conditions, and to pave the way towards better targeted (i.e. personalized) therapeutic interventions. This particularly applies to Autism Spectrum Disorder (ASD), which is a common neurodevelopmental condition that is clinically characterized by (i) atypical social communication and interaction, (ii) the presence of rigid, repetitive and stereotyped behaviors, and (iii) atypical sensory processing (2). These core ASD symptoms emerge alongside atypical anatomical development of several neurocognitive systems (3,4). Regional neuroanatomical differences in ASD, however, typically have small effect sizes (5), and reflect highly variable patterns of neurodevelopmental deviations across individuals (6). This makes it inherently difficult to link atypical brain structure to molecular and pathophysiological mechanisms.

Few studies to date have explored the genomic mechanisms underpinning atypical neurodevelopment in ASD. Notably, a recent study by Romero-Garcia et al. (2019) demonstrated that differences in cortical thickness (CT) during childhood are robustly associated with genes involved in synaptic transmission pathways that are known to be downregulated in the post-mortem ASD cortex (7,8). This study represents an important step towards 'closing the gap' between molecular and macroscopic pathology in ASD, and highlights the potential of *in vivo* neuroimaging markers for patient stratification and subtyping. However, the study only examined a narrow range of individuals across the autism spectrum, i.e. 10-year-olds with an average full-scale IQ (FSIQ). It therefore remains unknown if – and how – these findings generalize to the wider autism phenotype that is highly heterogeneous not only between individuals meeting diagnostic criteria (9), but also within individuals across development (10).

In the current study, we explored inter-individual differences in CT and their potential molecular underpinnings in a large, clinically diverse sample of ASD individuals and controls within the EU-AIMS Longitudinal European Autism Project (LEAP; www.aims-2-trials.eu) (11). This study provides deep phenotypic assessments and genotypic data of more than 700 individuals, including males and females between 6

and 30 years, with a wide range of intellectual abilities and varying degrees of symptom severity (9). Rather than exclusively focusing on 'core' ASD regions (i.e. regions with a significant between-group difference), we based our investigation on local (i.e. regional) and global (i.e. cortex-level) neuroanatomical deviations from the neurotypical range of CT in our sample. A similar approach has previously been used to assess neuroanatomical heterogeneity in ASD (6,12), and for the biologically-driven stratification of ASD into putative subtypes (13). Here, we used the technique to (i) identify a wider set of brain regions where being a neuroanatomical outlier significantly impacts on the probability (i.e. risk) of ASD in our sample, and (ii) to link patterns of neuroanatomical variability to ASD symptomatology and genetic risk factors.

More specifically, leveraging the spatial gene expression data of the Allen Human Brain Atlas (AHBA; (14)), we tested the hypothesis that brain regions associated with high neuroanatomical variability in ASD express more than expected genes that (i) are enriched for rare (15) or common ASD risk variants (16), and/or (ii) are abnormally expressed in ASD (8,17,18). Moreover, using a virtual histology approach (19), we probed the potential molecular mechanisms underpinning clinical heterogeneity in ASD via patterns of neurodevelopmental deviations that differed between distinct sensory ASD subgroups (20). We focused on sensory symptoms as these have previously been highlighted as promising candidates in parsing heterogeneity in ASD (21). Moreover, sensory features have aetiologically been linked to an imbalance between excitation and inhibition (E/I) (22,23), which provides a reasonable conceptual framework for hypothesis testing.

Materials and Methods

Participants

This study utilized data provided by the EU-AIMS Longitudinal European Autism Project (LEAP), a multicenter transdisciplinary study on stratification biomarkers for ASD (www.eu-aims.eu). A comprehensive description of the sample has been published elsewhere (9). In brief, the total sample for which usable structural MRI data was available included N=360 ASD individuals and N=279 controls, split in N=274 typically developing (TD) participants and N=25 individuals with mild intellectual disability (ID) (14 males and 15 females defined by FSIQ between 50 and 74), between the ages of 6 and 30 years (Table 1, SF1). A full list of in- and exclusion criteria, clinical assessments, and medication status is provided in the Supplement. An independent ethics committee approved the study. Written informed consent was obtained for all participants.

MRI Data Acquisition

All participants were scanned with an MRI scanner operating at 3T at 6 different sites (University of Cambridge and King's College London, UK; Mannheim University, Germany; Radboud University and Utrecht University, Netherlands; Rome University, Italy). High-resolution structural T1-weighted volumetric images were acquired with full head coverage, at 1.2mm thickness with 1.2x1.2mm in-plane resolution (see ST2 for details).

Cortical Surface Reconstructions using FreeSurfer

Usable structural MRI data was initially available for N=709 individuals in the LEAP sample. FreeSurfer v6.0.0 software (http://surfer.nmr.mgh.harvard.edu/) was used to derive models of the cortical surface for each T1-weighted image. These well-validated and fully automated procedures have been described extensively elsewhere (24, 25). Each reconstructed surface underwent strict quality assessments (see Supplement), resulting in a final sample of N=639. We examined measures of CT, which represent the closest distance from the outer (i.e. pial) to the inner (i.e. white)

matter boundary at each vertex on the tessellated surface (26), smoothed using a 15-mm kernel (see Supplement for details). For each participant, we also computed mean CT across the cortex (C_0).

Surface-based statistical analyses of CT

Statistical analyses were conducted using the SurfStat toolbox (http://www.math.mcgill.ca/keith/surfstat) for Matlab (R2017b; MathWorks) and R for Statistical Computing (www.r-project.org). Vertex-wise between-group differences in CT (Y) were examined by regression of a general linear model (GLM) with (i) diagnostic group, sex, and acquisition site as fixed-effects factors (see Supplement for site effects), and (ii) linear and quadratic age, FSIQ, und CT_0 as continuous covariates, i.e.

$$Y_i=\beta_0+\beta_1 Group+\beta_2 Sex+\beta_3 Age+\beta_4 Age^2+\beta_5 FSIQ+\beta_6 Site+\beta_7 CT_0+\epsilon_i$$

, where ϵ_i is the residual error at vertex i. Between-group differences were estimated from the coefficient β_1, normalized by the standard error. All continuous covariates were mean centred across groups. Corrections for multiple comparisons were performed using 'random field theory' (RFT)-based cluster analysis for non-isotropic images with a cluster-based significance threshold (p_{clust})<0.05 (2-tailed, (27)). Effect sizes associated with each model term were assessed using *Cohen's f*, where a value of 0.1, 0.25, and 0.4 indicates a small, medium, and large effect respectively. At each vertex, we also used a *Levene's test* to assess between-group differences in CT variability at a False Discovery Rate (FDR)-corrected p-value (p_{adj})<0.05. Brain-behavior correlations were examined using *Pearson's r*.

To quantify neuroanatomical deviations from the neurotypical CT range, we fitted a GLM within the neurotypical controls without ID that included age, sex, FSIQ, site, and CT_0 as predictors (X) (see Supplement for effects of ID and sex). The model coefficients (β_{TD}) were subsequently utilized to predict CT for ASD individuals and ID-controls ($\hat{Y}=X\beta_{TD}$). The resulting residuals ($res=Y-\hat{Y}$) were centered and scaled based on the neurotypical CT distribution, thus expressing all data in unit standard deviations of the predicted neurotypical mean (z_{res}). Based on these deviations, we identified vertex-level outliers defined as CT values falling outside the neurotypical

90% Prediction Interval ($PI_{90\%}$) (see Supplement on PI threshold). This resulted in an *n*-by-*p* matrix of either zeros (inside $PI_{90\%}$) or ones (outside $PI_{90\%}$), where *n* denotes participants and *p* vertices. Based on the outlier matrix, vertex-wise estimates of the model's in-sample (i) sensitivity, i.e. probability of being a neuroanatomical outlier given an individual has ASD ($p(PI_{90\%,out}|ASD)$), (ii) specificity, i.e. probability of being inside the neurotypical $PI_{90\%}$ for non-ASD individuals ($p(PI_{90\%,in}|TD)$), (iii) positive predictive value (PPV), i.e. probability of ASD for individuals outside the neurotypical $PI_{90\%}$ ($p(ASD|PI_{90\%,out})$), and (iv) negative predictive value (NPV), i.e. probability of not having ASD for individuals inside the neurotypical $PI_{90\%}$ ($p(TD|PI_{90\%,in})$) were identified. At each vertex, we also compared the sample prevalence of ASD (pre-test probability) with the probability of ASD given an individual falls outside the neurotypical $PI_{90\%}$ (post-test probability).

The individuals' accumulated degree of neuroanatomical atypicality was summarized in a *subject-level total neuroanatomical atypicality index* (tAI_s), which indicates the percentage of vertices outside the neurotypical $PI_{90\%}$ per individual. The tAI_s were computed based on (i) all vertices on the cortical surface, and (ii) within an outlier mask that only included vertices with a significant χ^2-enrichment of ASD outside the neurotypical $PI_{90\%}$ (i.e. $p(ASD|PI_{90\%,out})>p(ASD|PI_{90\%,in})$). The tAI_s were subsequently used for the comparison between groups using a *t-test* for independent samples ($p<0.05$), and for the prediction of diagnostic categories using a logistic regression model. Moreover, using *Pearson's r*, we examined the global impact of neuroanatomical deviations on ASD symptomatology across DSM-5 symptom domains. Last, we tested the hypothesis that the individual's neuroanatomical load is significantly correlated with the polygenic risk for ASD, and other psychiatric conditions.

Genetic analyses

Genome-wide polygenic scores (PGS_{genome}) for ASD (16) and a variety of other psychiatric conditions and phenotypic traits (e.g. ADHD (28), schizophrenia (29), depression (30), and epilepsy (31)) were derived as outlined in the Supplement. In addition, using the PRSet function in PRSice-2 (https://www.prsice.info (32)), we

derived gene set-based polygenic scores (PGS_{set}) for ASD (using the GWAS summary statistics by (16)) across gene sets that are highly expressed within brain areas highlighted by our neuroimaging findings (see Supplement for details). To this aim, we performed a gene expression decoding analysis (GEDA) within Neurosynth and NeuroVault (33) to identify genes whose spatial expression patterns resembles our neuroimaging findings (see Supplement for details). In brief, this analysis utilizes the gene expression data from the Allen Human Brain Atlas (AHBA (14)) to statistically assess the spatial correlation between our neuroimaging maps (*t*-map, *Cohen's f*-map, χ^2-outlier maps) and the patterns of expression for each of 20,787 protein coding genes. To do so, the six AHBA donor brains are initially co-registered with the neuroimaging data to bring normalized gene expression values into transcriptomic alignment with the FreeSurfer surface overlays (see SF20). A linear model is then constructed for each donor brain, where the slopes encode the spatial correlation between each gene's expression pattern and the values contained in the statistical maps at each probe (i.e. sampling site). The slopes are then subjected to a one-sample *t-test* to identify genes whose expression patterns are consistently (i.e. across donor brains) highly similar to the imaging maps.

The resulting gene lists for each statistical map were thresholded at $p<0.01$. We chose this liberal threshold as this analysis did not constitute a hypothesis test *per se*, but rather a selection step to provide a list of candidate genes. This list was subsequently tested for enrichment with genes previously implicated in ASD in genetic and transcriptomic studies. At the genetic level, this included ASD risk genes with *de-novo* and rare variants (15), and GWAS-significant ASD risk genes with common variants (16). At the level of differential gene expression, we tested gene-lists that are (i) differentially expressed (i.e. upregulated/downregulated) in post-mortem cortical tissue (17), and in specific neuronal cell types in ASD (18), and (ii) genes of differentially expressed co-regulated modules in ASD (8,34). We also included the ASD-gene list compiled by the SFARI database (categories S,1,2,3 downloaded November 2020 from https://gene.sfari.org/). Notably, these gene sets are partially overlapping (see SF22 for number of total and intersecting genes).

In addition, we tested for an enrichment of genes underpinning typical brain development via the human brain transcriptome dataset provided by Kang et al. (2011),

which covers transcriptome profiles of 16 different brain regions from embryonic development to late adulthood (35). For this purpose, 2D heatmaps representing the time course of gene expression across different brain regions were created based on the module 'eigengene', as implemented in the MAGMA pipeline (https://github.com/SheenYo/MAGNET (36)). All enrichment testing was performed using the GeneOverlap package in R (10.18129/B9.bioc.GeneOverlap), which generated enrichment Odds Ratios (OR), hypergeometric *p*-values, and FDR-corrected *p*-values (p_{adj}). Only comparisons with p_{adj}<.05 were interpreted further. To establish the relative impact of differentially expressed genes (DEGs) and ASD risk genes on the individuals' degree of neurodevelopmental perturbation, we predicted *tAIs* using set-based PGS across (i) gene sets with atypical expression that were significantly enriched in the χ^2-outlier map (PGS_{DGE}), and (ii) ASD risk genes with common and *de novo* variants (PGS_{risk}) (15,16) (see ST4 for details).

Neuroanatomical differences and genetic underpinnings of clinical ASD subgroups

To relate distinct clinical ASD phenotypes to different patterns of neuroanatomical deviations, we stratified ASD individuals based on the severity and profile of sensory symptoms. The subgroups were originally derived by (20) using a factor mixture modelling approach across questionnaire items of the Short Sensory Profile (SSP (37), see ST5 for summary scores), which resulted in three groups of 'sensory low' (N=209), 'sensory moderate' (N=37), and 'sensory severe' (N=18) ASD individuals in our sample. At each vertex, an F-test for the main effect of sensory subgroup was performed based on the individuals' standardized CT deviations (z_{res}) using an RFT-corrected *p*-value<0.05. Given the link between atypical sensory processing and E/I imbalance (22), we employed a virtual histology approach to relate regional differences between ASD subgroups to those in cell-specific gene expression (19). To this end, a GEDA of the resulting F-map was performed as outlined above. The list of significant decoded genes was subsequently tested for cell-type enrichment based on the Single-Cell Transcriptomic Atlas of Human Neocortical Development during Mid-gestation (38), which – among others – allowed us to test for an enrichment of genes representing different subtypes of excitatory and inhibitory cells.

Results

Participant Demographics

Overall, groups were matched for age, total brain volume, and CT_0 (see Table 1). However, ASD individuals had a significantly lower full-scale IQ (M=98.8, SD=20) than controls (M=104.8, SD=18, $t(617)$=-3.93, p<0.001). Our sample included more males with ASD than male controls ($\chi^2(1)=4.824, p<0.02$).

Vertex-wise between-group differences in cortical thickness

Individuals with ASD had increased CT relative to controls in the (i) bilateral anterior cingulate cortex (ACC, approximate Brodmann areas [BA] 24/33), (ii) bilateral anterior temporal lobes (BA 20/21/22/38/41/42), (iii) left lingual gyrus (BA 18/19), and (iv) right posterior cingulate cortex (PCC, BA 23/31). By contrast, decreased CT in ASD was observed in the left dorsolateral prefrontal cortex (DLPFC, BA 4/6/8/9) and precentral gyrus (BA 4/6), the right parahippocampal and fusiform gyrus (BA 18/19/34/37), the left temporal pole (BA 20/38), and in the right pre- and postcentral gyrus (BA 4/6) (see Figure 1a,b and ST2). In these brain regions, measures of CT within the ASD group were also significantly correlated with measures of symptom severity across DSM-5 symptom domains (Figure 1g,h).

Vertex-level effect sizes (*Cohen's f*) for the main effect of group were small overall, ranging from 0 to 0.174 across the cortex (M=0.042,SD=0.032, Figure 1c), which is consistent with small mean differences and significantly increased CT variability within the ASD group (Figure 1e,f). Largest effect sizes were observed in the bilateral temporal lobes and the ACC, as well as in anterior medial frontal and occipital regions (Figure 1d). Effect sizes for the main effect of group were also relatively low compared to the effects of other model terms such as total brain volume (M=0.477,SD=0.012), age (M=0.405,SD=0.168), and acquisition site (M=0.279,SD=0.130) (Figure 1c), each of which displayed a unique pattern of spatial variability (SF2).

Vertex-level deviations from the neurotypical distribution of CT

Based on the neurotypical $PI_{90\%}$ in our sample, we initially identified vertex-level neuroanatomical outliers. At any given vertex, maximally 22.2% of ASD individuals fell outside the neurotypical range (M=11.2%,SD=2.5% across vertices), with the highest proportion of outliers being observed in the bilateral temporal and medial prefrontal regions (Figure 2a, see SF26 for standard residual error). Moreover, there was considerable inter-individual variability with regards to the pattern and direction of neuroanatomical deviations observed, with some individuals displaying predominantly positive deflections, negative deflections, or a mixture of both (SF6).

Among all individuals outside the neurotypical $PI_{90\%}$, the probability of ASD was very high (i.e. positive predictive value (PPV)), with some vertices displaying a PPV of up to 95% (M=65.6%, SD=5.8%) (Figure 2b). More specifically, taking into account the sensitivity, specificity, and ASD sample prevalence (i.e. 0.56), we found that being a neuroanatomical outlier increased the risk of a diagnosis of ASD by as much as 38.2% in some brain regions (M=7.1%, SD=5.95%, Figure 2d). Consequently, there were a number of vertices with a significant χ^2-enrichment of ASD outside the neurotypical $PI_{90\%}$, which highlights brain regions where (i) the risk of ASD is significantly modulated by being a neuroanatomical outlier, and (ii) where ASD individuals have a higher 'outlier' probability than controls (see χ^2-outlier map in Figure 2e).

ASD individuals also had a significantly larger total degree of neuroanatomical abnormality (tAI_s) across the cortex ($t(609)=-7.123$,$p<0.001$), and within the χ^2-map of significant neuroanatomical outliers ($t(634)=-14.316$,$p<0.001$) (Figure 2f,g). This difference was sufficient to separate groups at an overall accuracy of 74.96% (64.79% across the cortex) in our sample (*Odd Ratio*=1.292, β_{tAI}=0.256, $p<0.001$), with a sensitivity of 76.94% and a specificity of 72.40% (PPV=78.25%, NPV=70.88%), where the negative predictive value (NPV) indicates the probability of not having ASD for individuals inside the neurotypical $PI_{90\%}$. Notably, 84% of the ID controls were identified as neuroanatomical outliers. Thus, while our tAI_s-model seems highly sensitive to neurodevelopmental deviations, the detected outliers are not specific to ASD but may include other conditions associated with atypical neurodevelopment. Measures of tAI_s were also significantly correlated with symptom severity in the ADI

social domain (r=0.142,p_{adj}<0.05), the SRS-2 (r=0.18,p_{adj}<0.05), the RBS-R (r=0.25,p_{adj}<0.05), and the SSP (r=0.25,p_{adj}<0.05).

Gene expression decoding and enrichment analyses

To link CT variability to etiological mechanisms, we utilized the AHBA to identify genes with a spatial pattern of expression resembling our imaging maps. This resulted in a set of N=546, 408, and 662 significant genes for the *t*-map of between-group differences, *Cohen's f*-map of statistical effects, and χ^2-map of neuroanatomical outliers, respectively (p<0.01, Figure 3a). Within these gene sets, we found an enrichment for genes known to be associated with ASD, and particularly for genes with differential gene expression (DGE) during childhood and adolescence (Figure 3b). More specifically, in the *Cohen's f*- and χ^2-map, we observed a significant enrichment for gene sets that are upregulated in ASD, namely M16.up (34), CTX.M9.up (8), CTX.M19.up (8), ASD.DEGs.up (17), and CTX.M20.up (8) (p_{adj}<0.05). These gene sets have previously been linked to Gene Ontology (GO) terms representing immune/inflammatory response and the development/regulation of cell differentiation (8, 17, 34). In contrast, the *t*-map of significant between-group differences showed a high expression of genes that are downregulated in ASD, e.g. M12.down (34) and ASD.DEGs.down (17), with represent genes underlying synaptic functioning and transmembrane transporter activity. There was no significant over-representation of ASD risk genes representing common (15) or rare and *de novo* variants (16).

Furthermore, we found an enrichment for gene co-expression modules underpinning typical brain development during the first decades of life (35). We observed a significant enrichment for co-expression modules representing 'synaptic transmission' (Module 2,15,14) and 'cell adhesion signaling' (Module M16), which have their highest level of expression during childhood and adolescence (35), and for 'nuclear function' Module 21 (Figure 3d). In agreement with the analysis of ASD-related genes, the χ^2-outlier map also displayed a high expression of genes in 'immune response' Module 4, which is highly expressed during childhood and adolescence (OR=7.46,p_{adj}<0.001). Module 4 was also significantly associated with the ASD genotype in our sample (β_{M4}=0.374,p_{adj}<0.01) (see Gene-Set Analysis in the

Supplement). Odds Ratios for all modules and adjusted *p*-values are displayed in Figure 3c.

Correlations between tAI$_s$ and polygenic scores for ASD

Based on the gene set-based PGS (PGS$_{set}$), we established that genetic variation in DEGs and co-expression modules enriched in the χ^2-outlier map (i.e. PGS$_{DGE}$) explained a larger percentage of neuroanatomical outliers (*tAIs*) than PGS$_{set}$ across ASD risk genes with common and *de novo* variants (i.e. PGS$_{ASD.risk}$) ($F(1)=5.99, p<0.05$ vs. $F(1)=1.39, p=0.23$, respectively) when the main effect of group was accounted for ($F(1)=142.56, p<0.001$) (Figure 3e). The number of ASD risk genes with common and *de novo* variants was, however, small overall, and only included a total of 1,455 SNPs in our sample (see ST4). We therefore also examined the association between genotype and phenotype based on genome-wide PGS. Measures of *tAIs* were significantly correlated with the genome-wide risk for ASD ($r=0.11, p_{adj}<0.05$), ADHD ($r=0.2, p_{adj}<0.01$), depression ($r=0.16, p_{adj}<0.01$), schizophrenia ($r=0.11, p_{adj}<0.05$), and neuroticism ($r=0.16, p_{adj}<0.01$) (Figure 3f). Thus, while patterns of CT are enriched for genes known to be implicated in ASD, composite measures of neuroanatomical atypicality significantly correlated not only with the risk for ASD, but also for other neurodevelopmental conditions.

Differences in neuroanatomical deviations across different sensory subgroups

Vertex-level neurodevelopmental CT deviations from the neurotypical mean (z_{res}) differed significantly between sensory symptom subgroups. There was a significant main effect of subgroup in the right premotor cortex (PMC) and supplementary motor area (SMA) (BA 6/8, $F_{max}=6.239, p_{clust}<0.05$), which included the fontal eye fields (Figure 4a,b). Here, ASD individuals in the 'moderate' and sensory 'severe' subgroups displayed a significantly larger proportion of negative CT deviations compared to 'sensory low' individuals ($\chi^2(2)=10.131, p<0.01$) (Figure 4c). Neuroanatomical deviations in this region were also significantly correlated with the 'movement sensitivity' subdomain of the SSP ($r=-0.186, p_{adj}<0.01$), which was the subdomain that differed the most between subgroups (Effect Size=2.52) (20) (Figure 4d). Last, we

found the *F*-map for the main effect of sensory subgroup to be enriched for genes expressed in excitatory neurons in the developing cortex (38) (Figure 4e), including an enrichment for migrating excitatory neurons (OR=6.057,p_{adj}<0.05) and excitatory neurons in deep layer 1 and 2 (OR=2.43 and 2.13, respectively, p_{adj}<0.05, Figure 4f).

Discussion

Our study confirms that ASD is accompanied by significant between-group differences in CT (e.g. in fronto-temporal and cingulate regions) that reflect highly individualized patterns of neurodevelopmental deviations overall. In addition to these 'core' ASD brain areas, we identified a wider spatially distributed network of regions where ASD individuals, and ID controls, manifested as neuroanatomical outliers. This network of regions was enriched for genes known to be upregulated in ASD during childhood and adolescence. Moreover, within this network, the individuals' total degree of neuroanatomical abnormality was significantly correlated with measures of symptom severity, as well as with the polygenic risk for ASD and other psychiatric conditions. Last, we demonstrate that distinct clinical ASD subgroups display different patterns of neurodevelopmental deviations, which map onto specific cell types in the developing cortex. Our study thus provides novel insights into the genetic and neurobiological mechanisms underpinning heterogeneity in ASD.

Our finding of significant between-group differences in CT aligns with previous investigations into the neuroanatomical underpinnings of ASD, where both increased and decreased CT has been reported predominantly in fronto-temporal and fronto-parietal regions (39–41). In these regions, measures of CT also undergo an abnormal developmental trajectory in ASD (42,43), with differences being most prominent during childhood and adolescence and diminishing during adulthood (44). The results of most prior studies in samples of N<200 have been highly variable with regards to the direction and pattern of CT differences observed. However, a more consistent picture is now emerging across an increasing number of large-scale investigations (N>500) – which typically exclusively report increased CT in ASD (e.g. (5,44). Our results thus differ from these latter studies in that we also observed CT reductions in the DLPFC and precentral gyrus. This discrepancy may be due partly to differences in sample

characteristics, preprocessing pipelines, and/or the composition of the GLM, e.g. we covaried for mean CT to account for global differences related to brain size (also see SF11). However, the pattern of effect sizes for the main effect of group is remarkably similar across studies (see also (12)), with the largest effects being reported in the STS, DLPFC, and PCC. Thus, taken together, the data from our study and others strongly supports the hypothesis that CT and related aspects of the vertical organization of the cortex (e.g. cortical lamination (45)) are atypical in ASD, and particularly in brain regions that are functionally linked to symptoms and traits (reviewed in (4)).

ASD individuals also manifested as neuroanatomical outliers in a number of brain regions that were not highlighted by the main effect of group, e.g. medial prefrontal regions, where falling outside the neurotypical $PI_{90\%}$ was associated with increased ASD risk in our sample. Within these brain regions, the pattern and degree (i.e. percentage) of vertex-level outliers varied considerably across individuals, with larger tAI_s being indicative of more severe symptoms across DSM-5 domains. However, being a neuroanatomical outlier based on tAI_s was not specific to ASD. For instance, 84% of the non-ASD individuals with mild ID fell outside the neurotypical range, and tAI_s not only correlated with the genome-wide polygenic risk for ASD (16) but also for ADHD (28), depression (30), and schizophrenia (29) – phenotypes which are also genetically correlated with ASD (16). Thus, while our 'composite' measure of neuroanatomical atypicality may be highly sensitive to deviations from the neurotypical range, the detected outliers are not specific to diagnostic labels, and may detect other conditions associated with atypical neurodevelopment. In a next step, we therefore also examined whether the patterns of CT differences we observe are linked to ASD etiology.

Similar to Romero-Garcia (2019), we found the *t-map* of statistical between-group differences to be enriched for genes and co-expression modules that are downregulated in the ASD cortex, which in turn code for synaptic and neuronal proteins (8). Some of these modules (e.g. M12 (34)) show a significant overrepresentation of known ASD-associated genes, which are typically expressed during early (i.e. prenatal) brain development and regulate gene expression at various developmental stages (15,46). In contrast, the χ^2-map of neuroanatomical outliers (and

Cohen's f effect size map) was enriched predominantly for genes upregulated in ASD, which map onto immune/inflammatory pathways (34) that are most active in the first decades of life (8). Set-based PGS across sets that are differentially expressed in ASD, and during typical brain development, also explained a larger proportion of tAI_S variability than PGS computed on ASD risk genes (15,16). This highlights that the *t*-map and χ^2-outlier map are intrinsically different, i.e. the *t*-map reflects 'group-level' differences, whereas the χ^2-map detects outliers within the neurotypical CT range and can therefore accommodate highly variable patterns of neuroanatomical deviations across individuals. Our findings thus agree with the notion that the effects of ASD-susceptibility genes on the brain are pleiotropic, mediated via gene regulatory mechanisms during childhood and adolescence, which result in highly individualized neuroanatomical patterns or 'fingerprints'. The examination of these patterns, rather than group differences, may therefore hold the key to stratifying and subtyping ASD.

To this aim, we further established that differences in neuroanatomical deviations are associated with distinct clinical ASD phenotypes that differed in the severity and pattern of sensory symptoms (20). These differences were primarily observed in brain regions subserving sensorimotor control (47), which has been reported to be dysregulated in ASD (48). In these brain regions, ASD individuals with moderate and severe sensory symptoms displayed predominantly negative CT deflections (i.e. >72%) compared to individuals with low sensory symptoms. ASD individuals might therefore be stratified into biologically more homogeneous subgroups not only based on their absolute phenotypic difference, but also depending on how – and to what extent – they deviate from the neurotypical mean. Brain regions that differed the most between subgroups, were also enriched for genes expressed in excitatory neurons in the developing cortex (38). Despite our sample size not allowing for the direct comparison between genotypes and phenotypes, our findings therefore support the notion that a disrupted E/I balance (22,49) might underpin some autism phenotypes (50), and link sensory symptoms to excitatory neurons in the developing cortex. Here, it is important to note that we examined measures of CT exclusively. Evidence suggests, however, that different aspects of the cortical architecture have distinct genetic determinants (51), contrasting phylogeny (52), and differing developmental trajectories (43). It will therefore be important to repeat our analyses using different

morphometric features (e.g. measures of surface area and/or cortical gyrification) to further characterize the complex etiology and neuropathology of ASD.

There are a number of additional limitations. Most importantly, as we did not perform an unbiased out-of-sample validation, our study is based on in-sample estimates of the neurotypical CT range rather than population norms. Even though we have examined the robustness of our results across variable models (see Supplement), it will be important to establish the model's generalization performance in the future using independent samples not only of ASD individuals but also neurotypical controls. Moreover, we examined neuroanatomical outliers based on the GLM, which made it possible to link group-differences to patterns of neuroanatomical deviations within the same framework. Future work is therefore needed to replicate our findings using alternative 'normative modelling' approaches, e.g. employing Gaussian Process Regression (13), that are not confined to linear relationships exclusively. Also, we examined measures of CT exclusively. Last, our GEDA was based on the AHBA, which is the most comprehensive gene-expression atlas to date. However, the AHBA is based on adult donors exclusively and provides a coverage that is significantly lower than the spatial resolution of our neuroimaging data. We therefore acknowledge the importance of repeating the analyses in high-resolution age-specific gene-expression datasets, once these become available, to corroborate the important link between molecular and macroscopic pathology in ASD.

References

1. Wardenaar KJ, Jonge P de: Diagnostic heterogeneity in psychiatry: towards an empirical solution. Bmc Med 2013; 11:201

2. Association AP: Diagnostic and statistical manual of mental disorders (5th ed.). 5th ed. Arlington, American Psychiatric Pub, 2013

3. Ecker C, Bookheimer SY, Murphy DGM: Neuroimaging in autism spectrum disorder: brain structure and function across the lifespan. Lancet Neurology 2015; 14:1121-1134

4. Amaral DG, Schumann CM, Nordahl CW: Neuroanatomy of autism. Trends Neurosci 2008; 31:137–145

5. Bedford SA, Park MTM, Devenyi GA, et al.: Large-scale analyses of the relationship between sex, age and intelligence quotient heterogeneity and cortical morphometry in autism spectrum disorder. Mol Psychiatr 2020; 25:614–628

6. Zabihi M, Oldehinkel M, Wolfers T, et al.: Dissecting the heterogeneous cortical anatomy of autism spectrum disorder using normative models. Biological Psychiatry Cognitive Neurosci Neuroimaging 2018; 4:567–578

7. Romero-Garcia R, Warrier V, Bullmore ET, et al.: Synaptic and transcriptionally downregulated genes are associated with cortical thickness differences in autism. Mol Psychiatr 2019; 24:1053–1064

8. Parikshak NN, Swarup V, Belgard TG, et al.: Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. Nature 2016; 540:423–427

9. Charman T, Loth E, Tillmann J, et al.: The EU-AIMS Longitudinal European Autism Project (LEAP): clinical characterisation. Mol Autism 2017; 8:27

10. Lord C, Bishop S, Anderson D: Developmental trajectories as autism phenotypes. Am J Medical Genetics Part C Seminars Medical Genetics 2015; 169:198–208

11. Loth E, Charman T, Mason L, et al.: The EU-AIMS Longitudinal European Autism Project (LEAP): design and methodologies to identify and validate stratification biomarkers for autism spectrum disorders. Mol Autism 2017; 8:24

12. Bethlehem RAI, Seidlitz J, Romero-Garcia R, et al.: A normative modelling approach reveals age-atypical cortical thickness in a subgroup of males with autism spectrum disorder. Commun Biology 2020; 3:486

13. Zabihi M, Floris DL, Kia SM, et al.: Fractionating autism based on neuroanatomical normative modeling. Transl Psychiat 2020; 10:384

14. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, et al.: An anatomically comprehensive atlas of the adult human brain transcriptome. Nature 2012; 489:391–399

15. Satterstrom FK, Kosmicki JA, Wang J, et al.: Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism. Cell 2020; 180:568-584.e23

16. Grove J, Ripke S, Als TD, et al.: Identification of common genetic risk variants for autism spectrum disorder. Nat Genet 2019; 51:431-444

17. Gandal MJ, Zhang P, Hadjimichael E, et al.: Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. Science 2018; 362:eaat8127

18. Velmeshev D, Schirmer L, Jung D, et al.: Single-cell genomics identifies cell type-specific molecular changes in autism. Science 2019; 364:685-689

19. Patel Y, Shin J, Drakesmith M, et al.: Virtual histology of multi-modal magnetic resonance imaging of cerebral cortex in young men. Neuroimage 2020; 218:116968

20. Tillmann J, Uljarevic M, Crawley D, et al.: Dissecting the phenotypic heterogeneity in sensory features in autism spectrum disorder: a factor mixture modelling approach. Mol Autism 2020; 11:67

21. Uljarević M, Baranek G, Vivanti G, et al.: Heterogeneity of sensory features in autism spectrum disorder: Challenges and perspectives for future research. Autism Res 2017; 10:703-710

22. Rubenstein JLR, Merzenich MM: Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003; 2:255-267

23. Puts NAJ, Wodka EL, Harris AD, et al.: Reduced GABA and altered somatosensory function in children with autism spectrum disorder. Autism Res 2017; 10:608-619

24. Dale AM, Fischl B, Sereno MI: Cortical Surface-Based Analysis I. Segmentation and Surface Reconstruction. Neuroimage 1999; 9:179-194

25. Fischl B, Sereno MI, Dale AM: Cortical Surface-Based Analysis II: Inflation, Flattening, and a Surface-Based Coordinate System. Neuroimage 1999; 9:195-207

26. Fischl B, Dale AM: Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc National Acad Sci 2000; 97:11050-11055

27. Worsley KJ, Marrett S, Neelin P, et al.: A unified statistical approach for determining significant signals in images of cerebral activation. Hum Brain Mapp 1996; 4:58–73

28. Demontis D, Walters RK, Martin J, et al.: Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. Nat Genet 2019; 51:63–75

29. Ripke S, Neale BM, Corvin A, et al.: Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014; 511:421–427

30. Howard DM, Adams MJ, Clarke T-K, et al.: Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. Nat Neurosci 2019; 22:343–352

31. Epilepsies ILAEC on C: Genetic determinants of common epilepsies: a meta-analysis of genome-wide association studies. Lancet Neurology 2014; 13:893–903

32. Choi SW, Mak TS-H, O'Reilly PF: Tutorial: a guide to performing polygenic risk score analyses. Nat Protoc 2020; 15:2759–2772

33. Gorgolewski1 KJ, Fox AS, Chang L, et al.: Tight fitting genes: Finding relations between statistical maps and gene expression patterns. F1000 Posters. 2014;5:1607.

34. Voineagu I, Wang X, Johnston P, et al.: Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 2011; 474:380–384

35. Kang HJ, Kawasawa YI, Cheng F, et al.: Spatio-temporal transcriptome of the human brain. Nature 2011; 478:483–489

36. Yousaf A, Duketis E, Jarczok T, et al.: Mapping the genetics of neuropsychological traits to the molecular network of the human brain using a data integrative approach. Biorxiv 2018; 336776

37. Tomchek SD, Huebner RA, Dunn W: Patterns of sensory processing in children with an autism spectrum disorder. Res Autism Spect Dis 2014; 8:1214–1224

38. Polioudakis D, Torre-Ubieta L de la, Langerman J, et al.: A Single-Cell Transcriptomic Atlas of Human Neocortical Development during Mid-gestation. Neuron 2019; 103:785–801.e8

39. Hyde KL, Samson F, Evans AC, et al.: Neuroanatomical differences in brain areas implicated in perceptual and other core features of autism revealed by cortical thickness analysis and voxel-based morphometry. Hum Brain Mapp 2010; 31:556–566

40. Hadjikhani N, Joseph RM, Snyder J, et al.: Anatomical Differences in the Mirror Neuron System and Social Cognition Network in Autism. Cereb Cortex 2006; 16:1276–1282

41. Ecker C, Ginestet C, Feng Y, et al.: Brain Surface Anatomy in Adults With Autism: The Relationship Between Surface Area, Cortical Thickness, and Autistic Symptoms. Jama Psychiat 2013; 70:59–70

42. Wallace GL, Dankner N, Kenworthy L, et al.: Age-related temporal and parietal cortical thinning in autism spectrum disorders. Brain 2010; 133:3745–3754

43. Ecker C, Shahidiani A, Feng Y, et al.: The effect of age, diagnosis, and their interaction on vertex-based measures of cortical thickness and surface area in autism spectrum disorder. J Neural Transm 2014; 121:1157–1170

44. Khundrakpam BS, Lewis JD, Kostopoulos P, et al.: Cortical Thickness Abnormalities in Autism Spectrum Disorders Through Late Childhood, Adolescence, and Adulthood: A Large-Scale MRI Study. Cereb Cortex 2017; 27:1721–1731

45. Pan Y-H, Wu N, Yuan X-B: Toward a Better Understanding of Neuronal Migration Deficits in Autism Spectrum Disorders. Frontiers Cell Dev Biology 2019; 7:205

46. Courchesne E, Gazestani VH, Lewis NE: Prenatal Origins of ASD: The When, What, and How of ASD Development. Trends Neurosci 2020; 43:326–342

47. Mosconi MW, Sweeney JA: Sensorimotor dysfunctions as primary features of autism spectrum disorders. Sci China Life Sci 2015; 58:1016–1023

48. Hannant P, Tavassoli T, Cassidy S: The Role of Sensorimotor Difficulties in Autism Spectrum Conditions. Front Neurol 2016; 7:124

49. Sohal VS, Rubenstein JLR: Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. Mol Psychiatr 2019; 24:1248–1257

50. Oliveira B, Mitjans M, Nitsche MA, et al.: Excitation-inhibition dysbalance as predictor of autistic phenotypes. J Psychiatr Res 2018; 104:96–99

51. Panizzon MS, Fennema-Notestine C, Eyler LT, et al.: Distinct Genetic Influences on Cortical Surface Area and Cortical Thickness. Cereb Cortex 2009; 19:2728–2735

52. Rakic P, Swaab DF: Defects of neuronal migration and the pathogenesis of cortical malformations. Prog Brain Res 1988; 73:15–37

Acknowledgements

We gratefully acknowledge the contributions of the EU-AIMS LEAP Group: Jumana Ahmad, Sara Ambrosino, Bonnie Auyeung, Tobias Banaschewski, Simon Baron-Cohen, Sarah Baumeister, Christian F. Beckmann, Sven Bölte, Thomas Bourgeron, Carsten Bours, Michael Brammer, Daniel Brandeis, Claudia Brogna, Yvette de Bruijn, Jan K. Buitelaar, Bhismadev Chakrabarti, Tony Charman, Ineke Cornelissen, Daisy Crawley, Flavio Dell'Acqua, Guillaume Dumas, Sarah Durston, Christine Ecker, Jessica Faulkner, Vincent Frouin, Pilar Garcés, David Goyard, Lindsay Ham, Hannah Hayward, Joerg Hipp, Rosemary Holt, Mark H. Johnson, Emily J.H. Jones, Prantik Kundu, Meng-Chuan Lai, Xavier Liogier D'ardhuy, Michael V. Lombardo, Eva Loth, David J. Lythgoe, René Mandl, Andre Marquand, Luke Mason, Maarten Mennes, Andreas Meyer-Lindenberg, Carolin Moessnang, Nico Mueller, Declan G.M. Murphy, Bethany Oakley, Laurence O'Dwyer, Marianne Oldehinkel, Bob Oranje, Gahan Pandina, Antonio M. Persico, Barbara Ruggeri, Amber Ruigrok, Jessica Sabet, Roberto Sacco, Antonia San José Cáceres, Emily Simonoff, Will Spooren, Julian Tillmann, Roberto Toro, Heike Tost, Jack Waldman, Steve C.R. Williams, Caroline Wooldridge, and Marcel P. Zwiers. This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115300 for the project EU-AUIMS and No 777394 for the project AIMS-2- TRIALS. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and AUTISM SPEAKS, Autistica, SFARI. The primary contact for the EU-AIMS LEAP Group is Declan G. Murphy (Email: pa-dmurphy@kcl.ac.uk). CE gratefully acknowledges support from the German Research Foundation (DFG) under the Heisenberg Programme (EC480/1-1 and EC480/2-1). DGM also acknowledges support from the NIHR Maudsley Biomedical Research Centre.

Disclosures

Jan Buitelaar has been in the past 3 years a consultant to/member of advisory board of/and/or speaker for Janssen Cilag BV, Takeda/Shire, Roche, Novartis, Medice, Angelini, and Servier. He is not an employee of any of these companies and not a stock

shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, and royalties. Sven Bölte discloses that he has in the last 5 years acted as an author, consultant or lecturer for Shire, Medice, Roche, Eli Lilly, Prima Psychiatry, GLGroup, System Analytic, Ability Partner, Kompetento, Expo Medica, and Prophase. He receives royalties for textbooks and diagnostic tools from Huber/Hogrefe, Kohlhammer and UTB. Tobias Banaschewski has served in an advisory or consultancy role for Actelion, Hexal Pharma, Lilly, Medice, Novartis, Oxford outcomes, Otsuka, PCM scientific, Shire and Viforpharma. He received conference support or speaker's fee by Medice, Novartis and Shire. He is/has been involved in clinical trials conducted by Shire and Viforpharma. He received royalties from Hogrefe, Kohlhammer, CIP Medien, and Oxford University Press. The present work is unrelated to the above grants and relationships. Andreas Meyer-Lindenberg has received consultant fees from American Association for the Advancement of Science, Atheneum Partners, Blueprint Partnership, Boehringer Ingelheim, Daimler und Benz Stiftung, Elsevier, F. Hoffmann-La Roche, ICARE Schizophrenia, K. G. Jebsen Foundation, L.E.K Consulting, Lundbeck International Foundation (LINF), R. Adamczak, Roche Pharma, Science Foundation, Sumitomo Dainippon Pharma, Synapsis Foundation–Alzheimer Research Switzerland, and System Analytics, and has received lectures fees including travel fees from Boehringer Ingelheim, Fama Public Relations, Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Janssen-Cilag, Klinikum Christophsbad, Göppingen, Lilly Deutschland, Luzerner Psychiatrie, LVR Klinikum Düsseldorf, LWL Psychiatrie Verbund Westfalen-Lippe, Otsuka Pharmaceuticals, Reunions i Ciencia S. L., Spanish Society of Psychiatry, Südwestrundfunk Fernsehen, Stern TV, and Vitos Klinikum Kurhessen. Will Spooren and Lindsey Mark Ham are employees at F. Hoffmann-La Roche Ltd. Julian Tillmann is a consultant for F. Hoffmann-La Roche Ltd. Simon Baron Cohen discloses that he has in the last 5 years acted as an author, consultant or lecturer for Shire, Medice, Roche, Eli Lilly, Prima Psychiatry, GLGroup, System Analytic, Ability Partner, Kompetento, Expo Medica, Clarion Healthcare, and Prophase. He receives royalties for textbooks and diagnostic tools from Huber/Hogrefe, Kohlhammer, and UTB. Tony Charman has received research grant support from the Medical Research Council (UK), the National Institute for Health Research, Horizon 2020 and the Innovative

Medicines Initiative (European Commission), MQ, Autistica, FP7 (European Commission), the Charles Hawkins Fund, and the Waterloo Foundation. He has served as a consultant to F. Hoffmann-La Roche Ltd and Servier. He has received royalties from Sage Publications and Guilford Publications. Declan Murphy has received consultancy fees from Roche and Servier, and grant support from the Medical Research Council (UK), the National Institute for Health Research, Horizon 2020 and the Innovative Medicines Initiative (European Commission).

Christine Ecker, Charlotte Pretzsch, Anke Bletsch, Caroline Mann, Tim Schaefer, Sara Ambrosino, Carolin Moessnang, Sarah Baumeister, Flavio Dell'Acqua, Dorothea L. Floris, Mariam Zabihi, Andre Marquand, Emily Jones, Luke Mason, Antonio Persico, Sarah Durston, Simon Caron-Cohen, Eva Loth, Guillaume Dumas, Christian F. Beckmann, Christian Beckmann, Jumana Ahmad, Bonnie Auyeung, Carsten Bours, Michael Brammer, Daniel Brandeis, Claudia Brogna, Yvette de Bruijn, Bhismadev Chakrabarti, Ineke Cornelissen, Daisy Crawley, Jessica Faulkner, Vincent Frouin, Pilar Garcés, David Goyard, Hannah Hayward, Joerg Hipp, Rosemary Holt, Mark H. JohnsonPrantik Kundu, Meng-Chuan Lai, Xavier Liogier D'ardhuy, Michael V. Lombardo, David J. Lythgoe, René Mandl, Maarten Mennes, Nico Mueller, Bethany Oakley, Laurence O'Dwyer, Marianne Oldehinkel, Bob Oranje, Gahan Pandina, Barbara Ruggeri, Amber Ruigrok, Jessica Sabet, Roberto Sacco, Antonia San José Cáceres, Emily Simonoff, Roberto Toro, Thomas Bourgeron, Heike Tost, Jack Waldman, Steve C.R. Williams, Caroline Wooldridge, and Marcel P. Zwiers reported no biomedical financial interests or potential conflicts of interest.

Figure Captions

Figure 1. Vertex-wise between-group differences in cortical thickness (CT). (**A**) Clusters with significantly increased (orange to yellow) and decreased (blue to cyan) CT in ASD relative to controls ('random field theory' (RFT)-based cluster corrected, $p<0.05$, two-tailed). (**B**) *t*-test statistic for the contrast ASD vs. control (un-thresholded). (**C**) Effect sizes associated with individual model terms across the cortex. (**D**) Spatially-distributed pattern of effects (*Cohen's f*) for the main effect of group. (**E**) Vertices with significantly increased variance in CT in ASD relative to controls resulting from a *Levene's test* of homogeneity of variances ('false discovery rate' (FDR)-corrected $p<0.05$). (**F**) Absolute vertex-level differences in CT variability between groups, (**G**) Clusters with significant bivariate Pearson correlation coefficients between CT and measures of symptom severity subdivided into Domain A and B of the 'Diagnostic and Statistical Manual of Mental Disorders' (DSM-5) within the ASD group. (**H**) Brain-behaviour correlations between clusters with significant differences in CT and measures of symptom severity subdivided into DSM-5 Domain A and B symptoms within the ASD group. Correlation coefficients marked in bold survive an FDR-corrected *p*-value <0.05. L: left hemisphere, R: right hemisphere, CT_0: mean cortical thickness , ACC: anterior cingulate cortex, STS: superior temporal sulcus, PCC: posterior cingulate cortex, *tAIs*: subject-level total neuroanatomical abnormality index, ADI: Autism Diagnostic Interview-Revised, RRB: repetitive/restricted behaviour, ADOS.SA/ADOS.RRB: Autism Diagnostic Observation Schedule Calibrated Severity Score for Social Affect (SA) and Restricted and Repetitive Behaviours (RRB), SRS-2: Social Responsiveness Scale-2, RBS-R: Repetitive Behaviors Scale – Revised, SSP: Short Sensory Profile, which was reversely scored so that larger values indicate more severe symptoms.

Figure 2. Vertex-level outlier statistics from the neurotypical range of cortical thickness (CT) predicted by age, sex, full-scale intelligence quotient (IQ), site, and mean CT across the cortex (CT_0). (**A**) Probability of falling outside the neurotypical 90% Prediction Interval ($PI_{90\%}$) given an individual has ASD. This equals the proportion of ASD individuals falling outside the neurotypical $PI_{90\%}$ at each vertex (i.e. sensitivity). (**B**) Prevalence (i.e. probability) of ASD among all individuals outside the neurotypical

$PI_{90\%}$. This equals the positive predictive value (PPV) of the model (C) Sensitivity (true positive rate), specificity (true negative rate), positive predictive value (PPV), and negative predictive value (NPV) of the neurotypical model across all vertices on the cortical surface. (D) Increases (yellow to red) and decreases (cyan to blue) in the post-test probability of ASD based on a pre-test probability that equals prevalence of ASD in our sample. Δ: difference. (E) Vertices with a significant χ^2-enrichment of ASD individuals outside the neurotypical $PI_{90\%}$. (F) Between-group differences in the individuals' total degree of neuroanatomical abnormality (tAI_s) quantified by the percentage of vertices outside the neurotypical $PI_{90\%}$ across the cortex, and (G) within the χ^2-enrichment mask. Subplots display the results of the logistic regression analysis, predicting diagnostic categories based on the individuals tAI_s. TN: true negative rate; FN: false negative rate; FP: false positive rate; TP: true positive rate; TD: typically developing controls; L: left hemisphere; R: right hemisphere. *Note*. TN, FN, FP and TP represent sample estimates rather than quantifying generalization performance.

Figure 3. Genomic underpinnings of neurodevelopmental deviations in cortical thickness (CT) in ASD based on (A) the *t-map* of statistical between-group differences in CT (see Figure 1a), the *Cohen's f* effect size map associated with the main effect of group (see Figure 1d), and the χ^2- map of neuroanatomical ASD outliers (see Figure 2e). (B) Significant Odds-ratios (OR) at an 'false discovery rate' (FDR)-corrected $p<0.05$ resulting from the gene set enrichment analyses for genes expressed in the different output maps. Gene sets were subdivided into sets with differential gene expression (DGE) in ASD, 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 labelled based on their original publication. up: upregulated expression in ASD, down: down-regulated expression in ASD, CTX: cortex, DEG: differentially expressed genes. (C) Set enrichment of genes mediating typical brain development as reported in the spatio-temporal transcriptome dataset provided by Kang et al. (2011) (35). Set names contain their respective co-expression module label (e.g. M1), followed by their functional description based on their GO term enrichment. (D) Spatio-temporal expression profiles of brain gene modules

significantly enriched in the χ^2-outlier map for Module 2 (left panel) enriched for genes implicated in synaptic transmission, and for Module 4 (right panel) enriched for immune response genes. The *x*-axis shows the developmental time frame (pcw: post conception weeks) and the *y*-axis shows the different brain regions; i.e. 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. (**E**) Gene set-based polygenic scores (PGS) across gene sets enriched in the χ^2-outlier map, and across ASD risk genes. Each bar represents the proportion of variance associated with ASD case-control status explained by the PGS within sets. DGE: differentially expressed genes in ASD, GE: genes expressed in typically developing brain, RG: ASD risk genes with common and *de novo* variants. The subplot shows the *p*-value associated with the impact of the combined PGS across DGE and GE gene sets (PGS_{DGE}, purple) on the individuals' total degree of neuroanatomical abnormality *(tAIs)* relative to the impact of PGS across ASD risk genes ($PGS_{ASD.risk}$, green). (**F**) Pearson correlations (*r*) between *tAIs* and genome-wide PGS for ASD and other neuropsychiatric conditions, as well as general phenotypic traits across groups. ADHD: attention deficit hyperactivity disorder, MDD: major depressive disorder, SCZ: schizophrenia, SWB: subjective well-being, BMI: body mass index, YearsEdu: years in education. PGS are annotated based on their phenotypical outcome (i.e. clinical diagnosis vs. general phenotype trait). *: FDR-corrected *p*-value < 0.05, **: FDR-corrected *p*-value < 0.01

Figure 4. Neuroanatomical differences between different ASD sensory subgroups. (**A**) Clusters with a significant main effect of subgroup ('random field theory' (RFT)-based cluster corrected, $p<0.05$, two-tailed). (**B**) *F*-test statistic for the main effect of subgroup (un-thresholded). (**C**) Upper panel: standardized deviations (i.e. residuals) from the neurotypical distribution of cortical thickness (CT) for the subgroups with low, moderate, and severe sensory symptoms. Lower panel: percentage of individuals with deviations falling below the neurotypical mean CT. (**D**) Pearson correlation

coefficients (*r*) between neuroanatomical deviations and Short Sensory Profile (SSP) subdomains at a 'false discovery rate' (FDR)-corrected $p<0.05$. TAC: tactile sensitivity, TSM: taste/smell sensitivity, MOV: movement sensitivity, USS: under-responsiveness/seeks sensation, AFL: auditory filtering, LEW: low energy/weak, VAS: visual/auditory sensitivity, SMA/PMC: significant cluster in the supplementary motor area and pre-motor cortex. The colorbar on top shows the subdomain effect size for separating the low from the severe sensory subgroup published in (20). *Note*. The SSP subdomain scores were reversely scored so that larger values indicate more severe symptoms. A negative correlation thus indicates that more negative neuroanatomical deviations are associated with more severe symptoms. (**E**) Schematic illustration of cell types in germinal zones of the developing cortex adapted from Polioudakis et al. (2019) (38). VZ: ventricular zone, iSVZ: inner subventricular zone, oSVZ: outer subventricular zone, IZ: intermediate zone, SP: subplate, Cpi: inner cortical plate, CPo: outer cortical plate, 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. (**F**) Cell-type enrichment Odds Ratios (ORs) and associated -log10(q)-values for genes sets expressed in the *F*-map. Cell-types are coloured and labeled based on Polioudakis et al. (2019) (also see Figure 4e). MP: mitotic progenitor, OPC: oligodendrocyte precursors, CGE/MGE: caudal and medial ganglionic eminence-derived interneurons, IP: intermediate progenitors, o/vRG: outer and ventricular radial glia

Table 1. Sample characteristics

Variable	TD				ASD				Significance	
	M	SD	Min	Max	M	SD	Min	Max	Statistic	*p*-value
Sex (% of male participants)	101F, 178M (63%)				101F, 295M (81.9%)				$\chi^2(1)=4.82$	$p<0.02$
Age	17.33	5.91	6.89	30.98	17.51	5.51	6.81	30.60	$t(576)=-0.40$	$p=0.69$
Full-scale IQ	104.79	18.25	50.00	142.00	98.86	19.72	40.00	148.00	$t(617)=3.93$	$p<0.01$
ADI-R										
Social Interaction	-	-	-	-	16.68	6.69	0	29	-	-
Communication	-	-	-	-	13.22	5.63	0	26	-	-
RRB	-	-	-	-	4.29	2.67	0	12	-	-
ADOS										
Total	-	-	-	-	6.07	2.64	2.00	10.00	-	-
SA	-	-	-	-	6.77	2.40	3.00	10.00	-	-
RRB	-	-	-	-	4.70	2.62	1.00	10.00	-	-
Total Brain Volume [litre]	1.19	0.13	0.69	1.73	1.19	0.13	0.65	1.86	$t(603)=-0.15$	$p=0.88$
Mean CT [mm^2]	2.67	0.11	2.41	2.98	2.68	0.13	2.30	3.37	$t(623)=-0.78$	$p=0.43$

Note. ASD: autism spectrum disorder, TD: typically developing, M: mean, SD: standard deviation, Min: minimum, Max: maximum, F: female, M: male, ADI-R: Autism Diagnostic Interview-Revised, SA: Social Affect, RRB: Restricted and Repetitive Behaviors