Reduced Brain Connectivity in Clinical and Dimensional Autism Phenotypes Beyond Familial Confounding – A Twin Study

Janina Neufeld (✉ janina.neufeld@ki.se)

Karolinska Institutet   https://orcid.org/0000-0002-7743-526X

Simon Maier

University of Freiburg Hospital: Universitatsklinikum Freiburg

Mirian Revers

Karolinska Institutet

Marco Reisert

University of Freiburg Hospital: Universitatsklinikum Freiburg

Ralf Kuja-Halkola

Karolinska Institutet

Tebartz van Elst Ludger

University of Freiburg Hospital: Universitatsklinikum Freiburg

Sven Bölte

Karolinska Institute: Karolinska Institutet

Abstract

Background

Previous studies on brain connectivity in clinical and dimensional autism have largely focused on selective connections and yielded inconsistent results. This study aimed to overcome these limitations. Global fiber tracking allowed a more unbiased assessment of white matter connectivity and utilizing a within-twin pair design introduced implicit control for genetic and environmental factors shared by twins and allowed conclusions regarding their impact.

Methods

The study examined the within-twin pair associations between structural brain connectivity of anatomically defined brain regions and both clinical autism spectrum diagnoses and dimensional autistic traits in 85 twin pairs (n=170; 56% monozygotic; 25 individuals with autism spectrum diagnosis). Structural connectivity was estimated using diffusion tensor imaging and linear regression models were fit, adjusted for IQ, other neurodevelopmental and psychiatric conditions and multiple testing.

Results

Overall, both clinical and dimensional autism phenotypes were associated with localized reductions in structural connectivity, despite comprehensively controlling for possible confounders, including all factors shared by twins. Twins fulfilling autism spectrum diagnostic criteria showed decreased brainstem-cuneus connectivity compared to their co-twins without the diagnosis. Further, twins with higher autistic traits showed decreased connectivity of the left hippocampus with the left fusiform and parahippocampal areas. These associations pointed into the same direction in mono- and dizygotic sub-cohorts, but were only significant in dizygotic twins.

Limitations

The recruitment approach of selecting primarily twin pairs discordant for autistic traits prevented a quantitative estimation of genetic and environmental contributions to brain correlates of clinical and dimensional autism. Further, assessing twins and excluding individuals with an IQ below 75 limited the generalizability of the findings. The statistical power allowed detecting medium-size or larger effects of dimensional autism. Finally, due the relatively small number of twin pairs discordant for a clinical autism, the results for clinical autism need to be interpreted with caution.

Conclusions

Reduced brainstem-cuneus connectivity might point towards alterations in low-level visual processing in clinical autism while reduced connectivity in networks crucial for visual and especially face processing seem to be more associated with dimensional aspects of autism. The results further suggest that the observed associations were potentially influenced by both genes and environment.

Background

Clinical autism, here defined as fulfilling diagnostic criteria for an autism spectrum disorder, is characterized by challenges in social and communication functioning along repetitive behaviors, restricted interests, and alterations in sensory processing (1). Clinical autism is associated with low employment rates, increased risk of anxiety and depressive disorders, and premature mortality (2). The heterogeneity of clinical autism and the dimensionality of autism-defining symptoms makes it challenging to establish reliable biomarkers for assessment and intervention purposes. Research indicates that clinical autism is the extreme end of continuously distributed autistic traits (3), here referred to as dimensional autism. Studying biomarkers in association with both dimensional and clinical autism is a meaningful approach because dimensional markers assess the quantity of a phenomenon and provide hence important additional information in addition to diagnostic markers. In this study, we therefore investigated brain connectivity in association with both, clinical and dimensional autism.

It is generally assumed that atypical brain development leading to altered brain connectivity underlies the clinical autism phenotypes (4). The nature of these connectivity alterations is however still largely unknown since neuroimaging findings have hitherto remained inconclusive. Early models of altered brain connectivity in clinical autism suggested overarching cerebral under-connectivity, particularly between distal cortical brain regions (see e.g. (6)). However, these models have been challenged by findings indicative of functional over-connectivity in individuals diagnosed with clinical autism (6, 7). This inconsistency can partly be explained by phenotypic variation of clinical autism and methodological heterogeneity (6). Furthermore, age seems to modulate autism-related connectivity atypicalities: A developmental model brain connectivity in clinical autism suggests wide-spread over-connectivity in early infancy, mirroring findings of early brain overgrowth, followed by altered neurodevelopmental trajectories with regional over- or under-connectivity later in life (7). Finally, environmental factors influencing the etiology of clinical autism, such as parental age or preterm birth (8), might modulate the brain-structural and functional alterations associated with the condition.

Structural connectivity correlates of clinical and dimensional autism

Structural brain connectivity is commonly studied using Diffusion Tensor Imaging (DTI), which takes advantage of the fact that water molecules diffuse along white matter fibers (i.e. anisotropically). A recent meta-analysis of voxel-based morphometry across 14 DTI studies, including 297 individuals diagnosed with clinical autism and 302 neurotypical (NT) individuals, revealed decreased Fractional Anisotropy (FA), indicative of reduced white matter integrity, in the left splenium of the corpus callosum and the right cerebral peduncle, possibly reflecting sensorimotor impairments (9). In contrast, a review of 16 studies applying 'tract based spatial statistics' (TBSS), where FA data from DTI images are projected onto a predefined skeleton of prominent fiber tracts, identified more wide-spread reductions in white matter connectivity in older children, adolescents and adults diagnosed with clinical autsim compared to matched controls (10). The uncinate and arcuate fasciculi, the inferior longitudinal fasciculus, the inferior

fronto-occipital fasciculus and the cingulum – tracts that are crucial for language and face/emotion processing, episodic memory, object recognition and attention control – were particularly affected (10). In a population-based sample of 604 6-to-10 year-old children, FA within the left superior longitudinal fasciculus and axial diffusion in the corpus callosum and the corticospinal tract were negatively associated with dimensional autism, suggesting that some autism-related changes in white matter microstructure might show a dose-like effect, increasing with increasing levels of dimensional autism (11).

Utility of twin designs for assessing autism brain biomarkers

Given the genetic heterogeneity of clinical and dimensional autism and the difficulties to control for all possible confounding variables, twin studies provide the unique opportunity to implicitly control for the genetic and environmental factors shared by twins. These familial factors might co-occur with (clinical) autism without being part of the condition's phenotype and might have biased the results of previous studies (12). Within-pair associations are adjusted for 100% of genetic factors in monozygotic (MZ) twins, except for post-twinning mutations, and on average half of the genetics in dizygotic (DZ) twins. Comparing associations within MZ vs DZ twins can thereby help to differentiate genetic and environmental influences on brain structure and function (13). For instance, a meta-analysis of 48 brain imaging twin studies in NT twins indicated strong genetic impact on cortical morphometric measures and FA of most brain structures, and environmental influence on cortical thickness of the uncus, left parahippocampal gyrus, and insula as well as FA in the callosal splenium (14). Interestingly, the latter structures have also been implicated in ASD (9, 10, 15).

Only a few studies assessed twin pairs discordant or concordant for clinical autism, showing, for instance, that twins fulfilling diagnostic criteria for clinical autsim and their co-twins who did not showed similar reductions in gray and white matter volume and cortical folding (e.g. 22,23). This suggests that these autism-related brain alterations might be largely genetic. However, results have varied between studies, most samples have been small and partly overlapping, and no within-pair comparisons have been performed. One recent larger twin study reported that cortical thickness and cerebellar white matter volume were more influenced by environmental factors in twins with clinical autism compared to NT twins, whereas the genetic and environmental influence on other brain-structural measures was similar in both twins with and without clinical autism (18). A previous twin study from our group revealed autism-related intrinsic functional brain connectivity alterations within twin pairs between core hubs of the salience network (19), while this study is the first assessing autism-related structural brain connectivity in twins.

Methods

In this study, we applied a global tracking approach, allowing reconstructing the entire white-matter connectome without making predetermined anatomical assumptions (20), and twin design for a

hypothesis-free assessment of autism-related structural brain connectivity alterations, unbiased by familial confounders. While within-twin pair analyses are perfectly controlled for age, within-pair association between autism and brain connectivity can be modulated by age (19). Hence, we investigated potential interaction effects between clinical and dimensional autism and age on brain connectivity in a follow-up analysis.

Participants

A total of 170 individuals (8-36 years) from the Roots of Autism and ADHD Twin Study Sweden (RATSS) (21) were included into the study. In RATSS, twins are predominantly recruited from the population-based Child and Adolescent Twin Study Sweden (CATSS) (22), prioritizing pairs being screened positively for significant autism symptom discordance based on a parent interview. This study included 44 MZ and 37 DZ twin pairs and 4 twin pairs where zygosity was undetermined, 25 individuals fulfilled diagnostic criteria for clinical autism, belonging to 17 clinical autism diagnosis discordant (6 MZ and 11 DZ twin pairs) and four concordant twin pairs (3 MZ and 1 DZ twin pair). Further 31 individuals had one or more other neurodevelopmental conditions (NDD) (primarily Attention Deficit Hyperactivity Disorder, ADHD, or specific learning disorders) and further 27 individuals had one or more other psychiatric diagnosis (mainly affective disorders). The remaining 87 included individuals were NT, defined as not fulfilling criteria for any NDD or psychiatric diagnosis. For autistic traits, 74 twin pairs (38 MZ, 36 DZ) differed in the exposure (by at least 1 point on the Social Responsiveness Scale-2, SRS-2) and 50 twin pairs (23 MZ and 27 DZ) differed by at least 7 points, which is above measurement error (23). Sample characteristics are summarized in Table 1. Of originally 420 individuals in the complete RATSS cohort, 230 had to be excluded mainly because they or their co-twin had missing or too low quality data (for a detailed description see the Exclusion procedure section in the **supplementary text, section 1.1**).

Table 1
Sample characteristics

	Total sample (N=170)	ASD (N=25)	other diagnoses* (N=58)	NT (N=87)
Female / male sex	96 / 74	13 / 12	22 / 36	87 / 48
MZ / DZ / unknown	88 / 74 / 8	11 / 13 / 1	28 / 27 / 3	49 / 34 / 4
NDD / psych.	31 (17)	7 / 11	31 / 41	-
Age range years	8-36	11-31	8-36	8-33
Mean age (SD)	19.32 (6.38)	17.4 (5.69)	19.28 (6.71)	19.9 (6.31)
Mean SRS-2 (SD)	33.38 (28.37)	79.40 (25.23)	31.74 (20.99)	21.25 (18.6)
Mean IQ (SD)	103.34 (14.42)	104.76 (19.14)	101.16 (13.32)	104.38 (13.56)

Note. *other diagnoses means here fulfilling any diagnoses other than ASD; MZ = monozygotic, DZ = dizygotic, NDD = fulfilling criteria for neurodevelopmental disorders other than ASD, psych. = fulfilling criteria for psychiatric diagnoses (primarily anxiety disorders and depression); NT = neurotypical (defined as not fulfilling diagnostic criteria for any of the assessed neurodevelopmental or psychiatric diagnoses)

Diagnostic assessment

Twins underwent comprehensive assessment according to the RATSS protocol (21), including first choice standardized diagnostic instruments for clinical autism, such as the 'Autism Diagnostic Interview – Revised' (ADI-R) and the 'Autism Diagnostic Observation Schedule' (ADOS or ADOS-2). Other NDD and psychiatric diagnoses were determined based on a multitude of sources, including the 'Kiddie Schedule for Affective Disorders and Schizophrenia', the 'Diagnostic Interview for ADHD in adults' and the 'Structured Clinical Interview for DSM-IV' (SCID, axis I). General intellectual ability was assessed with the Wechsler Intelligence Scale for Children or Adults, 4th Editions (WISC-IV/WAIS-IV) and the composite IQ score was calculated based on three verbal comprehension and three perceptual reasoning subtests.

Assessment of dimensional autism

Dimensional autism was assessed using the parent report SRS-2, see **supplementary text, section 1.2.** for psychometric characteristics), using total raw scores as recommended for research settings (23).

Image acquisition and processing

An approximately 50 minute MRI session in a 3 Tesla MR750 GE-scanner included a 5 minute T1-weighted Spoiled Gradient Echo anatomical scan (176 slices, TR = 8.2 sec, FOV = 240 mm, voxel size = .94 x .94 x 1.00 mm^3), and a diffusion imaging sequence with 60 spatial directions and a b-value of 1000 s/mm² (61 slices, TR = 8.0 sec, FOV = 220 mm, voxel size = 2.29 x 2.29 x 2.3 mm^3).

After quality control and pre-processing, streamline counts between 56 atlas regions (**Supplementary Table 1**) of the LBPA40 atlas (24) were extracted for each individual and normalized (please see **supplementary text, sections 1.3. and 1.4.**). After data reduction (see **supplementary text, section 1.5**), 903 connections were included. For these, the median raw streamline count across NT participants ranged between 35 and 5177 streamlines.

Statistical analyses

First we conducted explorative analyses on group differences and within-pair differences in dimensional autism and IQ, which are summarized in the **Supplementary text, sections 2.1** and **2.2**.

As the main analyses, we performed linear regressions in the generalized estimating equations framework with connectivity strength measured as streamline counts as dependent variable and clinical or dimensional autism as independent variables. Using an identity link function and conditioning on a unique twin pair id, we conducted within-pair analyses where each individuals were compared to their co-twins. Thus, confounding (and mediating) factors that are stable between the twins, i.e. all genetic and environmental factors shared by twins, were adjusted for by design (see e.g. 21). We fitted models for 903 selected connections, adjusting multiple comparisons using false discovery rate (FDR, Benjamini-Hochberg method), as well as IQ, NDD diagnosis other than clinical autism, and psychiatric diagnosis. Twin pairs discordant for the main predictor contribute directly to the estimate (76 pairs were discordant for dimensional autism and 17 for clinical autism) while the remaining pairs influenced the standard errors and affected the estimate indirectly if they were discordant for any of the covariates. Standardized estimates were calculated, which can be interpreted as effect size estimates. In a follow-up analysis, the interaction terms between clinical or dimensional autism and age were added to the models.

In order to complement our within-pair analysis, we also performed linear regressions across the cohort using the same statistical framework, treating twins as individuals but adjusting standard errors for twin clustering, and summarized these secondary results within the **Supplementary text, section 2.5**.

Results

Associations of clinical and dimensional aspects of autism and structural connectivity

The statistics of all within-pair associations with clinical or dimensional autism surviving the FDR correction are summarized in Table 2 and visualized in Figure 1.

Table 2
Within-pair associations between structural connectivity and ASD

Sample	Anatomical regions	β	SE	Z	p corr.	p uncorr.
ASD diagnosis						
MZ+DZ	brainstem - L cuneus	-.502	.115	-4.358	.012	$1.3*10^{-5}$
DZ		-.574	.121	-4.751	.002	$2.0*e^{-6}$
MZ		*-.467*	*.210*	*-2.222*	*1.000*	*.026*
Autistic traits						
MZ+DZ	L hippocapus - L parahyppocampal gyrus	-.362	.071	-5.136	$2.5*10^{-4}$	$2.8*10^{-7}$
DZ		-.510	.070	-7.320	$2.2*10^{-10}$	$2.5*10^{-13}$
MZ		*-.190*	*.127*	*-1.495*	*1.000*	*.135*
MZ+DZ	L hippocapus - L fusiform gyrus	-.396	.078	-5.112	$2.9*10^{-4}$	$3.2*10^{-7}$
DZ		-.389	.081	-4.785	.002	$1.7*10^{-6}$
MZ		*-.529*	*.160*	*-3.303*	*.863*	$9.6*10^{-4}$

Note. *β = standardized regression coefficient, SE = standard error, Z = z-statistic, p corr. = FDR-corrected p-value; significant associations (p < .05) marked in* bold. *The within-pair association estimate is directly influenced by twin pairs who differ in the exposure (ASD diagnosis or SRS-2) and indirectly by pairs differing only in covariates. For ASD diagnosis, 17 twin pairs (6 MZ and 11 DZ) differed in the exposure. For autistic traits, 74 twin pairs (38 MZ, 36 DZ) differed in the exposure (by at least 1 point on the SRS-2).*

Fulfilling diagnostic criteria for clinical autism was significantly associated with decreased structural connectivity between the brainstem and the left cuneus, both within all twin pairs (*corrected p value < .05*) and DZ twin pairs (11 discordant pairs, *corrected p-value < .01*). The association was also observable in MZ pairs (6 diagnosis discordant pairs), but did not survive the correction for multiple comparisons. The CIs of the DZ and MZ estimates overlapped.

There were two significant negative associations between dimensional autism and structural connectivity strength involving the hippocampus (*corrected p-values < .001*) that were similar in both the whole sample and the sub-sample of DZ twins (*corrected p-values < .01*), whereas the estimate was still negative but not significant in MZ twin pairs. Also here, the CIs of the DZ and MZ estimates overlapped.

The covariates effects are summarized in **Supplementary text, section 2.3**. Uncorrected Z-values of the associations between clinical or dimensional autism on connectivity are summarized in **Supplementary text, section 2.4** and visualized in **Supplementary Figures 1 and 2**.

Modulating effects of age on the association between ASD and structural connectivity

The interaction between age and clinical autism was significant for 13 connections. Among these, however, were none of the connections observed in the main analysis for clinical autism, but primarily intra-hemispheric fronto-occipital connections (see Table 3). These interaction effects were all negative, and no interactions between age and dimensional aspects of autism survived correction for multiple comparisons. The uncorrected Z-values of these interaction effects are visualized in **Supplementary Figures 3 and 4**.

Table 3
Interactions between ASD diagnosis and age that survived FDR correction

Anatomical regions	β (95% CI)	SE	Z	p corr.	p uncorr.
L sup. occipital g. − L postcentral g.	-.114 (-.157, -.071)	.022	-5.175	$2.1*10^{-4}$	$2.3*10^{-7}$
L mid. occipital g. − L precentral g.	-.128 (-.170, -.087)	.021	-6.004	$1.7*10^{-6}$	$1.9*10^{-9}$
L inf. occipital g. − L sup. front. g.	-.169 (-.234, -.103)	.033	-5.069	$3.6*10^{-4}$	$4.0*10^{-7}$
L inf. occipital g. − L mid. front. g.	-.181 (-.238, -.124)	.029	-6.253	$3.6*10^{-7}$	$4.0*10^{-10}$
L inf. occipital g. − L precentral g.	-.156 (-.219 -.093)	.032	-4.866	.001	$1.1*10^{-6}$
L fusiform g. − L mid. front. g.	-.119 (-.169 -.070)	.0025	-4.707	.002	$2.5*10^{-6}$
R inf. occipital g. − R sup. front. g	-.193 (-.254, -.132)	.031	-6.193	$5.3*10^{-7}$	$5.9*10^{-10}$
R inf. occipital g. − R mid. front. g.	-.141 (-.205 -.077)	.033	-4.323	.014	$1.5*10^{-5}$
R cuneus − R sup. front. g.	-.114 (-.166 -.062)	.027	-4.311	.015	$1.6*10^{-5}$
R cuneus − R mid. front. g.	-.115 (-.162 -.069)	.024	-4.833	.001	$1.4*10^{-6}$
R lingual g. − R sup. front. g.	-.122 (-.168 -.076)	.024	-5.167	$2.2*10^{-4}$	$2.4*10^{-7}$
R lingual g. − R mid. front. g.	-.126 (-.182 -.071)	.028	-4.448	.008	$8.7*10^{-6}$
R putamen − R angular g.	-.083 (.119 -.047)	.018	-4.538	.005	$5.7*10^{-6}$

Note. Statistics of the Age*ASD diagnosis interaction effects surviving FDR correction. *β = standardized regression coefficient, 95%CI = 95% confidence interval, SE = standard error, Z = z-statistic, p corr. = FDR-corrected p-value, p uncorr. = uncorrected p-value.*

Discussion

In this study, we investigated changes in brain connectivity associated with clinical and dimensional autism, using global fiber tracking and applying a within-twin pair design where familiar factors are implicitly controlled for. Both clinical and dimensional aspects of autism were associated with reduced connectivity beyond the influence of familial factors. However, different connectivity alterations appear to be relevant for the clinical autism based on present diagnostic algorithms compared to dimensional autism. The results are discussed in more detail below.

Clinical autism and structural connectivity

Twins with fulfilling diagnostic criteria for clinical autism had reduced white matter connectivity between the brainstem and the left cuneus compared to their co-twins without a diagnosis. While several lines of evidence suggest a brainstem involvement in clinical autism, the direct evidence for brainstem alterations from postmortem histological and in-vivo neuroimaging studies in humans remains limited (25), likely because the majority of brain imaging studies focused on predetermined cortical regions of interest. The brainstem hypothesis of autism suggests that atypical early brainstem development in clinical autism has cascading effects on cortical development, resulting in alterations in sensory processing that might be causal to other autism core symptoms (25). For instance, the superior colliculus of the brainstem and its interaction with cortical visual regions has been linked to visual exploration during visual search (26). Such changes in low-level visual processing could have a secondary effect on higher-order visual processing and cognition. The cuneus is an occipital brain region contributing to the dorsal visual stream, involved in form, motion and spatial processing and is a central, integrative hub within a functional visual brain network (27). This region has previously been implicated in clinical autism in a large study (394 individuals with clinical autism diagnosis and 473 controls), where lower effective (directed) connectivity of cuneus / precuneus to temporal brain regions involved in face processing to the was found in relation to both clinical autism diagnosis and autism symptom severity in the clinical group (28).

Our finding of reduced connectivity between the brainstem and cuneus in individuals clinical autism compared to their co-twins might indicate an atypical development of early aspects of the visual pathway in clinical autism, which in turn may influence low-level perception and, in consequence, social information processing. These alterations might be considered a trait marker of autism, i.e. a marker of the dichotomous presence of clinical diagnosis, regardless of symptoms severity.

Dimensional autism and structural connectivity

Our results suggest that twins with more pronounced autistic traits tend to have reduced white matter connections from the left hippocampus to the left parahippocampal gyrus and to the left fusiform gyrus. The left hippocampus is crucial for (episodic) memory (29) and is, via the parahippocampal gyrus, connected to the brain's Default Mode Network (30), which is believed to be involved in self-referential thinking (31). Connectivity between the hippocampus and the fusiform gyrus is crucial for facial emotional processing (32), and reduced connectivity between these regions (33) and altered microstructure of the hippocampus-fusiform pathway (more densely packed but thinner) (34) has been observed previously in individuals diagnosed with clinical autism compared to controls. The human

fusiform gyrus contains the fusiform face area (FFA) which is crucial for face perception (35). Since challenges in facial emotional processing are a core feature of clinical autism, many functional brain imaging studies on autism investigated the FFA during face processing. A meta-analysis of 50 fMRI studies concluded that the left FFA and the left parahippocampal gyrus are more strongly activated in individuals with clinical autism during social cognition tasks, most of which involving face stimuli (36). However, since the fusiform gyrus function is not restricted to face processing but includes for instance also object recognition and space processing (37), its connectivity to the hippocampus is also relevant for non-social visual processing, such as memory-guided visual exploration in visual search (38). Therefore, the reduced connectivity between hippocampus and fusiform gyrus in association with dimensional autism observed in this study might reflect alterations in visual processing, including but not restricted to facial emotional processing. These might be regarded as a state marker of autism, a marker of quantitative autistic trait severity.

Modulating effect of age

Some associations between clinical autism and connectivity between intra-hemispheric connections, involving primarily fronto-occipital connections, were significantly modulated by age (Table 3). These negative interactions indicate that the effect of clinical autism on brain connectivity between these regions decreased with increasing age, in line with developmental models of clinical autism (7, 19). These interaction effects should be interpreted with caution, due to the limited number of twin pairs discordant for clinical autism. Still – if validated in further studies – this is a clinically interesting observation in that it might reflect compensatory mechanisms affecting structural network organization, potentially taking place during adolescence and early adulthood.

Genetic vs environmental influences

In this study, genetic and environmental factors shared by twins are implicitly controlled. However, comparing associations between MZ and DZ sub-cohorts can allow conclusions regarding the influence of non-shared genetic and environmental factors, because MZ twins are largely genetically identical, while DZ twins share on average half of their genes. When splitting the sample into sub-samples of 46 MZ and 37 DZ twin pairs, the associations between clinical and dimensional autism and brain connectivity remained significant within DZ but not MZ twins. However, the estimates were quite similar and their CIs overlapped between MZ and DZ sub-cohorts, allowing no firm conclusions with respect to genetic influence on this association. For the latter, larger MZ and DZ samples might be necessary. Since the associations pointed into the same direction in both MZ and DZ twins but were statistically weaker in MZ twins, we speculate that both, genetic and non-shared environmental factors contributed to these within-pair associations.

Limitations

Focusing primarily on twin pairs with marked differences in autistic traits made this study more sensitive for detecting within-pair associations with autism phenotypes, but also prevented us from classic twin

modeling of the quantitative genetic and shared vs non-shared environmental effects since these estimated would have been biased.

Power analysis for our twin analysis is not straight forward, due to the non-independence of observations and due to our recruitment strategy, however, we aimed to approximate the questions using G*Power (version 3.1.9.2), assuming N = 76 pairs differing in autistic traits by at least one point on the SRS.2. While the sample is comparatively large for a neuroimaging twin study on autism, our power calculation indicated a power of 85.6 in order to detect medium sized effects ($\beta > .3$) at $\alpha = .05$.

A larger sample (N = 714 discordant twin pairs) would have been required to detect small effects ($\beta = .1$) at a power of 80%. Further, the FDR-correction for the relatively large number of tested connections (>900) might have increased the likelihood for type-II errors. For an overview over also sub-threshold effects (Z-maps), please see the supplementary material (**Supplementary Figures 1-4**). These revealed visually relatively similar patterns of both increased and decreased connectivity in association with both, clinical and dimensional autism, indicating that rather than differing fundamentally in their effects on overall connectivity pattern, clinical and dimensional autism might only differ in respect to their strongest associations with structural connectivity.

Our study had a wide age range and an even distribution of males and females, increasing the generalizability across ages and sexes, but this variability might on the other hand prevented us from detecting sub-group specific effects. In contrast, excluding individuals with an IQ<75 and individuals with insufficient brain imaging data quality has likely reduced the noise in the data, but restricted the sample largely to individuals in the normal IQ-range. Moreover, twin cohorts differ from non-twin cohorts in several ways. For instance, twins are more frequently born prematurely and suffer more often from growth restrictions (39). Hence, we cannot exclude the possibility that they also differ from non-twin samples in terms of brain connectivity.

Conclusions

Using a data-driven approach and a within-twin pair design, we found evidence for reduced connectivity between brainstem-occipital connectivity as a possible trait marker of autism, and reduced connectivity between regions involved in visual and especially face processing as a possible state marker of autism, beyond familial confounding and across sexes and ages. These associations were significant in DZ twins alone and attenuated in MZ twins despite pointing into the same direction, potentially indicating both genetic and environmental contributions. Negative interactions effects between clinical autism and age on brain connectivity might reflect compensatory processes.

Abbreviations

DZ
dizygotic

FA

fractional anisotropy

FFA

fusiform face area

FOV

field of view

IQ

intelligence quotient

MRI

magnetic resonance imaging

MZ

monozygotic

NDD

neurodevelopmental disorder

NT

neurotypical

TR

repetition time

Declarations

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders 5th ed. (DSM-5). American Psychiatric Publishing, Arlington, VA; 2013.

2. Levy A, Perry A. Outcomes in adolescents and adults with autism: A review of the literature. Res Autism Spectr Disord. 2011;5(4):1271–82.

3. Lundström S, Chang Z, Råstam M, Gillberg C, Larsson H, Anckarsäter H, et al. Autism spectrum disorders and autisticlike traits: similar etiology in the extreme end and the normal variation. Arch Gen Psychiatry. 2012;69(1):46–52.

4. Tebartz van Elst L, Riedel A, Maier S. Autism as a Disorder of Altered Global Functional and Structural Connectivity. Biol Psychiatry. 2016;79(8):626–7.

5. Just MA, Keller TA, Malave VL, Kana RK, Varma S. Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. Neurosci Biobehav Rev. 2012;36(4):1292–313.

6. Pua EPK, Bowden SC, Seal ML. Autism spectrum disorders: Neuroimaging findings from systematic reviews. Res Autism Spectr Disord. 2017;34:28–33.

7. Uddin LQ, Supekar K, Menon V. Reconceptualizing functional brain connectivity in autism from a developmental perspective. Front Hum Neurosci. 2013;7(7):Article 458.

8. Bölte S, Girdler S, Marschik PB. The contribution of environmental exposure to the etiology of autism spectrum disorder. Cell Mol Life Sci. 2019;76(7):1275–97.

9. Di X, Azeez A, Li X, Haque E, Biswal BB. Disrupted focal white matter integrity in autism spectrum disorder: A voxel-based meta-analysis of diffusion tensor imaging studies. Prog Neuropsychopharmacol Biol Psychiatry. 2018;82:242–8.

10. Ameis SH, Catani M. Altered white matter connectivity as a neural substrate for social impairment in Autism Spectrum Disorder. Cortex. 2015;62:158–81.

11. Blanken LM, Muetzel RL, Jaddoe VW, Verhulst FC, van der Lugt A, Tiemeier H, et al. White matter microstructure in children with autistic traits. Psychiatry Res Neuroimaging. 2017;263:127–34.

12. Mevel K, Fransson P, Bölte S. Multimodal brain imaging in autism spectrum disorder and the promise of twin research. Autism. 2014;1362361314535510.

13. McGue M, Osler M, Christensen K. Causal inference and observational research: The utility of twins. Perspect Psychol Sci. 2010;5(5):546–56.

14. Blokland GAM, Zubicaray GI de, McMahon KL, Wright MJ. Genetic and Environmental Influences on Neuroimaging Phenotypes: A Meta-Analytical Perspective on Twin Imaging Studies. Twin Res Hum Genet. 2012;15(3):351–71.

15. Uddin LQ, Menon V. The anterior insula in autism: under-connected and under-examined. Neurosci Biobehav Rev. 2009;33(8):1198–203.

16. Kates WR, Ikuta I, Burnette CP. Gyrification patterns in monozygotic twin pairs varying in discordance for autism. Autism Res. 2009;2(5):267–78.

17. Mitchell S, Reiss A, Tatusko D, Ikuta I, Kazmerski D, Botti J-A, et al. Neuroanatomic alterations and social and communication deficits in monozygotic twins discordant for autism disorder. Am J Psychiatry. 2009;166(8):917–25.

18. Hegarty JP, Pegoraro LFL, Lazzeroni LC, Raman MM, Hallmayer JF, Monterrey JC, et al. Genetic and environmental influences on structural brain measures in twins with autism spectrum disorder. Mol Psychiatry. 2019;1–11.

19. Neufeld J, Kuja-Halkola R, Mevel K, Cauvet É, Fransson P, Bölte S. Alterations in resting state connectivity along the autism trait continuum: a twin study. Mol Psychiatry. 2017;23(7):1659–65.

20. Reisert M, Mader I, Anastasopoulos C, Weigel M, Schnell S, Kiselev V. Global fiber reconstruction becomes practical. Neuroimage. 2011;54(2):955–62.

21. Bölte S, Willfors C, Berggren S, Norberg J, Poltrago L, Mevel K, et al. The roots of autism and ADHD twin study in Sweden (RATSS). Twin Res Hum Genet. 2014;17(03):164–76.

22. Anckarsäter H, Lundström S, Kollberg L, Kerekes N, Palm C, Carlström E, et al. The child and adolescent twin study in Sweden (CATSS). Twin Res Hum Genet. 2011;14(06):495–508.

23. Constantino JN, Gruber CP. Social responsiveness scale (SRS). Western Psychological Services Los Angeles, CA; 2005.

24. Shattuck DW, Mirza M, Adisetiyo V, Hojatkashani C, Salamon G, Narr KL, et al. Construction of a 3D probabilistic atlas of human cortical structures. NeuroImage. 2008;39(3):1064–80.

25. Dadalko OI, Travers BG. Evidence for Brainstem Contributions to Autism Spectrum Disorders. Front Integr Neurosci. 2018;12:47.

26. Gitelman DR, Parrish TB, Friston KJ, Mesulam M-M. Functional Anatomy of Visual Search: Regional Segregations within the Frontal Eye Fields and Effective Connectivity of the Superior Colliculus. NeuroImage. 2002;15(4):970–82.

27. Tomasi D, Volkow ND. Association between Functional Connectivity Hubs and Brain Networks. Cereb Cortex. 2011;21(9):2003–13.

28. Rolls ET, Zhou Y, Cheng W, Gilson M, Deco G, Feng J. Effective connectivity in autism. Autism Res. 2020;13(1):32–44.

29. Maguire E. Neuroimaging, memory and the human hippocampus. Rev Neurol (Paris). 2001;157(8-9 Pt 1):791–4.

30. Ward AM, Schultz AP, Huijbers W, Dijk KRAV, Hedden T, Sperling RA. The parahippocampal gyrus links the default-mode cortical network with the medial temporal lobe memory system. Hum Brain Mapp. 2014;35(3):1061–73.

31. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci. 2001;98(2):676–82.

32. Smith CD, Lori NF, Akbudak E, Sorar E, Gultepe E, Shimony JS, et al. MRI diffusion tensor tracking of a new amygdalo-fusiform and hippocampo-fusiform pathway system in humans. J Magn Reson Imaging Off J Int Soc Magn Reson Med. 2009;29(6):1248–61.

33. Chang Y-S, Owen JP, Desai SS, Hill SS, Arnett AB, Harris J, et al. Autism and sensory processing disorders: shared white matter disruption in sensory pathways but divergent connectivity in social-emotional pathways. PloS One. 2014;9(7):e103038.

34. Conturo TE, Williams DL, Smith CD, Gultepe E, Akbudak E, Minshew NJ. Neuronal fiber pathway abnormalities in autism: an initial MRI diffusion tensor tracking study of hippocampo-fusiform and amygdalo-fusiform pathways. J Int Neuropsychol Soc JINS. 2008;14(6):933–46.

35. Kanwisher N, McDermott J, Chun MM. The fusiform face area: a module in human extrastriate cortex specialized for face perception. J Neurosci. 1997;17(11):4302–11.

36. Patriquin MA, DeRamus T, Libero LE, Laird A, Kana RK. Neuroanatomical and neurofunctional markers of social cognition in autism spectrum disorder. Hum Brain Mapp. 2016;37(11):3957–78.

37. Weiner KS, Zilles K. The anatomical and functional specialization of the fusiform gyrus. Neuropsychologia. 2016;83:48–62.

38. Voss JL, Bridge DJ, Cohen NJ, Walker JA. A closer look at the hippocampus and memory. Trends Cogn Sci. 2017;21(8):577–88.

39. Gill P, Lende MN, Van Hook MD JW. Twin Births. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 [cited 2020 Nov 12]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK493200/

Figures

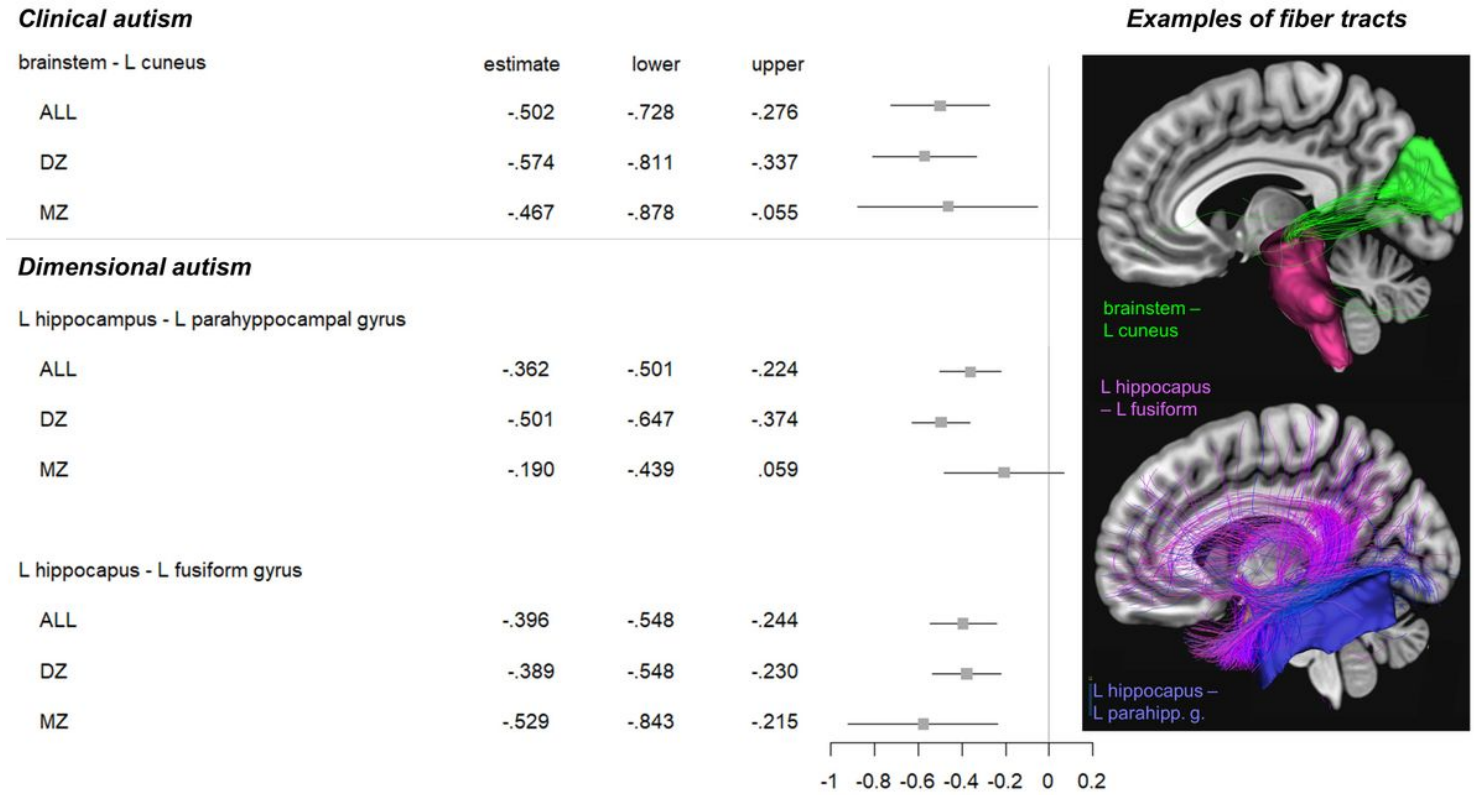| Clinical autism			
brainstem - L cuneus	estimate	lower	upper
ALL	-.502	-.728	-.276
DZ	-.574	-.811	-.337
MZ	-.467	-.878	-.055
Dimensional autism			
L hippocampus - L parahyppocampal gyrus			
ALL	-.362	-.501	-.224
DZ	-.501	-.647	-.374
MZ	-.190	-.439	.059
L hippocapus - L fusiform gyrus			
ALL	-.396	-.548	-.244
DZ	-.389	-.548	-.230
MZ	-.529	-.843	-.215

Figure 1

Within-pair associations that survived FDR-correction in the whole sample. ALL = within-pair association within the whole sample, DZ = within dizygotic twins, MZ = within monozygotic twins. Lower/upper = lower and upper bound of the 95% confidence interval. Note that although none of the associations survived correction within the MZ sub-sample, the confidence intervals do not cross the zero line for the association between autistic traits and the L hippocampus – L fusiform gyrus connection and for the association between ASD diagnosis and brainstem – L cuneus connection. The forest plots (left side) were created in RStudio3.5.1, using the package "forestplot". Examples of the according connections (right side) were created using functions within the NORA platform (http://www.nora-imaging.com).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterialMolAut.docx