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Age and Gender Effects on Intrinsic Connectivity in Autism Using Functional Integration and Segregation

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ABSTRACT

BACKGROUND: The objective of this study was to examine intrinsic whole-brain functional connectivity in autism spectrum disorder (ASD) using the framework of functional segregation and integration. Emphasis was given to potential gender and developmental effects as well as identification of specific networks that may contribute to the global results.

METHODS: We leveraged an open data resource (N = 1587) of resting-state functional magnetic resonance imaging data in the Autism Brain Imaging Data Exchange (ABIDE) initiative, combining data from more than 2100 unique cross-sectional datasets in ABIDE I and ABIDE II collected at different sites. Modularity and global efficiency were utilized to assess functional segregation and integration, respectively. A meta-analytic approach for handling site differences was used. The effects of age, gender, and diagnostic category on segregation and integration were assessed using linear regression.

RESULTS: Modularity decreased nonlinearly in the ASD group with age, as evidenced by an increase and then decrease over development. Global efficiency had an opposite relationship with age by first decreasing and then increasing in the ASD group. Both modularity and global efficiency remained largely stable in the typically developing control group during development, representing a significantly different effect than seen in the ASD group. Age effects on modularity were localized to the somatosensory network. Finally, a marginally significant interaction between age, gender, and diagnostic category was found for modularity.

CONCLUSIONS: Our results support prior work that suggested a quadratic effect of age on brain development in ASD, while providing new insights about the specific characteristics of developmental and gender effects on intrinsic connectivity in ASD.

Keywords: ABIDE, Age, Autism, Functional connectivity, Functional integration, Gender

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The dysconnectivity hypothesis of autism spectrum disorder (ASD) posits that the heterogeneous symptom presentation of social communicative deficits and restricted and repetitive behaviors and interests (1) reflects a neural systems disorder characterized by altered brain connectivity (2,3). In support of this model, there is a confluence of evidence that ASD is characterized by alterations in the functional organization of the brain, in particular, decreased connectivity between mesocorticolimbic, frontal, and posterior-temporal cortical systems that play key roles in processing social-affective information (4) as well as local overconnectivity and long-distance underconnectivity (5). Task-based functional magnetic resonance imaging studies have found altered connectivity in ASD during language comprehension (6), cognitive control (7), mentalizing (8), social processing (9), working memory (10), and visuospatial processing (11). Studies using functional connectivity of intrinsic networks during rest have found evidence in support of within-network underconnectivity, such as decreased frontal-posterior default network connectivity (12,13), decreased default mode network connectivity (14,15), and reduced functional connectivity within and between resting-state networks incorporating social brain regions, including the insula and amygdala within the default mode and salience networks, respectively, in ASD (16).

The precise nature of intrinsic brain connectivity in ASD remains difficult to characterize, owing, at least in part, to the wide variety of analytic methods used in the more than 70 published studies on this topic (17,18). The methods used have included seed-based studies (13), graph-theoretical approaches (19), self-organizing maps (20), independent component analysis (21), examination of intrinsic connectivity networks (22), and regional homogeneity approaches (23). Another obstacle has been the wide age range of participants in studies of intrinsic brain connectivity in ASD. To address this obstacle, there have been attempts to place the nature of intrinsic brain connectivity in ASD within a developmental framework (24). In this regard, there appears to be some degree of convergence that intrinsic brain connectivity in

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adolescents and adults with ASD is generally relatively reduced, whereas in younger children with ASD, it is generally increased, a pattern that appears to be consistent using regional homogeneity (25), wavelet correlation (26), and independent component analysis (27) approaches.

An additional obstacle to a fuller understanding of intrinsic brain connectivity differences in ASD is the underexplored potential influence of gender effects. This is particularly relevant given that a recent meta-analysis suggests that the male-to-female ratio in ASD is close to 3:1 (28) and that gender is recognized as a potential major source of heterogeneity in ASD neurobiology (29). Many smaller neuroimaging studies of ASD have excluded female subjects altogether or have covaried the effects of gender when female subjects are included. One exception is a recent study that used the first Autism Brain Imaging Data Exchange (ABIDE) dataset to examine gender differences with respect to intrinsic connectivity of the posterior superior temporal sulcus, a region with strong connections with brain regions that code for social information, and the posterior cingulate cortex, a core region of the default network (30). The investigators found gender effects wherein female individuals with ASD had high connectivity patterns that resembled patterns observed in typically developing male individuals, whereas connectivity in male individuals with ASD resembled the low connectivity patterns in typically developing female individuals. These preliminary findings suggest that gender exerts moderating effects on patterns of intrinsic connectivity in ASD.

The purpose of this study was to evaluate the joint developmental and gender effects on intrinsic connectivity using measures that assess functional segregation and integration. We used data from ABIDE, which contains brain imaging data collected from laboratories around the world. The ABIDE initiative now includes two large-scale collections: ABIDE I was compiled from 17 sites, containing 1112 datasets from 539 individuals with ASD and 573 typically developing control (TDC) individuals (age range 7–64 years and median age 14.7 years across groups) (31), and ABIDE II was compiled from 19 sites, containing 1114 datasets from 521 individuals with ASD and 593 TDC individuals (age range 5–64 years) (32). Together, the combined ABIDE I and ABIDE II datasets provide 2226 unique cross-sectional datasets allowing researchers to address unprecedented questions owing to the sample size afforded by these unique data repositories.

Our analytic approach applied the framework of functional segregation and integration to examine both gender and age differences in whole-brain intrinsic connectivity in participants selected from the ABIDE I and II datasets. Additionally, we probed these results to localize specific functional networks that contributed to any findings obtained at the whole-brain level. We used the meta-analytic approach of inverse variance weighted effect size estimates (33) to control for site difference for all analyses. Based on developmental models of intrinsic connectivity reviewed earlier, we expected to find a quadratic relationship between whole-brain segregation and integration and age in the ASD sample, relative to a linear pattern in the TDC sample (34). Finally, although we expected to find whole-brain differences in functional segregation and integration in ASD, we hypothesized that these differences would be pronounced in the somatosensory network (35–37) and/or the salience network (38–41). Based on previous work using the ABIDE I dataset on gender differences in ASD functional connectivity (30), we examine both main effects and interactions with age of gender on segregation and integration.

METHODS AND MATERIALS

Participants

Data from ABIDE I and II were used in this study. Anatomical and resting-state scans were acquired from 1060 individuals with ASD and 1166 TDC individuals. Each site confirmed ASD diagnosis through clinical judgment and/or standard diagnostic instruments (Autism Diagnostic Observation Schedule and/or Autism Diagnostic Interview–Revised). Full-scale IQ was collected using a variety of instruments, and the measure was then set to a standard scale among sites. For site-specific details on both diagnostic criteria and IQ, see http://fcon_1000.projects.nitrc.org/indi/abide/.

Site Exclusion Criteria

Both ABIDE I and II datasets were collected from a variety of sites, using a variety of different scanner protocols. A number of sites were excluded from the current analysis for the following criteria: 1) no female subjects were collected (nine sites), 2) no TDC individuals were collected (one site), and 3) the data at the site failed visual quality control (two sites). Demographic and motion data for each site are presented in Supplemental Tables S1 and S2, respectively.

Preprocessing

Preprocessing was performed using FSL version 5.0 (FMRIB Software Library; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) (42) as follows. Anatomical and functional scans were converted to left anterior superior alignment. Slice time correction was performed on each scan using FSL slicetimer. Rigid body motion correction was performed using FSL MCFLIRT; using the estimated motion parameters, 24-parameter regression was used to additionally correct for motion. Skull stripping was then performed using information from the T1 anatomical scans. The skull stripped functional images were then normalized to each participant's T1 scans using FSL FLIRT, the T1 scans were normalized to the Montreal Neurological Institute 152 2-mm standard image, and the functional scans were normalized to the Montreal Neurological Institute 152 space by combining those transformations. White matter and gray matter masks were generated by FSL FAST and regressed out from the functional signals. Finally, bandpass filtering was performed with the bounds 0.08 to 0.001 Hz. The preprocessed voxel-level time series were parcellated into the Power functional atlas (43), using 10-mm spheres centered around the provided coordinates. This atlas contains 264 functional regions of interest (ROIs) divided into 14 functional networks as follows: sensory/somatomotor hand, sensory/somatomotor mouth, cingulo-opercular task control, auditory, default mode, memory retrieval, ventral attention, visual, frontoparietal task control, salience, subcortical, cerebellar, dorsal attention, and undetermined. The 28 ROIs classified as being part of undetermined networks were omitted from subsequent analysis, and the final count of ROIs was 236.

The ROI correlation matrices were thresholded into binary undirected functional connectivity matrices before arriving at modularity and global efficiency measures, as these measures perform better on thresholded matrices rather than correlation (44). As a correlation is in a standard metric across participants, a universal thresholding procedure was applied, allowing average degree and density of edges to vary between participants, thus reflecting between-participant differences in connectivity. It is standard practice to report results across several putative thresholds when creating binary graphs from correlation matrices (45). To test for the sensitivity of the results to threshold choice, analyses were repeated using a sliding threshold, from 0.1 to 0.95 correlation, in steps of 0.025. Results for both modularity and global efficiency models were similar across a band of thresholding (0.375 to 0.525); as such, results are reported using a threshold of 0.45.

Motion Correction

Owing to the influence of motion on intrinsic connectivity (46), care needs to be taken to ensure that the effects of motion are minimized. Frame displacement was calculated as the sum of the absolute lag-1 differences in the six motion parameters. Scrubbing was applied to each participant's time series using a frame displacement threshold of 0.3 and removing 1 time point before and 2 time points after each time point that exceeded the threshold. Finally, participants with more than 30% data loss from scrubbing were removed from subsequent analysis. This, combined with the site exclusion criteria described above, resulted in a final sample of 709 individuals with ASD (118 female) and 878 TDC individuals (261 female). A model was fit examining mean frame displacement as predicted by gender and diagnostic category. This model demonstrated that the significant difference was that TDC individuals showed lower mean frame displacement overall and that TDC female individuals showed lower mean frame displacement than TDC male individuals. Details are presented in Supplemental Tables S3 and S4. While global signal regression (GSR) is a common approach for attenuating the influence of motion, we opted for scrubbing for a number of reasons. First, GSR induces negative correlations when conducting functional connectivity, and this would have a great impact on our results (47,48). Scrubbing reduces motion artifacts without resulting in systematic artifacts seen when GSR is used (49). Second, GSR may not be optimal when groups have different noise characteristics (50), which is highly likely in our sample of control and clinical individuals. Specific to the present analyses, GSR can mask between-group differences in connectivity, particularly when considering individuals with ASD and TDC individuals (51).

Analytic Procedure

Analysis of the thresholded binary functional connectivity matrices was performed in a series of steps:

1. Modularity and global efficiency were calculated per individual.
2. Two regression models were fit for each site with modularity and global efficiency data as dependent variables.
3. The resulting site-specific models were aggregated using the meta-analytic technique of inverse variance weighted effect size estimation.
4. A network knockout procedure was used to examine the performance of the first three steps when specific functional networks were excluded from the modularity and global efficiency calculations.

Each of these steps are explained below.

Modularity and Global Efficiency. Modularity (52) is a network statistic that indicates how segregated a priori defined communities are in a network. Used in neuroimaging research, it is a measure of the functional segregation of the brain (53). This value is contingent on an a priori defined set of communities. In the case of the current analysis, the 14 functional networks of the Power *et al.* (43) functional atlas were used as our prespecified communities.

Global efficiency (54) is a network statistic that is proportional to the average length of the shortest paths; values close to 1 represent high efficiency in information transfer, in that each node in the network can be quickly reached by any other node, whereas values close to 0 represent a more segregated network. Global efficiency, when used in a functional imaging context, represents whole-brain functional integration (53). In an important difference from modularity, it does not require a priori functional network definitions.

Regression Model. Once modularity and global efficiency were calculated for every individual, a linear regression model was fit to each site's data separately. This model was a fixed effects only model with a normal theory error and incorporated as main effects age, age-squared, gender, and diagnostic category; as second-order interactions, it included the interaction of age with gender, age with diagnostic category, age-squared with gender, and age-squared with diagnostic category; and finally two third-order interaction terms, age by gender by diagnostic category and age-squared by gender by diagnostic category.

Inverse Variance Weighted Effect Size Estimates. The effect estimates of the regression models from each site were aggregated using the meta-analytic technique of inverse variance weighted effect size (33). In this technique, the aggregate regression coefficient and variance are computed as follows:

$$\overline{\beta} = \frac{\sum \frac{\beta_i}{\sigma_i^2}}{\sum \frac{1}{\sigma_i^2}}, \; \overline{\sigma^2} = \frac{1}{\sum \frac{1}{\sigma_i^2}},$$

where β_i and σ_i^2 are the specific regression coefficient and variances from site i, respectively. This is a fixed effects method of aggregating effect estimates and assumes that there is a single population level value of the effect that needs to be estimated. If a site's value differs from that population level effect, this is due to random noise rather than systematic variation. This makes the assumption that the same population is under study at each site, and any differences in scanner

protocol or recruitment efforts (e.g., methods for obtaining those with diagnoses; systematic differences in symptom presentation across sites) result in statistical noise rather than meaningful differences. As each of the sites aimed at obtaining a representative sample of individuals with ASD, we view the assumptions of the fixed effect aggregation method as reasonable. Mechanistically, this aggregation method down-weights and discounts smaller sites and sites with noisier data, reducing the impact that these sites would have on full data analysis methods.

Network Knockout Analysis. To isolate the influence of specific networks on the results, a simple leave-one-out process was used where functional networks were removed from the analysis. The analysis pipeline, from computation of graph theory measures to aggregation of regression effects, was performed after each functional network was excluded from the data in turn. This procedure differs from a robustness analysis in that the nodes and edges removed for any given iteration were not random, but rather based on the pre-specified functional network definitions. The results from each model can be compared with the full network model by assessing if the aggregate regression coefficients of the leave-one-out model are significantly different from the full network model. This is accomplished by testing the difference between regression coefficients in the form of a *z*-test (55).

Supplementary Analysis

Two additional models were fit to the whole-brain network data and aggregated over sites using the method described above. The first additional model included full-scale IQ as a main effect. As some sites did not provide full-scale IQ, this reduced the overall sample size from 2226 to 1585 before motion correction, which is why these results are not the focus of the article. The second model restricted the age of the sample to younger than 33 years, which was chosen because it is 2 SD above the mean age. This choice enabled us to evaluate the potential influence of outliers in terms of age but greatly restricts the age span. These models are briefly discussed in Results.

RESULTS

Demographics

The demographics of the analyzed sample are presented in Table 1.

Group Invariant Connections

Figure 1 shows connectivity graphs for functional connections that occurred in more than 30% of the sample after the 0.45 correlation threshold was applied for the ASD and TDC samples. There are several similarities in patterns of connectivity: all groupings exhibited a high degree of connectivity in the visual cortex, denoted using yellow nodes, as well as some degree of connectivity in the prefrontal cortex. Finally, both groups exhibited a large set of interhemispheric connections, as would be expected. The brain networks were visualized with the BrainNet Viewer (http://www.nitrc.org/projects/bnv/) (56).

Table 1. Demographics by Gender and Diagnostic Category

	n	Age, Years	FIQ	SRS	FD	% Missing[a]
Male, ASD	591	14.53	106.64	91.93	0.077	0.073
Female, ASD	118	15.41	103.19	95.94	0.079	0.072
Male, TDC	617	14.73	112.78	19.96	0.071	0.058
Female, TDC	261	14.25	113.43	19.93	0.064	0.043

Values represent mean values within group.

ASD, autism spectrum disorder; FD, frame displacement (46); FIQ, full-scale intelligence quotient; SRS, Social Responsiveness Scale (62); TDC, typically developing control.

[a]Average number of time points removed at FD threshold of 0.3.

Age and Diagnostic Category Differences in Functional Integration and Segregation

Differences between ASD and TDC groups occurred in the relationship between age and both modularity and global efficiency. Specifically, the quadratic effect of age and the interaction between TDC status and age-squared were significant for both modularity and global efficiency (Table 2). The main negative quadratic effect for age on modularity represents the effect for male individuals with an ASD diagnosis and suggested that modularity first increases across age and then decreases ($\beta = -.0002$, $p = .0115$). Global efficiency, by contrast, decreased in the early years followed by an increase for individuals with ASD ($\beta = .0001$, $p = .0279$). The significant interaction of age squared with TDC status negated these effects, with TDC age demonstrating significant curvature in the opposite direction than seen with ASD age. This effectively brought the estimates of curvature on both measures to zero for TDC individuals. It is also important to note that TDC individuals had a linear effect for age that almost reached significance ($\beta = -.0034$, $p = .0523$), suggesting a lower slope for the relationship between age and modularity than seen in individuals with ASD.

Figures 2 and 3 show the plotted marginal effect curves imposed over the point cloud. These marginal lines demonstrate that in individuals with ASD, modularity had a clear negative quadratic effect with increases occurring up until the mean age of 16 and decreasing following that, whereas global efficiency had the opposite effect. For TDC individuals, modularity remained constant or increased, whereas global efficiency decreased.

Gender and Functional Integration and Segregation

There was largely a lack of gender-related findings with the notable exception of a marginally significant ($\beta = .0009$, $p = .07$) three-way interaction between diagnostic category, age squared, and gender on modularity. This trend-level finding suggests TDC female individuals had a slightly higher rate of modularity increase over development than did male individuals with ASD. However, owing to the marginal nature of this effect and the small sample size for studying gender effects, the magnitude of this effect should be interpreted with caution.

Localization of Effects

Network knockout was used to determine the contribution of each network to the above models. The only network whose removal completely suppressed an effect (operationalized as

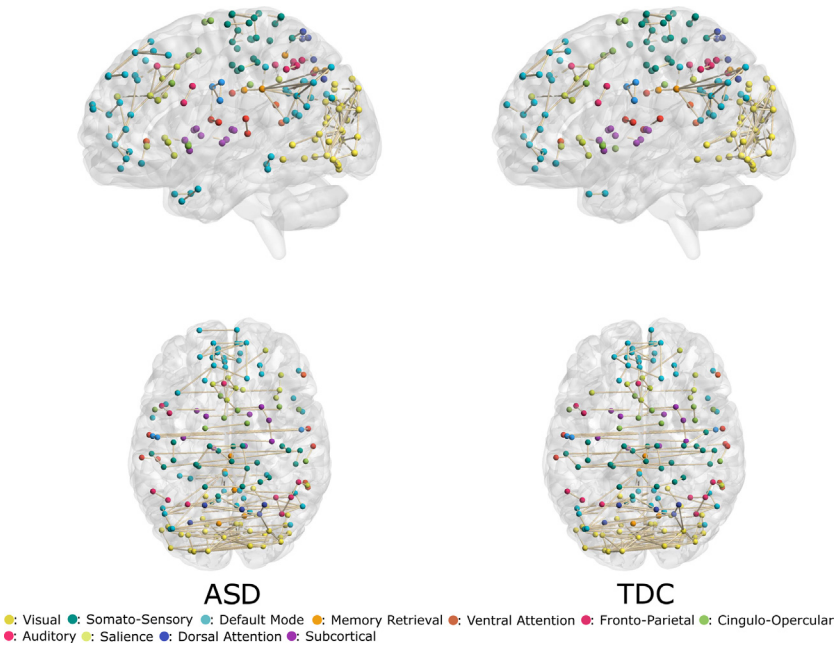

Figure 1. Connectivity patterns across diagnostic categories. All participants evidenced a high degree of connectivity within visual networks. The somatosensory network explained some of the age-related differences between individuals with autism spectrum disorder (ASD) and typically developing control (TDC) individuals, as removal of this network reduced the differences in modularity seen across age for male individuals with ASD and TDC individuals. Yellow nodes represent visual network regions, dark green nodes represent somatosensory regions, turquoise nodes represent default mode regions, orange nodes represent memory retrieval regions, dark orange nodes represent ventral attention regions, crimson nodes represent frontoparietal regions, green nodes represent cingulo-opercular task control regions, light red nodes represent auditory regions, light green nodes represent salience regions, dark blue nodes represent dorsal attention regions, and purple nodes represent subcortical regions.

the effect became nonsignificant across the majority of the thresholding band) was that of the somatosensory network. The removal of this network led to the nonsignificant effect of the interaction of age squared with diagnostic category on modularity. However, the removal of the somatosensory network did not affect the global efficiency models (Table 3).

Whereas the effect of age-squared was still significant, the effect of the TDC individuals by age squared interaction term became nonsignificant. However, the magnitude of this effect remained unchanged (β = .0002, p = .1025). Testing for significant differences between the full brain model and the model that omitted the somatosensory network indicated that the values of the regression coefficients were not significantly different between these models for any consistent interval of thresholding. This suggests that the presence of the somatosensory functional network provided a strong consistent effect (in that it reduced noise) on the curvature of modularity across age for TDC individuals when included in the calculation of modularity but had no major effect when included in the calculation of global efficiency. Finally, the marginal three-way interaction of diagnostic status, gender, and age squared remained marginally significant (β = .0009, p = .0646).

Overview of Supplementary Analysis

The supplementary analysis examining the inclusion of intelligence and restricted age range is included in the Supplement. Here we briefly describe the findings from those models. The inclusion of intelligence left the significant results the same as

Table 2. Marginal Inverse Variance Weighted Regression Coefficients

	Modularity			Global Efficiency		
	Estimate	*t* Value	*p* Value	Estimate	*t* Value	*p* Value
Intercept	0.2479[a]	38.2969[a]	.0000[a]	0.1639[a]	60.8504[a]	0[a]
TDC	0.0017	0.1847	.8534	0.0036	0.9082	.3638
Female	0.0116	0.6734	.5007	−0.007	−0.9929	.3207
Age	0.0012	1.003	.3159	−0.001	−1.7266	.0842
Age^2	−0.0002[a]	−2.5284[a]	.0115[a]	0.0001[a]	2.1986[a]	.0279[a]
TDC×Female	0.0034	0.1332	.8941	0.0071	0.663	.5074
TDC×Age	−0.0034[b]	−1.941[b]	.0523[b]	0.0001	0.1546	.8771
Female×Age	−0.0005	−0.1136	.9095	−0.0027	−1.2787	.201
TDC×Age^2	0.0002[a]	2.0913[a]	.0365[a]	−0.0001[a]	−2.478[a]	.0132[a]
Female×Age^2	−0.0001	−0.3783	.7052	0.0001	0.7746	.4386
TDC×Female×Age	0.003	0.5294	.5965	0.0035	1.3067	.1913
TDC×Female×Age^2	0.0009[b]	1.8122[b]	.0700[b]	−0.0001	−0.5176	.6047

TDC, typically developing control.
[a]$p < .05$.
[b]$p < .1$.

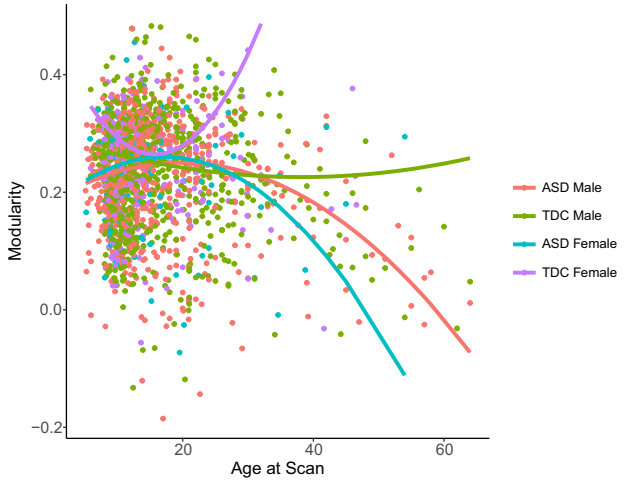

Figure 2. Modularity by age (marginal effect lines plotted). Individuals with autism spectrum disorder (ASD) had significantly more negative curvature in the relationship between age and modularity than typically developing control (TDC) individuals. There was a trend for female TDC individuals to have larger, positive curvature compared with male individuals with ASD.

the full data model, while additionally showing a significant diagnostic category by age interaction ($\beta = -.0044$, $p = .02$).

The models that excluded individuals older than 33 years of age substantially changed the pattern of significant results. The magnitude of most effects was unchanged, and many previously significant effects became nonsignificant. Furthermore, gender-related interactions between diagnostic category and age became significant for both modularity and global efficiency models. Removal of the upper age range in this case invokes a restriction of range, which is known to attenuate the significance of regression models (55). As the individuals removed from this analysis were not outliers in their values of modularity and global efficiency, this highlights the need to collect larger older samples with equal distributions of genders for researching brain connectivity in ASD.

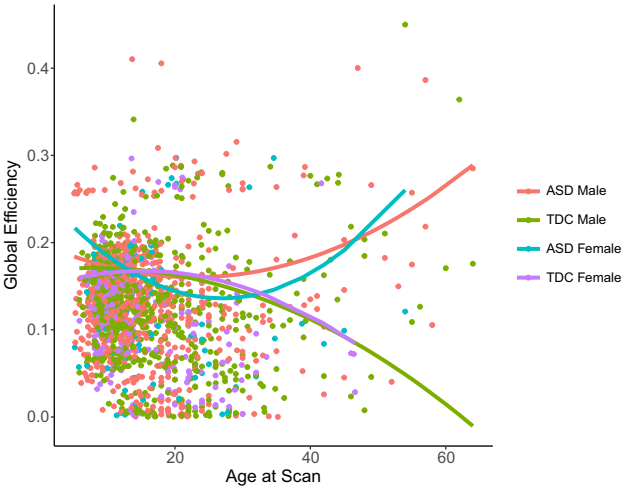

Figure 3. Global efficiency by age (marginal effect lines are plotted). Individuals with autism spectrum disorder (ASD) had significantly more negative curvature in the relationship between age and modularity than typically developing control (TDC) individuals. There were no gender differences for the general shape of the effects.

DISCUSSION

The goal of the present study was to examine the unique and multiplicative influences of development, gender, and ASD diagnosis on intrinsic functional connectivity using functional segregation and integration measures. We found that individuals with ASD had a distinct pattern of age-related functional connectivity relative to TDC individuals. In particular, the ASD group was characterized by an increase in regional segregation into distinct functional networks followed by a marked decrease in segregation across time. The opposite pattern was observed for TDC individuals, suggesting that segregation of functional networks persists into adulthood in typical development but not in ASD. A similar pattern was observed in connectivity integration results: individuals with ASD evidenced increased functional integration over development, whereas the opposite was observed for TDC individuals. These findings help to clarify prior work that showed hypoconnectivity in ASD for adults within various networks (12–15) as well as between networks (16), whereas the opposite has been found for children with ASD (25,26). Complementary to these prior findings, we observed that there was an overall pattern in adults with ASD of a lack of segregation of functional networks and a higher integration of the component ROIs in ASD. Thus, the hypoconnectivity within networks may partially be explained by a lack of differentiation in adulthood and increased connectivity with regions not in the intrinsic network.

Additionally, we observed that the differential relationship between modularity and age seen in the ASD sample was in large part due to the somatosensory network. This result from localization analysis suggests that the somatosensory network drives, at least in part, the increase in modularity across time seen in TDC individuals relative to individuals diagnosed with ASD. Thus, differentiation and specification of regions related to the somatosensory network appears to contribute greatly to functional connectivity changes across development. Recent findings have suggested that abnormal functional connectivity exists within the somatosensory cortex in individuals with ASD (36), and our findings support this conclusion. However, the knockout of the somatosensory cortex had no effect on the global efficiency models, which suggests that the differences in global efficiency between individuals with ASD and TDC individuals were not localized to the somatosensory cortex but rather were a more global whole-brain phenomenon.

We found trends toward significance for gender effects within the ASD group on modularity. In particular, TDC female individuals had higher quadratic effects of age on modularity that trended toward significantly different from male individuals with ASD. As higher-order interaction effects are notoriously low powered even for large samples, this result warrants mentioning (57). Additionally, our model aggregation method uses female individuals within each site, which further reduces power to detect effects. Should this effect be replicated in future work, it would be consistent with the hypothesis that female individuals with ASD require greater etiological risk

Table 3. Results for Somatosensory Network Knockout

	Modularity			Global Efficiency		
	Estimate	*t* Value	*p* Value	Estimate	*t* Value	*p* Value
Intercept	0.2463[a]	38.7272[a]	0[a]	0.1594[a]	58.4614[a]	0[a]
TDC	0.0015	0.1591	.8736	0.0036	0.9079	.364
Female	0.0082	0.493	.622	−0.007	−0.9844	.3249
Age	0.0011	0.9027	.3667	−0.0011	−1.9464	.0516
Age^2	−0.0002[a]	−2.4939[a]	.0126[a]	0.0001[a]	2.1417[a]	.0322[a]
TDC×Female	0.01	0.4053	.6853	0.007	0.6553	.5123
TDC×Age	−0.0025	−1.448	.1476	0.0002	0.2265	.8208
Female×Age	0.0013	0.2784	.7807	−0.0022	−1.0415	.2976
TDC×Age^2	0.0002	1.6327	.1025	−0.0001[a]	−2.4036[a]	.0162
Female×Age^2	−0.0001	−0.5004	.6168	0.0001	0.6479	.517
TDC×Female×Age	0.0003	0.0575	.9541	0.0035	1.3232	.1858
TDC×Female×Age^2	0.0009[b]	1.8482[b]	.0646[b]	−0.0001	−0.5854	.5583

TDC, typically developing control.
[a]$p < .05$.
[b]$p < .1$.

factors and genetic load than male individuals before reaching diagnostic thresholds of ASD (58–60). Future studies with larger samples of female individuals with ASD are needed to further clarify the effects of gender on the development of brain connectivity in ASD. This is further highlighted by the age-constrained analyses performed, which indicate that the inclusion of the older portion of the sample appears to in part control for the appearance of gender effects.

Our finding that the ASD group was characterized by a quadratic pattern of functional network segregation (i.e., an initial increase during adolescence followed by a decrease into adulthood) has a number of implications. First, it highlights the critical need to examine trajectories of development in ASD in large-scale datasets, given that smaller, cross-sectional studies would be insensitive to subtle changes over time within functional brain networks. The preponderance of cross-sectional studies in the literature also likely contributes to the contradictions in the cross-sectional ASD connectivity literature, with some studies reporting hyperconnectivity and other studies reporting hypoconnectivity (34,61). Additionally, these age-related findings highlight that there are likely critical developmental periods during which brain connectivity trajectories in ASD deviate from those of typically developing samples. True longitudinal datasets with dense sampling are needed to more fully delineate the precise nature of developmental trajectories of functional networks segregation in ASD through the life span.

There are several limitations to the current study. The ABIDE I and II datasets are cross-sectional, and so all developmental conclusions must be interpreted with caution. The ABIDE I and II datasets are significant resources for studying age-associated differences in functional connectivity, but a definitive study of individual trajectories of functional connectivity across the life span would require repeated scans within individuals. This study also highlights a limitation with multiple collection sites and scanner protocols in secondary analysis of magnetic resonance imaging data. Whereas our use of inverse variance weighted effect estimates helps to control for possible site-related spurious findings, it does so by combining within-site models and so reduces overall power to detect effects. This in part controls for findings driven by atypical sites, but it does not completely account for their influence. This is particularly salient when one is interested in low-prevalence phenomena such as female individuals with ASD. Despite these limitations, the present investigation leverages first-of-their-kinds ASD functional magnetic resonance imaging dataset to address the effects of gender and age on intrinsic brain connectivity in ASD.

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ARTICLE INFORMATION

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REFERENCES

1. American Psychiatric Association (2013): Diagnostic and Statistical Manual of Mental Disorders: DSM-5, 5th ed. Arlington, VA: American Psychiatric Association.

2. Li D, Karnath H-O, Xu X (2017): Candidate biomarkers in children with autism spectrum disorder: A review of MRI studies. Neurosci Bull 33:219–237.
3. Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V (2013): Default mode network in childhood autism: Posteromedial cortex heterogeneity and relationship with social deficits. Biol Psychiatry 74:212–219.
4. Vissers ME, Cohen MX, Geurts HM (2012): Brain connectivity and high functioning autism: A promising path of research that needs refined models, methodological convergence, and stronger behavioral links. Neurosci Biobehav Rev 36:604–625.
5. Schipul SE, Keller TA, Just MA (2011): Inter-regional brain communication and its disturbance in autism. Front Syst Neurosci 5:10.
6. Mizuno A, Liu Y, Williams DL, Keller TA, Minshew NJ, Just MA (2011): The neural basis of deictic shifting in linguistic perspective-taking in high-functioning autism. Brain 134:2422–2435.
7. Solomon M, Ozonoff SJ, Ursu S, Ravizza S, Cummings N, Ly S, *et al.* (2009): The neural substrates of cognitive control deficits in autism spectrum disorders. Neuropsychologia 47:2515–2526.
8. Mason RA, Williams DL, Kana RK, Minshew N, Just MA (2008): Theory of Mind disruption and recruitment of the right hemisphere during narrative comprehension in autism. Neuropsychologia 46:269–280.
9. Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA (2009): Atypical frontal-posterior synchronization of Theory of Mind regions in autism during mental state attribution. Soc Neurosci 4:135–152.
10. Koshino H, Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA (2008): fMRI investigation of working memory for faces in autism: Visual coding and underconnectivity with frontal areas. Cereb Cortex 18:289–300.
11. Damarla SR, Keller TA, Kana RK, Cherkassky VL, Williams DL, Minshew NJ, *et al.* (2010): Cortical underconnectivity coupled with preserved visuospatial cognition in autism: Evidence from an fMRI study of an embedded figures task. Autism Res 3:273–279.
12. Kennedy DP, Courchesne E (2008): The intrinsic functional organization of the brain is altered in autism. Neuroimage 39:1877–1885.
13. Weng SJ, Wiggins JL, Peltier SJ, Carrasco M, Risi S, Lord C, *et al.* (2010): Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. Brain Res 1313:202–214.
14. Assaf M, Jagannathan K, Calhoun VD, Miller L, Stevens MC, Sahl R, *et al.* (2010): Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. Neuroimage 53:247–256.
15. Cherkassky VL, Kana RK, Keller TA, Just MA (2006): Functional connectivity in a baseline resting-state network in autism. Neuroreport 17:1687–1690.
16. von dem Hagen EA, Stoyanova RS, Baron-Cohen S, Calder AJ (2013): Reduced functional connectivity within and between "social" resting state networks in autism spectrum conditions. Soc Cogn Affect Neurosci 8:694–701.
17. Hull JV, Jacokes ZJ, Torgerson CM, Irimia A, Van Horn JD, Aylward E, *et al.* (2017): Resting-state functional connectivity in autism spectrum disorders: A review. Front Psychiatry 7:205.
18. Mohammad-Rezazadeh I, Frohlich J, Loo SK, Jeste SS (2016): Brain connectivity in autism spectrum disorder. Curr Opin Neurol 29:137–147.
19. Redcay E, Moran JM, Mavros PL, Tager-Flusberg H, Gabrieli JD, Whitfield-Gabrieli S (2013): Intrinsic functional network organization in high-functioning adolescents with autism spectrum disorder. Front Hum Neurosci 7:573.
20. Wiggins JL, Peltier SJ, Ashinoff S, Weng S-J, Carrasco M, Welsh RC, *et al.* (2011): Using a self-organizing map algorithm to detect age-related changes in functional connectivity during rest in autism spectrum disorders. Brain Res 1380:187–197.
21. Starck T, Nikkinen J, Rahko J, Remes J, Hurtig T, Haapsamo H, *et al.* (2013): Resting state fMRI reveals a default mode dissociation between retrosplenial and medial prefrontal subnetworks in ASD despite motion scrubbing. Front Hum Neurosci 7:802.
22. Price T, Wee C, Gao W, Shen D (2014): Multiple-network classification of childhood autism using functional connectivity dynamics. Med Image Comput Comput Assist Interv 17(Pt 3):177–184.
23. Paakki JJ, Rahko J, Long X, Moilanen I, Tervonen O, Nikkinen J, *et al.* (2010): Alterations in regional homogeneity of resting-state brain activity in autism spectrum disorders. Brain Res 1321:169–179.
24. Uddin LQ, Supekar K, Lynch CJ, Khouzam A, Phillips J, Feinstein C, *et al.* (2013): Salience network-based classification and prediction of symptom severity in children with autism. JAMA Psychiatry 70:869–879.
25. Dajani DR, Uddin LQ (2016): Local brain connectivity across development in autism spectrum disorder: A cross-sectional investigation. Autism Res 9:43–54.
26. Supekar K, Uddin LQ, Khouzam A, Phillips J, Gaillard WD, Kenworthy LE, *et al.* (2013): Brain hyperconnectivity in children with autism and its links to social deficits. Cell Rep 5:738–747.
27. Nomi JS, Uddin LQ (2015): Developmental changes in large-scale network connectivity in autism. Neuroimage Clin 7:732–741.
28. Loomes R, Hull L, Mandy WPL (2017): What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry 56:466–474.
29. Lai MC, Lerch JP, Floris DL, Ruigrok AN, Pohl A, Lombardo MV, *et al.* (2017): Imaging sex/gender and autism in the brain: Etiological implications. J Neurosci Res 95:380–397.
30. Alaerts K, Swinnen SP, Wenderoth N (2016): Sex differences in autism: A resting-state fMRI investigation of functional brain connectivity in males and females. Soc Cogn Affect Neurosci 11:1002–1016.
31. Di Martino A, Yan C-G, Li Q, Denio E, Castellanos FX, Alaerts K, *et al.* (2014): The autism brain imaging data exchange: Towards a large-scale evaluation of the intrinsic brain architecture in autism. Mol Psychiatry 19:659–667.
32. Di Martino A, O'Connor D, Chen B, Alaerts K, Anderson JS, Assaf M, *et al.* (2017): Enhancing studies of the connectome in autism using the Autism Brain Imaging Data Exchange II. Sci Data 4:170010.
33. Lipsey MW, Wilson DB (2001): Practical Meta-analysis. Thousand Oaks, CA: Sage Publications.
34. Uddin LQ, Supekar K, Menon V (2013): Reconceptualizing functional brain connectivity in autism from a developmental perspective. Front Hum Neurosci 7:458.
35. Chen CP, Keown CL, Jahedi A, Nair A, Pflieger ME, Bailey BA, *et al.* (2015): Diagnostic classification of intrinsic functional connectivity highlights somatosensory, default mode, and visual regions in autism. Neuroimage Clin 8:238–245.
36. Khan S, Michmizos K, Tommerdahl M, Ganesan S, Kitzbichler MG, Zetino M, *et al.* (2015): Somatosensory cortex functional connectivity abnormalities in autism show opposite trends, depending on direction and spatial scale. Brain 138:1394–1409.
37. Nair A, Treiber JM, Shukla DK, Shih P, Müller RA (2013): Impaired thalamocortical connectivity in autism spectrum disorder: A study of functional and anatomical connectivity. Brain 136:1942–1955.
38. Green SA, Hernandez L, Bookheimer SY, Dapretto M (2016): Salience network connectivity in autism is related to brain and behavioral markers of sensory overresponsivity. J Am Acad Child Adolesc Psychiatry 55:618–626.e1.
39. Odriozola P, Uddin LQ, Lynch CJ, Kochalka J, Chen T, Menon V (2016): Insula response and connectivity during social and non-social attention in children with autism. Soc Cogn Affect Neurosci 11:433–444.
40. Uddin LQ, Menon V (2009): The anterior insula in autism: Under-connected and under-examined. Neurosci Biobehav Rev 33:1198–1203.
41. Yamada T, Itahashi T, Nakamura M, Watanabe H, Kuroda M, Ohta H, *et al.* (2016): Altered functional organization within the insular cortex in adult males with high-functioning autism spectrum disorder: Evidence from connectivity-based parcellation. Mol Autism 7:41.
42. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM (2012): FSL. Neuroimage 62:782–790.
43. Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, *et al.* (2011): Functional network organization of the human brain. Neuron 72:665–678.
44. Newman MEJ (2004): Analysis of weighted networks. Phys Rev E70:056131.
45. Fair DA, Cohen AL, Power JD, Dosenbach NU, Church JA, Miezin FM, *et al.* (2009): Functional brain networks develop from a "local to distributed" organization. PLoS Comput Biol 5:e1000381.

46. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2011): Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 59:2142–2154.
47. Anderson JS, Druzgal TJ, Lopez-Larson M, Jeong EK, Desai K, Yurgelun-Todd D (2011): Network anticorrelations, global regression, and phase-shifted soft tissue correction. Hum Brain Mapp 32:919–934.
48. Saad ZS, Gotts SJ, Murphy K, Chen G, Jo HJ, Martin A, *et al.* (2012): Trouble at rest: How correlation patterns and group differences become distorted after global signal regression. Brain Connect 2:25–32.
49. Ciric R, Wolf DH, Power JD, Roalf DR, Baum GL, Ruparel K, *et al.* (2017): Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. Neuroimage 154:174–187.
50. Murphy K, Fox MD (2017): Towards a consensus regarding global signal regression for resting state functional connectivity MRI. Neuroimage 154:169–173.
51. Gotts SJ, Saad ZS, Jo HJ, Wallace GL, Cox RW, Martin A (2013): The perils of global signal regression for group comparisons: A case study of autism spectrum disorders. Front Hum Neurosci 7:356.
52. Newman ME (2006): Modularity and community structure in networks. Proc Natl Acad Sci U S A 103:8577–8582.
53. Cohen JR, D'Esposito M (2016): The segregation and integration of distinct brain networks and their relationship to cognition. J Neurosci 36:12083–12094.
54. Latora V, Marchiori M (2001): Efficient behavior of small-world networks. Phys Rev Lett 87:198701.
55. Cohen J, Cohen P, West SG, Aiken LS (2003): Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences, 3rd ed. Mahwah, NJ: Erlbaum.
56. Xia M, Wang J, He Y (2013): BrainNet Viewer: A network visualization tool for human brain connectomics. PLoS One 8:e68910.
57. Heo M, Leon AC (2010): Sample sizes required to detect two-way and three-way interactions involving slope differences in mixed-effects linear models. J Biopharm Stat 20:787–802.
58. Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, *et al.* (2011): Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70:863–885.
59. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, *et al.* (2007): Strong association of de novo copy number mutations with autism. Science 316:445–449.
60. Werling DM, Geschwind DH (2013): Sex differences in autism spectrum disorders. Curr Opin Neurol 26:146–153.
61. Deen B, Pelphrey KA (2011): Large-scale cortical functional connectivity in children with autism spectrum disorders. Presented at the International Meeting for Autism Research, May 12–14, San Diego, California.
62. Constantino JN, Gruber C (2005): Social Responsiveness Scale (SRS). Los Angeles, CA: Western Psychological Services.