Title

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Journal

Brain, 145(1)

ISSN

0006-8950

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Publication Date

2022-03-29

DOI

10.1093/brain/awab204

Peer reviewed

BRAIN
ORIGINAL ARTICLE

Impact of autism genetic risk on brain connectivity: a mechanism for the female protective effect

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The biological mechanisms underlying the greater prevalence of autism spectrum disorder in males than females remain poorly understood. One hypothesis posits that this female protective effect arises from genetic load for autism spectrum disorder differentially impacting male and female brains.

To test this hypothesis, we investigated the impact of cumulative genetic risk for autism spectrum disorder on functional brain connectivity in a balanced sample of boys and girls with autism spectrum disorder and typically developing boys and girls (127 youth, ages 8–17). Brain connectivity analyses focused on the salience network, a core intrinsic functional connectivity network which has previously been implicated in autism spectrum disorder.

The effects of polygenic risk on salience network functional connectivity were significantly modulated by participant sex, with genetic load for autism spectrum disorder influencing functional connectivity in boys with and without autism spectrum disorder but not girls.

These findings support the hypothesis that autism spectrum disorder risk genes interact with sex differential processes, thereby contributing to the male bias in autism prevalence and proposing an underlying neurobiological mechanism for the female protective effect.

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Received November 21, 2020. Revised April 23, 2021. Accepted May 11, 2021. Advance access publication May 29, 2021

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Keywords: autism spectrum disorder; female protective effect; functional connectivity; imaging genetics; polygenic risk

Abbreviations: ASD = autism spectrum disorder; MNI = Montreal Neurological Institute; PRS = polygenic risk score; TD = typically developing

Introduction

Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental condition.[1] A substantial portion of genetic risk for ASD is estimated to derive from the additive effect of many common variants that are distributed across the genome and individually have very small effect sizes.[2–4] As a whole, genetic variation associated with ASD impacts neuronal and synaptic function, as well as synaptic plasticity, suggesting biological pathways whereby increased genetic risk for ASD affects brain connectivity.[5,6] Supporting this, both human post-mortem and *in vivo* neuroimaging studies have demonstrated that ASD is characterized by altered neural connectivity, which has been directly linked to individual ASD-associated common genetic variants in some studies.[7–10]

Notably, ASD is diagnosed approximately three to four times more frequently among males than females.[11,12] Although the exact discrepancy in prevalence rates between males and females may be affected by sex-specific social and diagnostic factors, a range of studies have shown that ASD is consistently less common among females than males; these studies include registry- and population-based analyses, as well as studies of siblings at high familial risk for ASD.[11–15] Sex differences in ASD prevalence are thought to be driven, at least in part, by a female protective effect, whereby females require an increased load of genetic and environmental risk factors to develop ASD compared to males.[13,14] Prior studies examining recurrence rates and ASD traits among multiplex families and twin pairs, as well as analyses investigating *de novo* mutation burden in ASD, have demonstrated a female protective effect with regards to both inherited and *de novo* genetic variation.[16–19] Previous work has also indicated that there is no overarching sex bias in the risk genes for ASD or their expression, suggesting that the posited female protective effect and associated sex differences in ASD prevalence are instead driven by the downstream impact of ASD risk genes.[14,20] One hypothesis proposes that the female protective effect specifically arises from ASD-associated genes interacting with the inherent sex differences present in the typical brain, such that genetic load for ASD differentially impacts male and female brains.[20] A prior study that assessed differential gene expression in the post-mortem human brain provided support for this hypothesis, demonstrating overlap between genes that have altered expression in ASD and genes involved in sexually dimorphic pathways.[20] In this study, altered gene expression in ASD was assessed in 32 subjects with and without ASD between the ages of 5 and 56 years, and in an overlapping dataset of 73 subjects with and without ASD between 2 and 82 years old; sex differences in gene expression were quantified in two sex-balanced samples of 10 participants between 13 and 56 years of age and in a sex-balanced sample of eight foetuses between 16 and 22 post-conception weeks.[20] To date, however, no studies in the *in vivo* human brain have tested the hypothesis that genetic load for ASD differentially impacts male and female brains or directly characterized the biological mechanisms of the female protective effect. We therefore examined for the first time the impact of cumulative polygenic risk for ASD on functional brain connectivity in boys and girls with and without ASD, allowing us to delineate the underlying neurobiological mechanisms of the female protective effect. We hypothesized that genetic load for ASD would differentially impact intrinsic functional connectivity in males with ASD compared to females with ASD, as well as in neurotypical males compared to neurotypical females.

Prior studies have found distributed functional connectivity alterations in ASD, as well as significant differences between males and females with ASD and atypical patterns of sex differences in ASD.[9,10,21–25] Among the functional connectivity networks that have been previously investigated in ASD, the salience network is of particular interest as it is hypothesized to contribute directly to the emergence of ASD traits.[26] The salience network is thought to play a role in orienting to salient stimuli.[26,27] Infants who later develop ASD show altered attention to and awareness of social and non-social sensory input, with such differences likely leading to an altered early environment for the infant and thereby possibly contributing to the development of ASD traits.[28–33] Supporting the importance of salience perception and the salience network to ASD, prior work examining resting-state salience network functional connectivity in a sample of 53 infants at high or low familial risk for ASD found altered salience network connectivity among 6-week-old infants at high familial risk for ASD compared to infants at low familial risk.[34] Salience network connectivity in these 6-week-old infants furthermore predicted subsequent individual trajectories in social attention to faces as measured with eye-tracking from 3 to 12 months, as well as future ASD traits on the Autism Observation Scale for Infants[35] at 12 months.[34] Paralleling these findings, numerous studies in individuals already diagnosed with ASD have found that salience network connectivity and activity are atypical in ASD, correlated with the magnitude of core ASD traits, and able to predict an ASD diagnosis at accuracies significantly above chance,[36–42] with prior work from our group likewise demonstrating significant resting-state salience network functional connectivity alterations in a sample of 75 boys with and without ASD between the ages of 8 and 17 years.[23] Notably, previous analyses focused on sex differences among individuals with and without ASD have also revealed that salience network functional connectivity significantly differs between neurotypical males and females, and such sex differences may be atypical among individuals with ASD.[23,43–46] Because of the robust evidence implicating the salience network in ASD, the current study focused on the relationship between polygenic risk for ASD and intrinsic salience network functional connectivity among males and females with and without ASD.

Materials and methods

Participants

Study participants were cognitively able youth with ASD or typically developing (TD) youth (ages 8–17) recruited from four collaborating sites (Harvard Medical School, Seattle Children's Research Institute, University of California Los Angeles and Yale University). All procedures complied with all site-specific ethical regulations. Informed assent and consent were obtained from all participants and their legal guardians, and the experimental protocol was approved by the Institutional Review Board at each participating site. All participants included in the ASD group were required to have a pre-existing diagnosis of ASD confirmed by a trained, research-reliable study clinician using the Autism Diagnostic Interview-Revised (ADI-R[47] and/or the Autism Diagnostic Observation Schedule, Generic or Second Edition; ADOS-G[48] or ADOS-2[49]), with virtually all participants meeting criteria on both (n = 57/61). Youth with ASD were additionally required to have no history of neurological disorders involving pathology above the brainstem (with the exception of uncomplicated non-focal epilepsy, with no active seizures within the last year). To be included in the TD group, youth were required to have no previously diagnosed developmental, neurological or psychiatric conditions, as well as no evidence of elevated ASD traits (i.e. a total t-score >65 on the Social Responsiveness Scale, Second Edition; SRS-2[50]). Exclusionary criteria for all study participants included an inability to comprehend scan instructions, any known genetic conditions, excessive head motion during the resting-state scan, insufficient high-quality resting-state data and any structural brain abnormalities. Participants were additionally excluded if they were the sibling of another subject in the study; the retained youth were chosen to match groups more closely on age, full-scale IQ, the number and percent of resting-state components labelled as motion/noise during preprocessing, mean relative head motion and, within the ASD group, ADOS-2 calibrated severity scores. As polygenic risk scores (PRSs) calculated based on data from one genetic ancestry do not generalize well to other ancestries,[51,52] our final analyses only included participants who were of the same ancestry as the genome-wide association study (GWAS) used to compute our PRSs[6]; that is, all included youth were of genetically ascertained European descent.

Our final sample included 127 youth: 30 boys with ASD, 38 TD boys, 31 girls with ASD and 28 TD girls. Assignment to the boy/male or girl/female group was based on parent-reported biological sex, confirmed using genotyped chromosomal sex; participant gender identity was not assessed. Descriptive statistics and two-tailed P-values for all four groups and between-group comparisons are reported in Table 1. Reported statistical comparisons were completed in R[53] using t-tests, chi-squared tests or the non-parametric equivalent as appropriate (i.e. when visual inspection indicated a non-normal data distribution for t-tests, or when there were fewer than five observations per cell for chi-squared tests). Within both ASD and TD groups, males did not significantly differ from their female counterparts in any of the following (all P-values > 0.2; Table 1 and Supplementary Table 1): mean relative head motion, age, handedness, total ASD traits (as quantified using SRS-2 Total Raw scores, SRS-2 Total T-scores and ADOS-2 Calibrated Severity Scores), the number and percent of resting-state components automatically classified as motion/noise[54,55] and full-scale IQ (as assessed using the second edition of the Differential Ability Scales,[56] the fourth edition of the Wechsler Intelligence Scale for Children,[57] or the first or second edition of the Wechsler Abbreviated Scale of Intelligence[58,59]); however, TD boys were slightly older than TD girls (P = 0.08). Within the male and female groups, youth with ASD did not significantly differ from their same-sex TD counterparts in any of the following (all P-values > 0.2; Table 1 and Supplementary Table 1): mean relative head motion, age, handedness and the number and percent of resting-state components automatically classified as motion/noise, except girls with ASD were slightly older than TD girls (P = 0.07). Youth with ASD exhibited lower full-scale IQs than their same-sex counterparts, although this difference only attained statistical significance in the girls (male: P = 0.10; female: P = 0.004). Full-scale IQ and age were thus included as covariates of non-interest in all neuroimaging analyses.

Genotyping and polygenic risk score derivation

DNA was extracted from whole-blood or saliva samples using standard protocols from the Gentra Puregene Blood DNA extraction kit (Qiagen) or the OraGene Collection Kit (DNA GenoTek). Genome-wide single nucleotide polymorphism (SNP) data were generated at Yale University or the UCLA Neuroscience Genomics Core according to standard manufacturer protocols using the Illumina Omni-1 or Omni-2.5-exome platforms (Illumina Inc.). Genotypic data were quality filtered (<5% missing per person/per SNP, >1% minor allele frequency, Hardy–Weinberg equilibrium

Table 1 Mean and standard deviation of sample descriptives

	ASD		TD		Male versus Female P-values		ASD versus TD P-values	
	Male	Female	Male	Female	ASD	TD	Male	Female
Sample size	30	31	38	28	–	–	–	–
Age (years)	13.45 ± 2.99	13.73 ± 2.45	13.69 ± 2.82	12.41 ± 2.95	0.69	0.08	0.73	0.07
Handedness (R/L)	27/3	30/1	35/3	25/3	0.35	0.69	1.00	0.34
Full-scale IQ	105.20 ± 15.82	100.77 ± 22.43	111.42 ± 15.01	115.75 ± 14.39	0.38	0.24	0.10	0.004
Scanner (HT/ST/SP/UT1/UT2/UP/YT)	5/2/4/9/7/0/3	4/8/6/0/3/2/8	5/2/9/6/8/0/8	5/3/9/0/6/2/3	0.002	0.14	0.55	0.30
Mean relative head motion (mm)	0.15 ± 0.21	0.16 ± 0.15	0.12 ± 0.08	0.13 ± 0.10	0.97	0.48	0.84	0.43
# of ICA motion/noise components	22.80 ± 5.47	21.13 ± 7.50	21.16 ± 6.50	19.61 ± 7.32	0.33	0.37	0.27	0.43
% ICA components removed	55.79 ± 9.76	52.81 ± 12.83	53.00 ± 9.41	50.01 ± 10.86	0.31	0.24	0.24	0.37
SRS-2 Total Raw	97.43 ± 31.00[b]	98.27 ± 32.00[a]	18.68 ± 14.30[a]	15.12 ± 11.34	0.92	0.28	<0.001	<0.001
SRS-2 Total T-Score	75.46 ± 12.28[b]	77.37 ± 11.21[a]	44.05 ± 5.98[a]	44.29 ± 4.84	0.54	0.87	<0.001	<0.001
ADOS-2 Calibrated severity score	6.66 ± 2.16[a]	6.27 ± 1.70[a]	–	–	0.44	–	–	–

Handedness: R = right, L = left; Scanner: HT = Harvard Trio, ST = Seattle Trio, SP = Seattle Prisma, UT1 = UCLA Trio 1, UT2 = UCLA Trio 2, UP = UCLA Prisma, YT = Yale Trio. ADOS-2 = Autism Diagnostic Observation Schedule, second edition. Reported P-values are two-tailed.

[a]Data missing from one subject.

[b]Data missing from two subjects.

$P > 10^{-7}$) in PLINK and used to confirm reported biological sex and familial relationships.[60] Genotypes that passed quality control were imputed to the Haplotype Reference Consortium reference panel (http://www.haplotype-reference-consortium.org/) using the Michigan Imputation Server (imputationserver.sph.umich.edu). Principal components reflecting genetic ancestry were calculated and categorical genetic ancestry assigned using the 1000 Genomes Project as reference.[61]

To quantify each participant's cumulative common genetic risk for ASD across the genome, we used PRSs. Briefly, PRSs are the weighted sum of a participant's risk alleles and are typically normally distributed in the population.[62] The use of PRSs to summarize aggregate genetic risk has been extensively validated in large-scale studies for a variety of conditions including ASD, although the individual-level discriminative ability of PRSs does not yet approach standards for clinical use.[6,62,63] Prior work has additionally established that PRSs for a range of brain-based disorders are significantly associated with variability in brain structure and function.[64–67] In the current study, PRSs for ASD were calculated based on recent ASD GWAS results from 18 381 individuals with ASD and 27 969 controls through the joint efforts of the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH) and the Psychiatric Genomics Consortium (PGC).[6] Genetic data from the current study were not included in this published GWAS, ensuring independence of the discovery and target sample. PRSice was used to compute PRSs based on a range of GWAS *P*-value thresholds.[68] In line with the known polygenicity of ASD, PRSs calculated at a *P*-value threshold of 0.5 explained the most variance in our case-control data and were used for all subsequent analyses.[2,6] PRS calculations and imaging genetics analyses included the first four principal components from our genome-wide data to further control for genetic ancestry. For supplemental analyses assessing the robustness of our neuroimaging findings to polygenic risk calculation method, polygenic risk was also computed in LDPred using a Bayesian approach to estimate posterior effect sizes of common genetic variants with the 1000 Genomes Project as reference.[69] All subsequent analyses focused on the relationship between aggregate genetic load and brain connectivity, similar to previous studies examining the impact of cumulative genetic risk on the brain, and in line with evidence of a stronger link between genetic variation and neural phenotypes than behavioural phenotypes.[64,70–72]

MRI acquisition and preprocessing

MRI data were acquired at each site on a Siemens 3 T Trio scanner using a 12-channel head coil or, after scanner upgrades, on a Siemens 3 T Prisma scanner using a 20-channel head coil. Eyes-open resting-state functional MRI scans were collected while participants viewed a fixation cross (Trio: repetition time = 2000 ms, echo time = 30 ms, field of view = 192 mm, 34 slices, slice thickness = 4 mm, in-plane voxel size = 3 × 3 mm, acquisition time = 5.5 min, or repetition time = 3000 ms, echo time = 28 ms, field of view = 192 mm, 34 slices, slice thickness = 4 mm, in-plane voxel size = 3 × 3 mm, acquisition time = 6 min; Prisma: repetition time = 2000 ms, echo time = 30 ms, field of view = 192 mm, 34 slices, slice thickness = 4 mm, in-plane voxel size = 3 × 3mm, acquisition time = 5.5 min). For registration purposes, we additionally acquired a high-resolution matched bandwidth scan that was coplanar to the functional MRI scan to ensure identical distortion characteristics (Trio: repetition time = 5000 ms, echo time = 34 ms, field of view = 192 mm, 34 or 36 slices, slice thickness = 4 mm, in-plane voxel size = 1.5 × 1.5 mm; Prisma: repetition time = 5000 ms, echo time = 35 ms, field of view = 192 mm, 34 slices, slice thickness = 4 mm, in-plane voxel size = 1.5 × 1.5 mm).

Resting-state functional MRI data underwent standard preprocessing using FMRIB's Software Library (FSL)[73] and Analysis of Functional NeuroImages,[74] including skull stripping, motion correction and smoothing with a 6 mm full-width at half-maximum Gaussian kernel. Participants' resting-state scans were linearly registered to their corresponding high-resolution matched bandwidth coplanar image using 6 degrees of freedom, before being linearly registered to the standard MNI152 2 mm standard brain using 12 degrees of freedom. To remove motion confounds and other sources of noise from the single-subject resting-state data, we completed several additional preprocessing steps. First, the automatic independent component classifier ICA-AROMA was used to regress out components labelled as motion or noise at the single-subject level, an approach which effectively controls for motion without needing to delete individual motion-contaminated volumes.[54,55] Second, we bandpass filtered the data ($0.01\,\text{Hz} < t < 0.1\,\text{Hz}$). Third, we included the following as nuisance regressors at the single-subject level: mean white matter time series, mean cerebrospinal fluid time series and mean global time series, as well as the temporal derivatives of these regressors.[75]

Intrinsic functional connectivity of the salience network was examined using a 5 mm radius seed located in the right orbital frontoinsula [Montreal Neurological Institute (MNI) coordinates: 38, 26, −10],[76] an approach which has previously been used to examine salience network functional connectivity in ASD.[23,41,77] Specifically, the mean time series extracted from this seed was correlated with that of every other voxel in the brain and the resulting correlations converted into connectivity *z*-scores using Fisher's *r*-to-*z* transform.

Statistical analysis

Group-level contrasts were conducted on salience network connectivity *z*-scores, calculated as described above, by using FSL's FMRIB's Local Analyses of Mixed Effects (FLAME 1 + 2), with a voxelwise threshold of $Z > 3.1$ and a corrected cluster threshold of $P < 0.5$ to correct for multiple comparisons based on current recommended standards in the field.[78] All analyses included site/scanner, full-scale IQ, age and the first four principal components obtained from our genome-wide data (reflecting genetic ancestry) as covariates of non-interest to control for within-group variability and/or group differences in these variables. We investigated the effect of polygenic risk for ASD on salience network functional connectivity by including demeaned PRSs in our group-level analyses as a covariate of interest and assessing their association with brain connectivity separately within each of our four groups (male ASD, female ASD, male TD, female TD). To test our hypothesis that the female protective effect arises from ASD risk genes interacting with sex differential processes, our planned contrasts assessed whether the relationship between demeaned PRSs and functional connectivity significantly differed between boys and girls with ASD (male ASD versus female ASD) or between TD boys and girls (male TD versus female TD); these comparisons allowed us to directly assess whether cumulative genetic load for ASD differentially affects connectivity in males and females with ASD, as well as in TD males and females (i.e. the interaction between genetic risk and sex was tested within each diagnostic group). Follow-up analyses were completed to confirm that our primary analyses examining the sex differential impact of cumulative genetic risk on functional connectivity were robust to head motion and site/scanner. Specifically, connectivity *z*-scores were extracted from those significant clusters where the effect of genetic load depended on participant sex. We then repeated our analyses using a

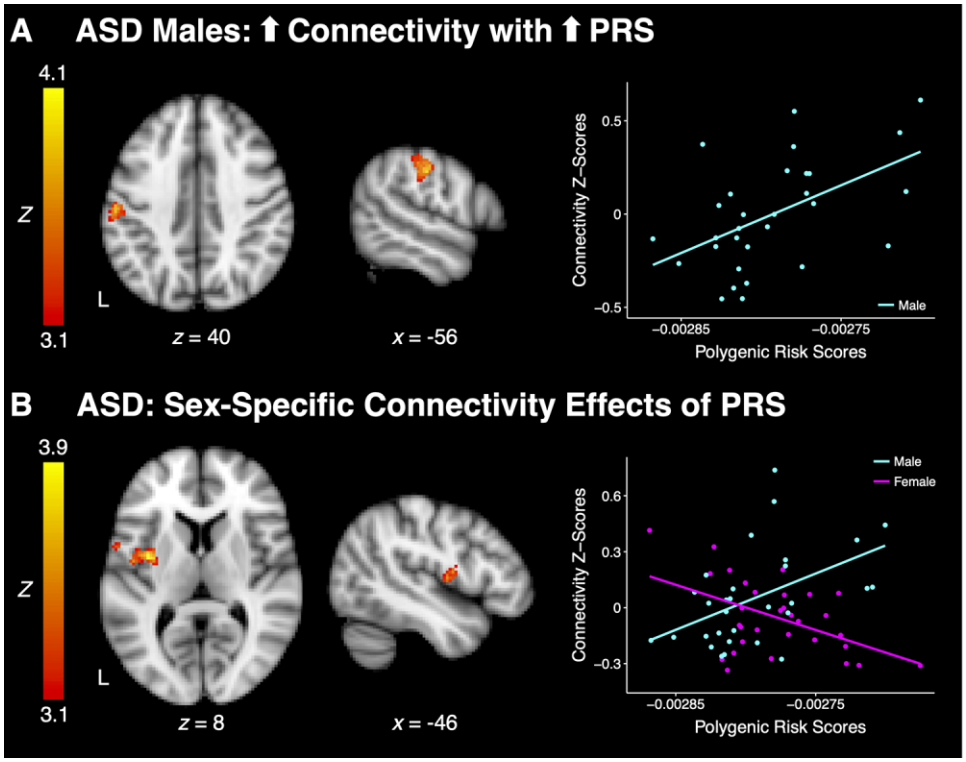

Figure 1 Effects of polygenic risk for ASD on salience network functional connectivity among youth with ASD. (**A**) In boys with ASD, greater polygenic risk was associated with increased functional connectivity between the salience network and the postcentral and supramarginal gyri. (**B**) When comparing boys and girls with ASD, distinct effects of polygenic risk were observed on salience network functional connectivity with the precentral gyrus and mid-insula. Graphs are for illustrative purposes only and represent the relationship between untransformed PRSs and mean connectivity z-scores extracted from each significant cluster at the *left*. L = left.

general linear model which was identical to our primary analyses, except the model was supplemented with mean relative motion as a nuisance covariate, or each site/scanner was excluded one at a time to assess the stability of results to site/scanner. Residuals from all regressions were confirmed to meet statistical assumptions of independence, normality and constant variance based on visual inspection of residual histograms and residual plots. Effect sizes for all group-level comparisons are reported as standardized regression coefficients (βs), reflecting that these comparisons were completed as regressions to allow for the inclusion of nuisance covariates.

Supplemental analyses were additionally completed to assess the robustness of our primary findings to polygenic risk calculation method. Extracted connectivity z-scores were analysed as described above, with polygenic risk estimated using Bayesian methods in LDPred. For completeness, supplemental analyses also investigated how the association between our main PRSs for ASD and salience network functional connectivity might differ across the whole-brain between boys with and without ASD (male ASD versus male TD) and between girls with and without ASD (female ASD versus female TD). Lastly, we also assessed the relationship between our main PRSs and functional connectivity in males and females across the whole-brain when collapsing across diagnostic groups (main effect of sex) and in youth with ASD and TD youth across participant sex (main effect of diagnostic group); these analyses also examined how the association between genetic load for ASD and functional connectivity may differ between males and females across diagnostic groups (interaction with sex) and between youth with ASD and TD youth regardless of participant sex (interaction with diagnostic group). Results from all additional analyses are presented in the Supplementary material.

Data availability

All subjects' neuroimaging data are available via the NIMH Data Archive (NDA) under collection ID 2021 (https://nda.nih.gov/edit_collection.html?id=2021) or via the Autism Brain Imaging Data Exchange (ABIDE) under the University of California Los Angeles collections (http://fcon_1000.projects.nitrc.org/indi/abide/abide_I.html; http://fcon_1000.projects.nitrc.org/indi/abide/abide_II.html).[45,46]

Results

To test the hypothesis that genetic risk for ASD differentially impacts the male and female brain, we analysed the association between polygenic risk and functional connectivity of the salience network in males and females with and without ASD. Among youth with ASD, we found that elevated genetic risk in boys with ASD was associated with increased functional connectivity between the salience network and regions involved in somatosensory processing only, including the postcentral gyrus and the supramarginal gyrus (Fig. 1A; MNI peak coordinates: –60, –30, 46; β = 0.76; max *Z*: 4.07). Girls with ASD exhibited no significant relationship between cumulative genetic load and salience network functional connectivity. A significant interaction was observed between sex and aggregate genetic risk in the ASD group, such that sex significantly modulated the relationship between polygenic risk and functional connectivity. Specifically, as aggregate genetic risk increased, boys with ASD exhibited stronger functional connectivity than girls with ASD did between the salience network and regions involved in sensorimotor processing, including the precentral gyrus and the mid-insula (Fig. 1B; MNI peak coordinates: –36, –4, 8; β = 1.19; max *Z*: 3.89). Follow-up analyses indicated that this result remained significant when using an alternate

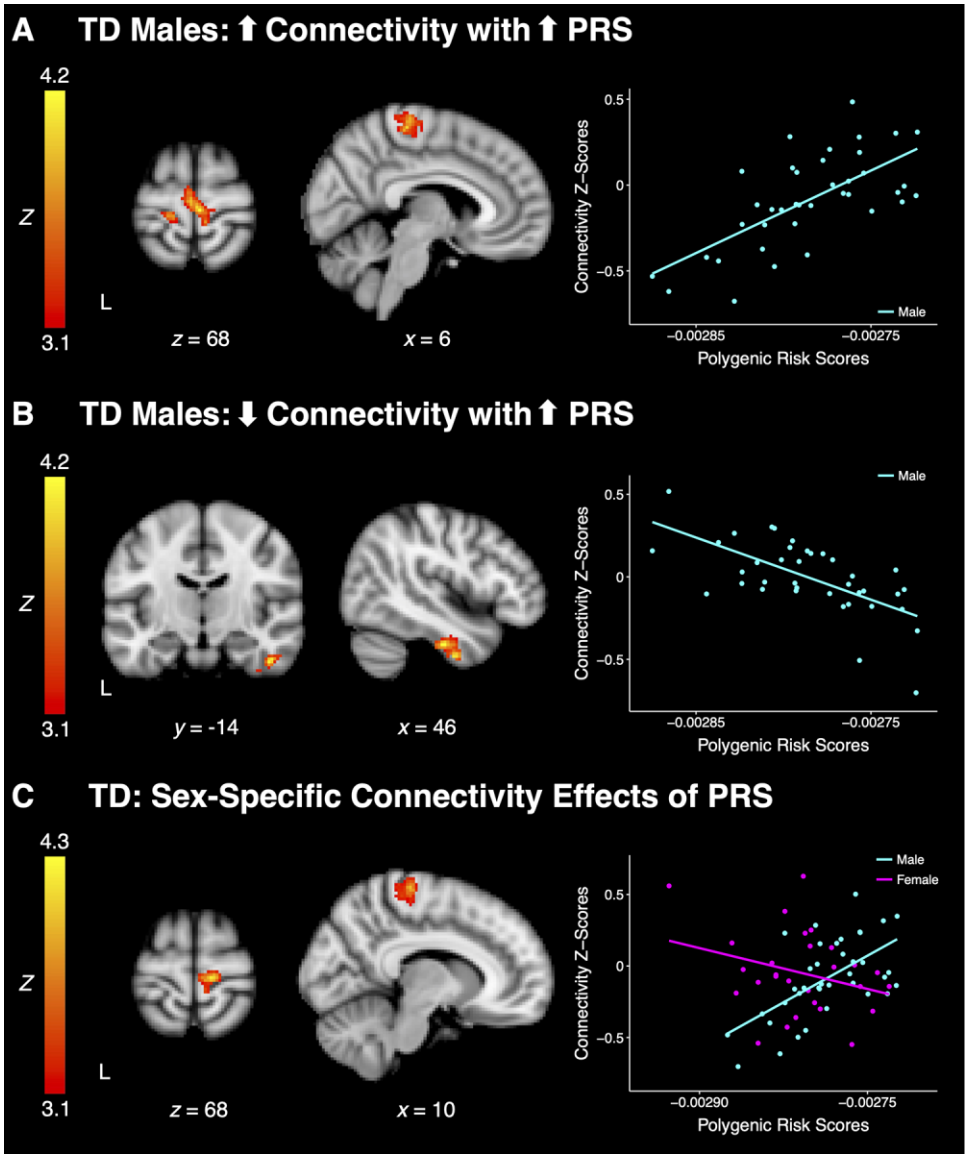

Figure 2 Effects of polygenic risk for ASD on salience network functional connectivity among TD youth. (**A**) In TD boys, greater polygenic risk was associated with increased functional connectivity between the salience network and the precentral and postcentral gyri. (**B**) In TD boys, increasing polygenic risk was related to weaker salience network functional connectivity with the inferior temporal gyrus. (**C**) When comparing TD boys and girls, distinct effects of polygenic risk were observed on salience network functional connectivity with the precentral gyrus. Graphs are for illustrative purposes only and represent the relationship between untransformed polygenic risk scores and mean connectivity z-scores extracted from each significant cluster at left. L = left.

approach to control for potential confounds resulting from head motion and when assessing the robustness of results by site/scanner (all *P*-values < 0.01; all βs = 0.96–1.26). Taken together, these findings indicate that genetic risk for ASD differentially impacts functional brain connectivity among males and females with ASD.

Similar to the relationship observed in the ASD cohort, TD boys demonstrated a significant relationship between cumulative genetic risk and functional connectivity. Analogous to boys with ASD, greater cumulative genetic risk among TD boys was related to stronger salience network functional connectivity with sensorimotor areas, including the precentral and postcentral gyri (Fig. 2A; MNI peak coordinates: 4, –26, 68; $\beta = 0.71$; max *Z*: 4.21). TD boys also showed weaker functional connectivity between the salience network and the inferior temporal gyrus as a function of increasing polygenic load (Fig. 2B; MNI peak coordinates: 46, –16, –32; $\beta = 0.55$; max *Z*: 4.24). In contrast to TD boys and similar to ASD girls, TD girls displayed no significant association between aggregate genetic risk for ASD and salience network connectivity. There was a significant interaction among TD youth between sex and cumulative genetic load. That is, participant sex significantly modulated the association between polygenic risk and functional connectivity in the TD group, such that TD boys displayed greater functional connectivity between the salience network and primary motor cortex (i.e. precentral gyrus) as a function of increasing genetic load compared to TD girls (Fig. 2C; MNI peak coordinates: 14, –26, 66; $\beta = 1.01$; max *Z*: 4.29). Results were identical when using a distinct approach to reduce potential head motion confounds and when assessing the stability of results by site/scanner (all *P*-values < 0.01; all βs = 0.93–1.10). As a whole, these results demonstrate that the impact of polygenic risk for ASD is dependent on participant sex not only among ASD youth, but also among TD youth.

Several additional analyses were conducted for completeness. First, we assessed that our primary findings remained significant when using a distinct method to calculate polygenic risk (Supplementary material). Second, we examined how the relationship between aggregate genetic risk for ASD and salience network connectivity might differ between boys with and without ASD, as well as between girls with and without ASD (Supplementary material). Third, we investigated the association between genetic load for ASD and salience network connectivity when collapsing across diagnostic group or participant sex, including how genetic risk's association with functional connectivity may differ between males and females across diagnostic groups and between ASD and TD groups regardless of participant sex (Supplementary material). These additional results are included in the Supplementary material.

Discussion

As a whole, our results serve as an important proof of concept by providing the first *in vivo* evidence that cumulative, genome-wide risk for ASD differentially impacts the male and female brain. This pattern of results is consistent with the hypothesis that the interaction of ASD risk genes with sex-specific biology drives the previously demonstrated male bias in ASD prevalence,[11,12] with such an interaction resulting in the sex-dependent associations between genetic risk and functional brain connectivity observed in the current study. Our findings are also in line with a previous study of gene expression in the post-mortem human brain, whereby genes with ASD-associated alterations in expression were found to overlap with genes that have differential patterns of expression as a function of sex.[20] Prior investigations of gene expression have also demonstrated that both male individuals and individuals with ASD display reduced expression of genes associated with neural and synaptic function, as well as increased expression of genes related to inflammatory and neuroimmune processes.[20,79] Gene expression patterns or other neurobiological differences in the developing male brain may thus provide the background for ASD-associated genetic risk to have more penetrant effects in males than females, ultimately resulting in the known greater prevalence of ASD among males.[11,12] Our data support this hypothesis that genetic risk for ASD may have more penetrant effects on the male brain, as boys demonstrated a stronger relationship between ASD genetic risk and functional connectivity than girls, irrespective of diagnosis.

The neural regions that exhibited a sex-dependent effect of genetic load on salience network functional connectivity are involved in sensorimotor processing.[80,81] Altered sensory processing and repetitive motor behaviours are included in the diagnostic criteria for ASD, and such sensorimotor differences are hypothesized to causally contribute to the emergence of ASD.[82–84] Indeed, infants at high familial risk for ASD exhibit significant salience network hyperconnectivity with sensorimotor cortices, and stereotyped and repetitive motor behaviours are one of the earliest behavioural manifestations of ASD to emerge.[34,85,86] Notably, previous studies have demonstrated that females exhibit lower levels of restricted and repetitive behaviours, which includes sensory and motor symptoms, compared to their male counterparts.[82,87–89] Our finding that girls are relatively shielded from the impact of polygenic risk on salience network functional connectivity with sensorimotor regions thus raises the intriguing possibility that girls may exhibit fewer repetitive behaviours and ultimately be less likely to receive an ASD diagnosis, precisely because this neural circuitry is protected. This proposed multiscale mechanism should be directly assessed in future infant studies by specifically testing whether such neural sensitivity to cumulative genetic risk for ASD mediates the emergence of restricted and repetitive behaviours.

The current study is, to the best of our knowledge, the largest neuroimaging genetics study to date focused on polygenic risk for ASD among individuals with ASD. Future analyses should investigate the replicability of current results when using yet larger samples and complementary approaches for assessing genetic risk, such as familial relatedness to individuals with diagnosed ASD or elevated ASD traits.[90–92] Additionally, the current study focused on the salience network because of the robust evidence implicating it in ASD and its hypothesized role in the emergence of ASD traits.[26] However, other neural systems may also contribute to ASD.[9,93,94] Future large-scale investigations should thus expand on the current proof-of-concept study by examining the effect of polygenic load for ASD on a range of additional brain networks.

In sum, our findings are the first to demonstrate *in vivo* that the neural impact of genome-wide risk for ASD is significantly modulated by biological sex, supporting the hypothesis that the female protective effect is driven by the interaction of ASD risk genes with sex differential biology and thereby proposing a neurobiological mechanism for the female protective effect.

Funding

This work was supported by the National Institute of Mental Health (award numbers R01MH100028 to K.A.P., R01MH117982 to M.D., F31MH110140 to K.E.L., K00MH119663 to L.M.H. and F32MH105167 to S.A.G.), the National Institute of Neurological Disorders and Stroke (award number T32NS048004 to K.E.L.), the National Science Foundation (Graduate Research Fellowship award number 1650604 to N.T.P.), the University of California Los Angeles Dissertation Year Fellowship Program and the ARCS Foundation. We are also grateful for the generous support from the Brain Mapping Medical Research Organization, Brain Mapping Support Foundation, Pierson-Lovelace Foundation, The Ahmanson Foundation, Capital Group Companies Charitable Foundation, William M. and Linda R. Dietel Philanthropic Fund and Northstar Fund. Research reported in this publication was also partially supported by the National Center for Research Resources and by the Office of the Director of the National Institutes of Health under award numbers C06RR012169, C06RR015431 and S10OD011939. The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

Appendix I

Full details of the GENDAAR Consortium are provided in the Supplementary material.

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