A neurogenetic analysis of female autism

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Females versus males are less frequently diagnosed with autism spectrum disorder (ASD), and while understanding sex differences is critical to delineating the systems biology of the condition, female ASD is understudied. We integrated functional MRI and genetic data in a sex-balanced sample of ASD and typically developing youth (8–17 years old) to characterize female-specific pathways of ASD risk. Our primary objectives were to: (i) characterize female ASD (n = 45) brain response to human motion, relative to matched typically developing female youth (n = 45); and (ii) evaluate whether genetic data could provide further insight into the potential relevance of these brain functional differences. For our first objective we found that ASD females showed markedly reduced response versus typically developing females, particularly in sensorimotor, striatal, and frontal regions. This difference between ASD and typically developing females does not resemble differences between ASD (n = 47) and typically developing males (n = 47), even though neural response did not significantly differ between female and male ASD.

For our second objective, we found that ASD females (n = 61), versus males (n = 66), showed larger median size of rare copy number variants containing gene(s) expressed in early life (10 postconceptual weeks to 2 years) in regions implicated by the typically developing female functional MRI contrast. Post hoc analyses suggested this difference was primarily driven by copy number variants containing gene(s) expressed in striatum. This striatal finding was reproducible among n = 2075 probands (291 female) from an independent cohort.

Together, our findings suggest that striatal impacts may contribute to pathways of risk in female ASD and advocate caution in drawing conclusions regarding female ASD based on male-predominant cohorts.

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Introduction

Males are diagnosed with autism spectrum disorder (ASD) at rates of around four males to every one female.\(^1\) Although non-biological factors such as ascertainment bias likely contribute to this skewed ratio,\(^2\)\(^-\)\(^4\) differential prevalence estimates are likely to remain at \(\sim 3:1\) even after accounting for these factors,\(^5\) signalling a robust aetiological role for sexually dimorphic biological processes. Investigating sex differences in ASD brain function, genetics, and gene-brain interactions is thus a critical step in delineating the systems biology of ASD. However, previous work on this question has been hindered by minimal inclusion of ASD females (ASDf).

Much work has focused on characterizing differences in ASD brain function relative to that of typically developing individuals. Emphasis is often placed on the neural bases of social perception, a multifaceted process that includes selectively attending to socially relevant cues, and processing/derviving appropriate information from these stimuli.\(^6\)\(^,\)\(^7\) The perception of biological motion—signals conveyed by the human face, eyes, hands, and body—is an important category within social perception.\(^8\) Previous research indicates that typically developing individuals possess specialized neural systems for processing human motion,\(^9\) which appear impacted in ASD.\(^8\)\(^,\)\(^10\) Specifically, posterior superior temporal sulcus (pSTS) is selectively recruited during biological motion perception\(^11\) and has shown reduced response to human motion in ASD.\(^12\)\(^-\)\(^14\) Members of our team previously characterized the under-response of this region and others (e.g. amygdala, fusiform gyrus) to human motion as constituting a ‘neural signature’ of ASD.\(^13\) However, thus far, both this and other functional MRI samples used to examine this question have all skewed predominantly male, ranging from ratios of 4:1 to 7:1,\(^12\)\(^-\)\(^14\) raising the possibility that this pattern does not best characterize ASDf.

The female protective effect (FPE) hypothesis suggests several reasons why deviations from neurotypical social perception might, themselves, differ for ASDf versus ASD males (ASDm). This hypothesis posits that greater aetiological load is necessary for ASD expression in girls and women due to female-specific protective factors that lend resilience to the development of an autistic phenotype.\(^15\)\(^,\)\(^16\) Such factors are presumed to operate regardless of diagnostic status, also influencing typically developing females (TDf) but not typically developing males (TDm). In ASDf, these factors mean that a larger magnitude aetiological ‘push’ is required for the autism phenotype to manifest. Thus, a core prediction of the FPE hypothesis is that ASDf will demonstrate greater genetic burden than ASDm, supported by evidence of ASDf carrying larger, more frequent, and more deleterious mutations on average than those found in ASDm.\(^17\)\(^-\)\(^19\)

While predictions regarding sex-differential genetic load follow in a comparatively straightforward manner from the FPE hypothesis, what can be expected regarding brain functional differences is less clear. If aetiological burden is assumed to translate in relatively direct fashion into later neuroendophenotype, then ASDf might be expected to exhibit higher magnitude deficits than ASDm in terms of brain response to social perceptual stimuli. Alternatively, greater aetiological load might not necessarily lead to more severe impacts than found in ASDm, as female protective factors might act to dampen the impact of these ‘hits’. In the latter case, female and male ASD profiles might not appear significantly different.

Objective differences in ASDf versus ASDm social perceptual response are not the only potential reason why profiles of deviation from sex-typical patterns might diverge between female and male ASD. Typically developing females and males might process social stimuli differently; for example, typically developing females might recruit additional mechanisms related to female resilience factors. Initial neuroimaging
work suggests sex-differential brain response among neurotypical adults viewing human motion. Applied to autism, even if ASDf and ASDm displayed an underlying similarity in brain response, this pattern might have a different ‘meaning’ for females versus males, in that it would indicate a deviation from expected function for their sex. These considerations suggest that examining how ASDf differ from TDF (as well as how ASDf differ from ASDm, and TDF from TDM) will provide new insights that cannot be extrapolated from existing analyses contrasting male-pre-dominant samples of autistic versus typically developing individuals. However, observed differences in brain response at a single time point (particularly in later development) could be due to genetically-mediated processes operating from very early stages of development and/or to experiences over the lifespan, both of which may differ as a function of sex. Combining genetic and neuroimaging information from the same individual, so that data about brain function constrain genetic analyses, could allow for more informed interpretation of neuroimaging results.

To better understand the ASDf neuroendophenotype of social perception, as well as potential genetic contributions to this profile, we over-sampled ASDf to obtain a well-matched cohort of ASD and typically developing youth at a sex ratio of ∼1:1 [the ‘GENDAAR’ (Gender Exploration of Neurogenetics and Development to Advance Autism Research) project]. Youth (8–17 years old) participated in genotyping, neuroimaging, and behavioural phenotyping. We describe results from functional MRI of brain response while viewing point-light displays of coherent (BIO) or scrambled (SCRAM) whole-body human motion in both ASD and sex-, age-, head motion, and IQ-matched typically developing youth from the GENDAAR cohort (n = 207, full functional MRI sample; n = 184, matched functional MRI sample; n = 250, genotyping sample; Table 1). We note that while Kaiser and colleagues used the same paradigm to assess differences between typically developing and ASD subjects, the present study allows for advantages over the previous work in terms of a nearly 4-fold increase in functional MRI sample size among ASD generally and a more than 9-fold increase in ASDf specifically, allowing us to conduct sex difference analyses not possible previously. Sex was defined as sex assigned at birth; no assessment of gender identity was conducted at this time point. We pursued two primary research objectives.

First (Objective 1), we sought to characterize a functional MRI-based profile of ASDf response to socially meaningful motion, defined as brain regions in which TDF displayed a stronger response to the experimental contrast (BIO > SCRAM) than ASDf. We expected that the resulting map would differ from previous reports of typically developing versus ASD differences in male-pre-dominant samples. Given little previous research on this question, we aimed to describe the nature of these differences. To contextualize this profile further, we also examined contrasts for ASDf versus ASDm, TDF versus TDM, and TDM versus ASDm.

Second (Objective 2), we integrated genetics and functional MRI data to explore to what degree the TDF > ASDf profile identified in Objective 1 might reflect, at least in part, an aetiological mechanism relevant specifically to ASD development in females (versus exclusively reflecting correlates of differing social experiences). We tested several complementary questions. First (Objective 2A), we predicted that ASDf versus ASDm would exhibit greater mutation load [defined as larger median size of rare genic copy number variants (CNVs)] in genes expressed both (i) in brain regions represented in the functional MRI-defined TDF > ASDf map; and (ii) during prenatal and infant development (‘candidate genes’). Second (Objective 2B), given the novelty of the dataset and the large number of regions represented in the TDF > ASDf map, we conducted exploratory tests to determine which brain region(s) seemed to be driving effects observed in Objective 2A. Given previous work demonstrating greater genetic load in ASDf versus ASDm generally, we hypothesized (Objective 2C) that potential genetic impacts to brain regions implicated in the TDF > ASDf map, especially those that seemed to be drivers of the ASDf > ASDm CNV size difference (Objective 2B), would contribute to this load differential more so than genetic impacts broadly defined. Finally, due to the exploratory nature of the tests conducted under Objectives 2B and 2C, we examined whether these findings would replicate (Objective 2D) in an independent ASD cohort.

Materials and methods

Sample

We analysed data from Wave 1 of the GENDAAR project, collected across four sites (Yale University, Harvard University/Boston Children’s Hospital, University of California Los Angeles, and University of Washington/Seattle Children’s Research Institute). Male and female youth (8–17 years old) were recruited for inclusion in the ASD or typically developing group. Unaffected siblings of ASD participants were also recruited but were not included in Objective 1 analyses given insufficient n’s for the planned statistics; however, siblings were genotyped and used to filter out CNVs in Objective 2. Parents provided informed written consent; children provided written assent. Procedures were conducted in compliance with the sites’ IRBs and the Declaration of Helsinki. Exclusionary criteria for all groups included: full-scale IQ (FSIQ) ≤ 70 as estimated via the Differential Ability Scales, Second Edition General Conceptual Ability Standard Score; twin status; active tic disorder that would interfere with imaging; pregnancy; metal in the body; active seizures within the past year; or current use of any benzodiazepine, barbiturate, or anti-epileptic (Supplementary material). Other medication use must have been stable for over 6 weeks. The Supplementary material describes group-specific exclusionary criteria.

Diagnostic confirmation

ASD diagnoses were confirmed by expert clinicians using the Autism Diagnostic Observation Schedule, Second Edition
**Table 1** Phenotypic and data quality characteristics of the samples, by sex and group

|                          | Female | Male | Sex Diff. | Typically developing | Sex Diff. | Omn. Diff. | Pair. Diff. |
|--------------------------|--------|------|-----------|----------------------|-----------|------------|-------------|
|                          | ASD    | ASD  |           | ASD                  | TD        |            |             |
|                          |        |      | n = 46   | n = 48               | n = 54    | n = 59     |             |
|                          |        |      | n = 46   | n = 48               | n = 54    | n = 59     |             |
| Age, m                   | 161.21 | 157.51 | 36.80 | 0.595               | 153.62    | 37.37      | 0.267       |
| FSIQ                     | 101.61 | 104.12 | (18.09)| 0.530               | 111.13    | (14.37)    | 0.037       |
| SRS-2                    | 93.74  | 97.49 | (26.01)| 0.530               | 16.57     | (11.81)    | 0.705       |
| ADOS                     | 6.63   | 7.56 | (1.84) | 0.013               |           |            |             |
| ADI-R: A                 | 18.91  | 19.44 | (5.19) | 0.638               |           |            |             |
| ADI-R: B                 | 15.78  | 16.62 | (4.48) | 0.332               |           |            |             |
| ADI-R: C                 | 5.83   | 6.60 | (2.64) | 0.161               |           |            |             |
| Max aRMS                 | 1.21   | 1.59 | (0.99) | 0.067               | 1.04      | (0.81)     | 0.783       |
| Max rRMS                 | 0.87   | 1.08 | (0.78) | 0.203               | 0.70      | (0.68)     | 0.633       |
| MO, n                    | 7.96   | 8.54 | (5.99) | 0.612               | 6.85      | (5.21)     | 0.382       |

**Matched functional MRI sample (n = 184)**

|                          | Female | Male | Sex Diff. | Typically developing | Sex Diff. | Omn. Diff. | Pair. Diff. |
|--------------------------|--------|------|-----------|----------------------|-----------|------------|-------------|
|                          | ASD    | ASD  |           | ASD                  | TD        |            |             |
|                          |        |      | n = 45   | n = 47               | n = 45    | n = 47     |             |
| Age, m                   | 161.95 | 158.77 | (36.14)| 0.648               | 158.25    | (35.48)    | 0.539       |
| FSIQ                     | 102.29 | 104.45 | (18.15)| 0.592               | 108.56    | (14.26)    | 0.634       |
| SRS-2                    | 92.83  | 98.11 | (25.97)| 0.379               | 16.69     | (11.23)    | 0.861       |
| ADOS                     | 6.58   | 7.35 | (1.86) | 0.011               |           |            |             |
| ADI-R: A                 | 18.80  | 19.49 | (5.23) | 0.543               |           |            |             |
| ADI-R: B                 | 15.64  | 16.66 | (4.53) | 0.246               |           |            |             |
| ADI-R: C                 | 5.69   | 6.62 | (2.67) | 0.092               |           |            |             |
| Max aRMS                 | 1.22   | 1.55 | (0.96) | 0.110               | 1.06      | (0.81)     | 1.25        |
| Max rRMS                 | 0.87   | 1.05 | (0.76) | 0.286               | 0.68      | (0.66)     | 0.166       |
| MO, n                    | 7.98   | 8.38 | (5.95) | 0.729               | 7.00      | (5.24)     | 0.179       |

**CNV sample (n = 250)**

|                          | Female | Male | Sex Diff. | Typically developing | Sex Diff. | Omn. Diff. | Pair. Diff. |
|--------------------------|--------|------|-----------|----------------------|-----------|------------|-------------|
|                          | ASD    | ASD  |           | ASD                  | TD        |            |             |
|                          |        |      | n = 61   | n = 65               | n = 65    | n = 59     |             |
| Age, m                   | 150.77 | 148.28 | (36.38)| 0.685               | 149.62    | (37.64)    | 0.223       |
| FSIQ                     | 98.43  | 100.58 | (16.46)| 0.489               | 111.74    | (14.98)    | 0.462       |
| SRS-2                    | 93.70  | 92.89 | (29.00)| 0.880               | 17.11     | (11.85)    | 0.526       |
| ADOS                     | 6.57   | 7.31 | (1.82) | 0.023               |           |            |             |
| ADI-R: A                 | 18.79  | 20.40 | (4.79) | 0.085               |           |            |             |
| ADI-R: B                 | 15.92  | 16.97 | (4.28) | 0.158               |           |            |             |
| ADI-R: C                 | 5.87   | 6.48 | (2.74) | 0.198               |           |            |             |

Values are mean (standard deviation, SD) for male/female. Mean differences among all four groups are calculated by F-test and, where F < 0.10, followed up by pair-wise comparisons with Tukey’s HSD test; Welch’s t-tests on the pair-wise comparisons can be found in the Supplementary material. Mean differences within diagnostic category are calculated by t-test. P-values of F < 0.05 and P < 0.10 are indicated by bold and italics, respectively. ADI-R = Autism Diagnostic Interview-Revised; ADOS = Autism Diagnostic Observation Schedule; ADOS-2 = Social Responsiveness Scale version 2; FSIQ = Full Scale IQ; Max aRMS = maximum absolute relative root mean squared motion; Max rRMS = maximum absolute relative root mean squared motion; m = months; MO = motion outliers; Omn. = omnibus; Pair. = pairwise; Sig. = significant; SRS-2 = Social Responsiveness Scale version 2; TYP = typically developing.

**Full and matched functional MRI samples**

Table 1 describes characteristics of the full sample (n = 207; 46 ASDf, 48 ASDm, 54 TDf, 59 TDm) meeting phenotyping and MRI data quality inclusion criteria. The Supplementary...
material and Supplementary Table 5 describe within-subject magnetic resonance quality assurance. The full sample of TDf and TDm did not differ on any key metrics (age, FSIQ, and MRI quality; Table 1) and consequently this sample is used in tests of TDf versus TDm functional MRI differences. Matching was implemented to resolve head motion and/or FSIQ differences between groups for other planned functional MRI contrasts (Supplementary material and Supplementary Table 5). Table 1 describes the resulting matched functional MRI sample \((n = 184)\). We note that, within each group, no sex differences emerged in scores on the Child Behavior Checklist (CBCL)\(^{25}\) empirically-based syndrome or DSM-oriented scales, for either the full or matched functional MRI samples, suggesting relatively similar levels of comorbidity; as expected, ASD had higher CBCL scores than typically developing youth on almost every scale (Supplementary Table 6).

### Neuroimaging data collection and analysis

#### Imaging sites

Initially, all sites scanned on a Siemens 3 T Tim Trio magnet. Partway through data collection, two imaging centres (Sites B and C) upgraded to the Siemens 3 T Prisma Fit. Site was included as a nuisance regressor in all group-level functional MRI analyses, with data acquired pre- and post-upgrade considered to come from different sites. See Supplementary material and Supplementary Table 7 for intersite quality assurance details.

#### Experimental paradigm

Participants viewed blocks (six per condition, 24 s each) of coherent point-light displays of whole-body human motion (BIO) interleaved with scrambled (SCRAM) versions of these displays over a total of 144 2-s volumes, with 10 additional volumes of final fixation. The BIO displays were initially developed and described by Klin et al.\(^{26}\) and depict child-friendly movements such as pat-a-cake and waving. As refined by Kaiser and colleagues\(^{3,5}\) for functional MRI implementation, the BIO > SCRAM contrast allows for assessment of brain activity associated with biological motion perception, controlling for visual motion perception generally (Supplementary Videos 1 and 2).

#### Imaging protocol

Whole-brain images were collected on a Tim Trio with 12-channel head-coil (Sites A.Trio, B.Trio, C.Trio, D.Trio, \(n = 131\)) or Prisma Fit with 20-channel head-coil (Sites B.Prisma, C.Prisma, \(n = 76\)). See Supplementary material for sequence parameters and Supplementary Fig. 3 for functional coverage.

#### Image analysis

Neuroimaging analysis used FSL v.5.0.8 (fmrib.ox.ac.uk/fsl) tools within the LONI Pipeline v.6.3 environment\(^{27}\); additional analysis used R v.3.4.0-1. See Supplementary material for pre-statistics and individual subject level processing.

Group-level mixed-effects analyses were conducted on the BIO > SCRAM contrast of parameter estimates images (COPEs). The full sample was used to calculate the mean BIO > SCRAM effect within each group (ASDf, ASDm, TDf, TDm; Supplementary material). Group difference analyses tested pairwise comparisons within sex (e.g. TDf > ASDf) and within group (e.g. ASDf > ASDm). We considered testing a traditional Sex \(\times\) Diagnosis interaction effect in an analysis of all individuals in the matched functional MRI sample (Table 1). However, because the TDf and ASDm groups demonstrated significant differences in head motion even after matching (Supplementary material), we were concerned that effects detected in such an analysis might prove partially attributable to lower head motion in TDf. We therefore decided that the most conservative option was to ensure that the TDf and ASDm groups were never included in the same model.

The full sample was used for TDf versus TDm comparisons and the matched sample for other comparisons. As recommended by Eklund et al.,\(^{28}\) these analyses used non-parametric permutation inference, via FSL’s Randise\(^{29}\) with threshold-free cluster-enhancement (TFCE)\(^{30}\), 10 000 permutations, corrected \(P < 0.05\). Analyses were limited to regions for which the ‘greater than’ group (e.g. for TDf > TDm, the TDf group) demonstrated a mean group BIO > SCRAM \(z\)-value > 0, as we were not interested in relative differences in deactivation. Age (grand-mean centred), estimated FSIQ (grand-mean centred), Social Responsiveness Scale-2 (SRS-2) total raw score (group-centred) and site (factor-effect dummy-coded) were included as covariates. For ASDf \(\leq\) ASDm analyses, ADOS-2 Calibrated Severity Score (grand-mean centred) was included as an additional covariate (Supplementary material).

### Genetics analyses

#### Genotyping

One hundred and twenty-seven ASD individuals (61 female) were genotyped using the HumanOmni2.5M BeadChip (Illumina). All DNA samples were hybridized and scanned on the Illumina iScan to minimize batch effects and variation. All subjects had a genotyping call rate > 95%. Genotyping data were analysed by PLINK v1.07\(^{31}\) using the forward strand and confirmed the reported sex and sibling relationships of all subjects. One ASDm was removed for failing to pass quality metrics of the CNV detection algorithms, leaving 126 probands. One hundred and thirty typically developing subjects (67 female) were also genotyped. Four TDm genotyping chips failed; two TDf failed to pass CNV quality metrics, leaving 124 typically developing subjects (see Table 1 and Supplementary Table 6 for genotyping sample characteristics). ASDf and ASDm were balanced on race/ethnicity as were TDf and TFm (Supplementary material and Supplementary Fig. 4).

#### Copy number variant detection

CNV detection (duplications and deletions) was performed using three algorithms: PennCNV v1.0.4, QuantiSNP v1.1, and GNOsis, as previously described.\(^{32}\) Analysis and merging of CNV predictions used CVnVision. All rare genic CNVs \((\geq 50\%\) of CNV at \(\leq 1\%\) frequency in the Database of Genomic Variants; \(^{33}\) hereafter, CNVs) predicted by at least PennCNV and QuantiSNP and having a CVnVision pCNV of \(\leq 0.001\) — i.e. those considered high-quality predictions\(^{33}\) — were obtained for further analysis. For the 37 ASD subjects who had unaffected sibling(s) with available genotyping data \((n = 40\) siblings), we further specified that rare CNVs in a proband should only include those not shared by their sibling(s). Subsequent analyses combined duplications and deletions to maximize the
number of CNVs available for examination; separating the two types led to low numbers and no significant effects.

**Gene expression levels**

Gene-level human brain expression data (Platform GPL5175, Affymetrix GeneChip Human Exon 1.0 ST Array), generated as part of the BrainSpan project (hbatlas.org) were downloaded from the NCBI GEO database (accession number GSE25219) as log2-transformed signal intensity values. Values ≥ 6 in at least one sample are considered positive brain expression. Affymetrix uses background probes with matching guanine-cytosine content for background correction for all array probes.

**Creation of spatiotemporal regions of interest and identification of candidate genes**

We expected that hypotheses on human motion in some regions represented in the functional MRI-defined TDF > ASDf map might relate not simply to differing social experiences over time, but could instead (or in addition) relate to a possible aetiological mechanism (i.e. mutation load) operating during the developmental window during which ASD is thought to onset, that is, from the prenatal period up to ~2 years. To test this prediction, we used the BrainSpan exon-array transcriptome dataset to categorize CNVs in our sample based on the spatiotemporal expression of gene(s) they contained during human brain development. Related approaches using this resource have been used by a number of groups. To create a candidate gene set, we selected genes expressed during early development [10 postconceptual weeks (pcw) to 2 years] in brain regions (n = 11) that were significant in the TDF > ASDf functional MRI contrast and for which BrainSpan data were available (Fig. 2A). These 11 regions included: right/left (R/L) dorsolateral prefrontal cortex (DFC), primary motor cortex (M1C), primary somatosensory cortex (S1C), striatum (STR), ventrolateral prefrontal cortex (VFC), and right superior temporal cortex (R-STC) (Supplementary material).

We chose the summed total size of CNVs per individual to represent mutation load since CNV size has been associated with deleteriousness in previous ASD genetics studies, and this parameter had the widest data distribution in our cohort. The distribution of number of CNVs or number of genes contained within CNVs per individual was limited in this dataset and did not yield significant differences between ASDf and ASDm (Supplementary Table 3). We did not restrict our analysis to CNVs containing genes previously associated with ASD to avoid removing potentially pathogenic CNVs not yet identified. The Supplementary material describe ASD-associated genes contained by CNVs in GENDAAR and replication cohorts.

**Permutation testing strategy**

Our sample contained subjects with CNV sizes that were statistically outlying; we retained these subjects for analysis given that these large CNVs might be aetiologically meaningful. We used the median rather than the mean as our summary statistic for CNV size given its greater robustness to outlying influence. The female minus male (F – M) difference (ASDf – ASDm or TDF – TDm) in median summed total CNV size was calculated by first obtaining the median CNV size for each group and then taking the difference between the two medians. All tests were conducted with custom Perl (v.5.24.1) scripts that used 10 000 permutations and P < 0.05, with P-values based on the number of iterations that yielded the actual F – M difference in median CNV size or greater. Fewer occurrences of such a difference led to lower P-values.

Permutation testing was conducted both by permuting the sex label of ‘person-sets’ of CNVs found within individuals and by permuting the sex label of CNVs independently of individuals. We defined a person-set of CNVs as all CNVs within an individual meeting certain criteria, the sizes of which are summed to give the total CNV size for that individual; person-set criteria varied by test (Supplementary material). We did not have parental information (de novo versus inherited) to help determine which of multiple CNVs within an individual may contribute to ASD. Therefore, we prioritized permuting by person-sets (versus by CNVs independent of individuals) as this would constitute a more conservative set of tests; we primarily rely on these results in drawing inferences. A given person-set of CNVs was selected only once within an iteration of a permutation test.

We also conducted control analyses to test the significance of the sex difference in median size of CNVs containing gene(s) not expressed in a region of interest (Supplementary material).

**Replication in the Simons Simplex Collection**

Rare, genic proband CNVs identified in the Simons Simplex Collection (SSC), were analysed and filtered using the same criteria as for our original dataset (Supplementary Table 4C and F). SSC CNVs were included regardless of inheritance status, and we again specified that CNVs in a proband should only include those not shared by their unaffected sibling(s) (n = 2282 siblings). Permutation analyses run in the SSC cohort largely paralleled those conducted in the GENDAAR cohort. However, in SSC, the sex ratio is skewed (SSC M: F 6:1; GENDAAR both 3:1). Therefore, we performed an additional permutation test controlling for dataset size.

**Data availability**

Raw and processed data, scripts, and workflows are available for this project through the National Database for Autism Research at ndar.nih.gov, DOI: 10.15154/1478424; NeuroVault (neurovault.org/collections/BZOJTKVI/); and the Open Science Framework (https://osf.io/mgzny) (Supplementary material).

**Results**

**Objective 1: functional MRI**

TDF ≤ TDm, ASDf ≤ ASDm, TDm ≤ ASDm, and TDF ≤ ASDf contrasts were tested. Significant group differences in BIO > SCRAM response were observed for TDF > TDm and TDF > ASDf. All other contrasts were non-significant. For mean within-group effects, see Supplementary material, Supplementary Fig. 1 and Supplementary Table 1.

**Typically developing females > ASD females**

TDF showed stronger response than ASDf in primarily parietal, posterior temporal, and posterior frontal regions, with
Figure 1 Group differences in response to the BIO > SCRAM contrast, controlling for FSIQ, age, site, and SRS total raw score. T-statistic images of regions showing a significantly [permutation-based \( P < 0.05 \) (corrected) with TFCE, \( n_{\text{permutations}} = 10,000 \)] greater BIO > SCRAM response for one group than another (A: TDF > ASDf; B: TDF > TDM) are displayed on 3D renderings of the MNI standard brain in neurological convention, with MNI coordinates provided. Box plots depict the distribution of subject-specific per cent signal change (% signal \( \Delta \)) for the BIO > SCRAM contrast, and are clustered by subject group and panelled by brain region. (C) Binary mask indicating regions of overlap between A and B, displayed as axial slices labelled by MNI z-coordinate at top left. Box plot elements: centre line = median; notches = confidence interval \([\pm 1.58 \times \text{IQR} / (n)]\) around the median; box limits = interquartile range (IQR; 25th to 75th percentile); whiskers = extend to largest/smallest value no further than \(1.5 \times \text{IQR}\) from box limit; points = outliers. aIPS = anterior intraparietal sulcus; BA = Brodmann area; CO = central operculum; IFG = inferior frontal gyrus; MFG = middle frontal gyrus; PCG = paracingulate; pITG = posterior inferior temporal gyrus; PMd = dorsal premotor cortex; PMv = ventral premotor cortex; PrG = precentral gyrus; pSTS = posterior superior temporal sulcus; R = right; S1C = primary somatosensory cortex; S2C = secondary somatosensory cortex; SFG = superior frontal gyrus; SPL = superior parietal lobule; TP = temporal pole.
The largest statistical effect sizes (Cohen’s $d > 0.90$) observed in right-lateralized ventral premotor cortex (PMv), M1C, parietal white matter, and putamen, and in bilateral DFC and central operculum. Additional regions with a relatively high COPE value (>20) but a medium statistical effect size included right-lateralized secondary somatosensory cortex, superior parietal lobule (SPL), and left temporal pole (Fig. 1A and Table 2).
Typically developing females > typically developing males

TDF exhibited increased BIO > SCRAM activation versus TDM in a variety of bilateral parietal and frontal regions. The largest statistical effect sizes were observed in right-lateralized anterior insula (anterior insula; Cohen’s $d = 0.79$) and DFC (Cohen’s $d = 0.84$); additional regions with a relatively high COPE value (> 20) but a medium statistical effect size

| Macroanatomical site                                      | Cyt     | Hem | x    | y    | z    | t    | d    | COPE | P    |
|----------------------------------------------------------|---------|-----|------|------|------|------|------|------|------|
| Right fronto-temporo-parietal cluster                    |         |     |      |      |      |      |      |      |      |
| PMv/PreCG BA6                                            | R       | 54  | -6   | 46   | 4.34 | 0.91 | 26.29| 0.005|
| M1/C/PreCG BA4a                                          | R       | 54  | -6   | 40   | 4.34 | 0.91 | 21.79| 0.005|
| Putamen                                                  | R       | 26  | 14   | -6   | 4.31 | 0.91 | 12.55| 0.007|
| Parietal WM/SLPA                                         | R       | 18  | -44  | 44   | 4.65 | 0.98 | 11.86| 0.012|
| Frontal pole/Frontal WM/DFC                              | R       | 34  | 34   | 22   | 4.44 | 0.94 | 13.92| 0.010|
| COper                                                    | R       | 44  | 2    | 12   | 4.32 | 0.91 | 11.36| 0.009|
| PMd/PreCG BA6                                            | R       | 30  | -18  | 58   | 4.20 | 0.91 | 12.63| 0.011|
| PoCG/S1C/Corticospinal tract                             | R       | 12  | -34  | 70   | 3.73 | 0.79 | 16.59| 0.012|
| Posterior temporal fusimotor cortex                      | R       | 40  | -14  | -28  | 4.02 | 0.85 | 14.66| 0.019|
| S2C/POper                                                | R       | 60  | -22  | 22   | 3.56 | 0.75 | 23.05| 0.010|
| M1/C/PreCG BA4a                                          | L       | -14 | -32  | 64   | 3.44 | 0.73 | 13.87| 0.019|
| Callosal body                                            | L       | 16  | -30  | 26   | 3.85 | 0.81 | 10.98| 0.017|
| Ventroposterior thalamus                                 | R       | 14  | -22  | 2    | 3.27 | 0.69 | 9.43 | 0.019|
| pSTG/Temporal WM                                         | R       | 52  | -30  | 8    | 3.61 | 0.76 | 12.44| 0.019|
| SPL/PoCG/S1C                                            | L       | -16 | -42  | 64   | 3.63 | 0.77 | 11.15| 0.020|
| SPL/SLOC                                                 | R       | 16  | -64  | 62   | 3.26 | 0.69 | 35.43| 0.026|
| Planum polare/Optic radiation                            | R       | 40  | -4   | -18  | 3.36 | 0.71 | 13.43| 0.016|
| pITG                                                     | R       | 50  | -30  | -22  | 3.73 | 0.79 | 9.06 | 0.019|
| Left fronto-parietal cluster                             |         |     |      |      |      |      |      |      |      |
| M1/C/PreCG BA4p                                          | L       | -48 | -2   | 26   | 4.11 | 0.87 | 12.96| 0.011|
| Anterior corona radiata                                  | L       | -26 | 12   | 20   | 4.02 | 0.85 | 7.40 | 0.013|
| PMd/SFG BA6                                              | L       | -6  | 14   | 54   | 3.82 | 0.81 | 16.79| 0.017|
| POper/Parietal WM                                         | L       | -36 | -40  | 18   | 4.23 | 0.89 | 7.70 | 0.012|
| Paracingulate gyrus                                      | R       | 6   | 18   | 40   | 4.24 | 0.89 | 17.68| 0.016|
| COper/Broca’s area/SLF                                   | BA44    |     |      |      |      |      |      |      |      |
| Callosal body                                            | L       | -12 | 14   | 28   | 3.68 | 0.78 | 9.95 | 0.019|
| Insula                                                   | L       | -38 | -10  | 6    | 3.86 | 0.81 | 10.86| 0.016|
| Callosal body                                            | L       | -24 | -52  | 28   | 3.79 | 0.80 | 7.75 | 0.016|
| Frontal pole/DFC                                         | L       | -36 | 40   | 24   | 4.68 | 0.99 | 17.15| 0.033|
| COper/S2C/POper                                          | OP1     |     |      |      |      |      |      |      |      |
| Anterior supramarginal gyrus/alPS                        | L       | -42 | -34  | 36   | 3.47 | 0.73 | 15.76| 0.019|
| Broca’s area/PreCG                                       | BA44    |     |      |      |      |      |      |      |      |
| Temporal Pole                                            | L       | -58 | 12   | -8   | 3.35 | 0.71 | 25.57| 0.030|
| SLF                                                      | L       | -22 | -38  | 42   | 3.49 | 0.74 | 7.15 | 0.019|
| IFG, pars opercularis/Frontal WM                         | R       | 40  | 16   | 20   | 2.77 | 0.58 | 11.65| 0.047| 34   |
| Optic radiation                                          | R       | 40  | -44  | 4    | 3.37 | 0.71 | 6.37 | 0.046| 33   |
| IFG, pars triangularis/Frontal WM                        | L       | -40 | 28   | 12   | 2.69 | 0.57 | 9.12 | 0.049| 10   |
| Callosal body                                            | L       | -10 | -38  | 18   | 2.98 | 0.63 | 8.38 | 0.048| 4    |

Coordinates are in MNI space. Statistical inference used 10 000 permutations and TFCE, corrected $P = 0.05$. For table creation purposes only, for clusters $k > 1000$ voxels, the cluster was further thresholded at $t > 3.00$ and a minimum extent of $k = 10$ prior to coordinate extraction and atlas query in order to yield more anatomically distinct subclusters. Where this procedure resulted in further clusters where $k > 1000$, additional local maxima were identified as necessary to describe the anatomical extent of activation. Macroanatomical labels are assigned from the Harvard-Oxford Cortical/Subcortical Structural Atlases and supplemented with macroanatomical and cytoarchitectonic labels from the Juelich Histological Atlas; additional white matter labels are provided by the Mars Parietal connectivity-based parcellation atlas and the JHU White-Matter Tractography and ICBM-DTI-81 White-Matter Labels Atlases. The dorsal/ventral premotor boundary is set at $z = 48$ per Tomassini et al. The dorsal/ventral precentral gyrus boundary is set at $z = 48$ per Tomassini et al. The anterior intraparietal sulcus; BA = Brodmann area; COPE = contrast of parameter estimates; COper = central operculum; Cyt = cytoarchitectonic; $d$ = Cohen’s $d$; IFG = inferior frontal gyrus; Hem = hemisphere; $k$ = voxel extent; L = left; pITG = posterior inferior temporal gyrus; PMd = dorsal premotor cortex; PMv = ventral premotor cortex; PoCG = postcentral gyrus; POper = parietal operculum; PreCG = precentral gyrus; pSTG = posterior superior temporal gyrus; R = right; S1C = primary somatosensory cortex; S2C = secondary somatosensory cortex; SLF = superior longitudinal fasciculus; SLOC = superior lateral occipital cortex; SPL = superior parietal lobule; $t$ = $t$-statistic; WM = white matter. SPLA is a white matter division from the Mars Parietal connectivity-based atlas.
included bilateral middle frontal gyrus (MFG) and right-lateralized anterior intraparietal sulcus (aIPS)/SPL, dorsal pre-motor cortex (PMd), and S1C (Fig. 1B and Table 3).

Post hoc analyses

Overlap between TDF > ASDf and TDF > TDM

As an exploratory step, we examined overlap between significant regions of TDF > ASDf and TDF > TDM to determine regions that might relate to TDF resilience in social perception. Overlap occurred in frontal and parietal regions including right anterior insula, DFC, inferior frontal gyrus (IFG), and MFG, and in bilateral aIPS and paracingulate (Fig. 1C and Supplementary Table 2).

Overlap between TDF > ASDf and TDM > ASDm

Contrary to expectations, TDM > ASDm did not produce significant results. We considered whether TDF > ASDf and TDM > ASDm might in fact be similar, with a lack of statistical power in the TDM > ASDm contrast preventing us from detecting this similarity. At trend level (corrected \( P < 0.10 \)), the TDM > ASDm contrast generated two small clusters in right corticospinal tract (Supplementary Fig. 2A); overlap between this map and that of TDF > ASDf was restricted to \( k = 10 \) voxels (Supplementary Fig. 2B). We also ran both the TDM > ASDm and TDF > ASDf contrasts again using less stringent thresholding procedures commonly used in previous literature (cluster-wise inference with \( z = 2.3 \) and corrected \( P < 0.05 \), i.e. FSL’s default thresholding prior to April 2017). Using these parameters, TDM > ASDm yielded a single cluster (\( k = 386; \) MNI peak: \( x = 48, y = –60, z = 8 \); Supplementary Fig. 2C) in right posterior caudal STS/middle temporal gyrus. This cluster was located posterior to right pSTS involvement in the TDF > ASDf contrast, with no overlap (Supplementary Fig. 2D).

Objective 2: integration of genetics and functional MRI data

Descriptive characteristics of rare genic CNV size in ASD sample

Table 1 summarizes the genotyping sample. As shown in Table 4, out of 61 ASDf, 116 CNVs in 53 unique individuals were identified, with a median summed total CNV size of 144 378 base pairs (bp) per individual (hereafter, ‘median size’); out of 65 ASDm, 114 CNVs in 48 unique individuals were identified, with a median size of 106 740 bp and an

| Macroanatomical site | Cyt | Hem | x | y | z | t | d | COPE | P | k |
|----------------------|-----|-----|---|---|---|---|---|-----|---|---|
| Bilateral superior frontal cluster | R | 18 | 8 | 50 | 3.82 | 0.72 | 8.46 | 0.027 |
| Superior frontal WM | R | 18 | 8 | 50 | 3.82 | 0.72 | 8.46 | 0.027 |
| MFG | R | 18 | 8 | 50 | 3.82 | 0.72 | 8.46 | 0.027 |
| PMd | R | 16 | –4 | 58 | 3.82 | 0.72 | 8.46 | 0.027 |
| Corticospinal tract | L | –30 | –6 | 38 | 3.82 | 0.72 | 8.46 | 0.027 |
| S1C | R | 40 | –8 | 28 | 3.82 | 0.72 | 8.46 | 0.027 |
| Insula | R | 34 | 20 | 4 | 3.82 | 0.72 | 8.46 | 0.027 |
| Superior parietal WM/SPLC/SPLD | R | 16 | –60 | 44 | 3.82 | 0.72 | 8.46 | 0.027 |
| aIPS/SPL | R | 16 | –60 | 44 | 3.82 | 0.72 | 8.46 | 0.027 |
| aIPS/SPLD | R | 16 | –60 | 44 | 3.82 | 0.72 | 8.46 | 0.027 |
| S1/Corticospinal tract | L | –48 | –2 | 28 | 3.82 | 0.72 | 8.46 | 0.027 |
| Frontal Pole/DFC | R | 40 | 42 | 24 | 4.44 | 0.84 | 20.39 | 0.043 |
| S1C/PoCG | R | 40 | 42 | 24 | 4.44 | 0.84 | 20.39 | 0.043 |
| M1C/PoCG | R | 40 | 42 | 24 | 4.44 | 0.84 | 20.39 | 0.043 |
| PoCG | R | 40 | 42 | 24 | 4.44 | 0.84 | 20.39 | 0.043 |
| PMv/Corticospinal tract | R | 32 | –10 | 44 | 3.36 | 0.69 | 10.86 | 0.046 |
| SPL/Superior parietal WM | L | –24 | –50 | 46 | 3.40 | 0.64 | 13.65 | 0.048 |
| Callosal body | L | –10 | 16 | 26 | 3.65 | 0.69 | 10.86 | 0.046 |
| PMd/SPLC | BA6 | 26 | –4 | 70 | 3.00 | 0.56 | 17.60 | 0.048 |
| Optic radiation | R | 24 | –60 | 32 | 2.96 | 0.56 | 17.60 | 0.048 |

Coordinates are in MNI space. Statistical inference used 10 000 permutations and TFCE, corrected \( P = 0.05 \). See notes for Table 2 for details on table creation and atlases used for label assignment. aIPS = anterior intraparietal sulcus; BA = Brodmann area; COPE = contrast of parameter estimates; Cyt = cytoarchitectonic; \( d \) = Cohen’s \( d \); Hem = hemisphere; \( k \) = voxel extent; L = left; MFG = middle frontal gyrus; PMd = dorsal pre-motor cortex; PMv = ventral pre-motor cortex; PoCG = postcentral gyrus; PreCG = precentral gyrus; R = right; S1C = primary somatosensory cortex; SFG = superior frontal gyrus; SLF = superior longitudinal fasciculus; SPL = superior parietal lobule; t = t-statistic; WM = white matter; SPLC and SPLD are white matter divisions from the Mars Parietal connectivity-based atlas.
Table 4 Analysis of rare genic CNVs in GENDAAR cohort

| ASD sample characteristics | Female | | Male | | | | Total individuals with CNVs (n) | F–M difference in median total CNV size (bp) |
|---------------------------|--------|--------|------|--------|------|--------|-----------------|-----------------|
|                           | Individuals with CNVs (n) | CNVs (n) | Individuals with CNVs (n) | CNVs (n) | Total individuals with CNVs (n) | F–M difference in median total CNV size (bp) |
| CNVs containing any gene(s) | 53     | 116    | 48   | 114    | 101  | 37,638 |
| CNVs containing BrainSpan gene(s) | 51     | 88     | 44   | 87     | 95   | 4,350  |
| CNVs containing gene(s) expressed in R/L-STR | 40    | 62     | 34   | 48     | 74   | 89,999 |
| CNVs containing gene(s) not expressed in R/L-STR | 22    | 26     | 28   | 39     | 50   | 1002  |

Permutation tests for ASD sample

| Person-sets labelled female (n) | Person-sets sampled from (n) | Person-sets labelled male (n) | Person-sets sampled from (n) | Compared to (bp) | P    |
|--------------------------------|------------------------------|------------------------------|------------------------------|-----------------|------|
| Sex label shuffling of person-sets of CNVs containing gene(s) expressed in R/L-STR | 40                             | 74                           | 34                           | 74              | 89,999 | 0.0293 |
| Person-sets of all CNVs: sex labels randomized | 40                             | 101                          | 34                           | 101             | 89,999 | 0.0178 |
| Person-sets of all CNVs: sex labels preserved | 40                             | 53                           | 34                           | 48              | 89,999 | 0.0172 |
| Person-sets of CNVs containing BrainSpan gene(s): sex labels randomized | 40                             | 95                           | 34                           | 95              | 89,999 | 0.0048 |
| Person-sets of CNVs containing BrainSpan gene(s): sex labels preserved | 40                             | 51                           | 34                           | 44              | 89,999 | 0.0000 |
| Sex label shuffling of person-sets of CNVs containing gene(s) not expressed in R/L-STR | 22                             | 50                           | 28                           | 50              | 1002  | 0.4134 |
| Person-sets of CNVs containing gene(s) expressed in R/L-STR: sex labels randomized, sample size reduced to non-R/L-STR size | 22                             | 74                           | 28                           | 74              | 89,999 | 0.0533 |
| Person-sets of CNVs containing gene(s) not expressed in R/L-STR: sex labels preserved, sample size reduced to non-R/L-STR size | 22                             | 40                           | 28                           | 34              | 89,999 | 0.4955 |

Typically developing sample characteristics

| Individuals with CNVs (n) | CNVs (n) | Individuals with CNVs (n) | CNVs (n) | Total individuals with CNVs (n) | F–M difference in median total CNV size (bp) |
|---------------------------|--------|---------------------------|--------|-----------------|-----------------|
| CNVs containing gene(s) expressed in R/L-STR | 35     | 55                         | 36     | 70              | 71              | 58,136 |
| CNVs containing gene(s) not expressed in R/L-STR | 28     | 41                         | 25     | 33              | 53              | 1595  |

Permutation tests for typically developing sample

| Person-sets labelled female (n) | Person-sets sampled from (n) | Person-sets labelled male (n) | Person-sets sampled from (n) | Compared to (bp) | P    |
|--------------------------------|------------------------------|------------------------------|------------------------------|-----------------|------|
| Sex label shuffling of person-sets of CNVs containing gene(s) expressed in R/L-STR | 35                             | 71                           | 36                           | 71              | 58,136 | 0.0779 |
| Sex label shuffling of person-sets of CNVs containing gene(s) not expressed in R/L-STR | 28                             | 53                           | 28                           | 53              | 1595  | 0.4131 |

Tests use 10,000 permutations. P-values indicate how many iterations yielded the actual female minus male (F–M) difference in median total CNV size (value under ‘compared to’) or greater; P < 0.05 (bold) is a significant difference. CNV = copy number variant; R/L-STR = right and left striatum.

*Tests intended to determine likelihood that results in full sample are driven by outlier individual(s).

Randomly assigning sex labels of person-sets of CNVs containing R/L-STR, but reducing sample size [to reflect n’s of CNVs containing gene(s) not expressed in R/L-STR], it is still unlikely (5.3%) to obtain a difference ≥ 89,999 bp.

Preserving sex labels of person-sets in the reduced sample, a difference ≥ 89,999 bp is still likely (49.5%).
Objective 2A: do females with ASD versus males with ASD show larger median size of CNVs containing candidate genes?

Figure 2B shows the ASDf – ASDm difference in median size of CNVs containing candidate genes. For all 11 brain regions of interest combined, the difference in median size was 81 597 bp (Fig. 2B, solid red line). Shuffling the sex label of person-sets of CNVs containing gene(s) expressed in the combination of all 11 regions of interest 10 000 times produced $P < 0.05$, indicating that this ASDf – ASDm difference in median CNV size was significant (Supplementary Table 4A). By contrast, when comparing TDF to TDM, there was a trend toward greater median CNV size among TDM (TDF – TDM: –63 128 bp; Supplementary Table 4B).

Objective 2B: which regions of interest appear to drive the ASDf – ASDm difference?

As depicted in Fig. 2B, the ASDf – ASDm difference in median size of CNVs containing candidate genes appeared to be driven by two regions of interest in particular: R-STR (83 529 bp) and L-STR (84 184 bp). L-VFC (78 733 bp) and L-M1C (75 922 bp) also showed notable peaks for difference in median size but, unlike R/L-STR, did not exceed the all regions of interest size difference (Fig. 2B, left). Combining data for R/L-STR (two regions of interest) yielded the largest difference (89 999 bp; Table 4). Figure 2B (right) shows the distribution of size of CNVs containing gene(s) expressed in R/L-STR for ASDf and ASDm.

Sex-label shuffling of person-sets of CNVs containing gene(s) expressed in each of the 11 regions of interest produced $P < 0.05$ only for L-M1C, R-STR, L-STR, and the combination of R-STR (Table 4 and Supplementary Table 4A). While sex-label shuffling alone suggested that CNVs containing genes expressed in motor or striatal cortex might be driving the effect, further control analyses (eight total, described below under Objective 2C) were only passed unanimously by R/L-STR, whereas L-M1C failed one of the control tests; therefore we focus our attention in the remainder of the text on R/L-STR results. Full results of all control tests on L-M1C (and other regions of interest) are available in Supplementary Table 4.

As for the combination of all 11 regions of interest, there was a trend among TDM (versus TDF) toward greater median CNV size for R/L-STR (TDF – TDM: –58 136 bp) (Table 4, Fig. 2B and Supplementary Table 4B).

Objective 2C: are results attributable to increased CNV size in female ASD more generally?

Sixty-two ASDf and 48 ASDm CNVs (in 40 ASDf and 34 ASDm individuals) contained at least one gene expressed in R- or L-STR. CNVs often contained genes that were expressed in multiple brain regions, such that genes expressed in R/L-STR were not exclusively expressed in these two regions of interest, and some genes were expressed in brain regions other than these two regions of interest. The ASDf – ASDm difference in median size of CNVs containing gene(s) expressed in R/L-STR (89 999 bp) was significant when 40 person-sets of CNVs were labelled female and 34 male 10 000 times after being selected from the set of (i) all person-sets of CNVs containing any gene(s), sex labels randomized; or (ii) preserved; and from (iii) all person-sets of CNVs containing BrainSpan gene(s), sex labels randomized; or (iv) preserved (Table 4). Tests in which CNVs were permuted independently of individuals (rather than as person-sets) were also significant, as were all of the above tests for the combination of all 11 regions of interest (Supplementary Table 4).

To further test the specificity of our findings, we calculated the ASDf – ASDm difference in median size of CNVs containing BrainSpan gene(s) that were not expressed in R/L-STR from 10 pcw to 2 years (non-R/L-STR; Table 4). Twenty-six non-R/L-STR CNVs with a median size of 34 708 bp were identified in 22 ASDf, while 39 non-R/L-STR CNVs with a median size of 33 706 bp were identified in 28 ASDm. The ASDf – ASDm difference (1002 bp) in median size for these CNVs was not significant (Table 4). Additional tests indicated our findings were unlikely to be driven by outlier individuals (Table 4).

Objective 2D: does larger ASDf versus ASDm size of CNVs containing genes expressed during early striatal development replicate in an independent ASD cohort?

We determined the replicability of our results in an independent ASD cohort, the Simons Simplex Collection (SSC) (Table 5) shows $n$’s of SSC ASDf and ASDm carrying CNVs containing any gene(s), BrainSpan gene(s), and gene(s) expressed or not expressed in R/L-STR. In the SSC cohort, the ASDf – ASDm difference in median size of CNVs containing gene(s) expressed in R/L-STR was 45 133 bp. Shuffling the sex labels of person-sets of CNVs containing gene(s) expressed in R/L-STR 10 000 times produced $P = 0.0041$, indicating that the ASDf – ASDm difference in median size of CNVs containing genes expressed in R/L-STR was significant in SSC (Table 5); similar control tests to those run in the GENDAAR cohort for Objectives 2A–C were also largely significant (Table 5 and Supplementary Table 4C). As in the GENDAAR cohort, the ASDf – ASDm difference in median size of CNVs containing gene(s) not expressed in R/L-STR (2641 bp) was not significant ($P = 0.2595$; Table 5).

To mimic the ~1:1 sex ratio of CNVs in our cohort (given that the ratio of ASDm to ASDf CNVs in SSC is ~6:1), we randomly selected 40 person-sets of ASDf CNVs and 34 ASDm CNVs 10 000 times from the set of SSC CNVs containing gene(s) expressed in R/L-STR and compared the ASDf – ASDm size difference generated in these permutations to the size difference in the GENDAAR cohort (89 999 bp). Permutation testing yielded $P = 0.1235$, indicating that,
| ASD sample characteristics                  | Female                                | Male                                |
|--------------------------------------------|---------------------------------------|-------------------------------------|
|                                            | Individuals with CNVs (n) | CNVs (n) | Individuals with CNVs (n) | CNVs (n) | Total individuals with CNVs (n) | F–M difference in median total CNV size (bp) |
| CNVs containing any gene(s)                | 291                                   | 658      | 1784                        | 3664      | 2075                        | 27824                                |
| CNVs containing BrainSpan gene(s)          | 261                                   | 498      | 1573                        | 2807      | 1834                        | 19703                                |
| CNVs containing gene(s) expressed in R/L-STR | 195                                   | 313      | 1212                        | 1784      | 1407                        | 45133                                |
| CNVs containing gene(s) not expressed in R/L-STR | 137                                   | 183      | 819                         | 1023      | 953                         | 2641                                 |

**Permutation tests for ASD sample**

| Test Description                                                                 | Person-sets labelled female (n) | Person-sets sampled from (n) | Person-sets labelled male (n) | Person-sets sampled from (n) | Compared to (bp) | P       |
|----------------------------------------------------------------------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------|---------|
| Sex label shuffling of person-sets of CNVs containing gene(s) expressed in R/L-STR | 195                             | 1407                        | 1212                        | 1407                        | 45133           | 0.0041  |
| Person-sets of all CNVs: sex labels randomized                                  | 195                             | 2075                        | 1212                        | 2075                        | 45133           | 0.0013  |
| Person-sets of all CNVs: sex labels preserved                                   | 195                             | 291                         | 1212                        | 1784                        | 45133           | 0.1020  |
| Person-sets of CNVs containing BrainSpan gene(s): sex labels randomized         | 195                             | 1834                        | 1212                        | 1834                        | 45133           | 0.0013  |
| Person-sets of CNVs containing BrainSpan gene(s): sex labels preserved          | 195                             | 261                         | 1212                        | 1573                        | 45133           | 0.0211  |
| GENDAAR n\(^{s}\) and comparison value for SSC person-sets of CNVs containing gene(s) expressed in R/L-STR, sex labels preserved | 40                              | 195                         | 34                          | 1212                        | 89999           | 0.1235* |
| Sex label shuffling of person-sets of CNVs containing gene(s) not expressed in R/L-STR | 137                             | 953                         | 816                         | 953                         | 2641            | 0.2595  |

Tests use 10,000 permutations. P-values indicate how many iterations yielded the actual female minus male (F–M) difference in median total CNV size (value under ‘compared to’) or greater; \( P \leq 0.05 \) (bold) is a significant difference. CNV = copy number variant; R/L-STR = right and left striatum.

*Test indicates that when SSC sample size is limited to that of GENDAAR ASD sample, we obtain an ASDf – ASDm difference in median size of CNVs containing gene(s) expressed in R/L-STR equivalent or greater than that found in the GENDAAR sample 12.4% of the time.
in the SSC cohort, the ASDf – ASDm difference in median size of CNVs containing gene(s) expressed in R/L-STR was not significantly different from that of GENDAAR (Table 5).

**Discussion**

This ASDf-enriched sample has yielded a number of novel insights into female neuro-endophenotypes of social motion perception and potential contributors to female risk for ASD. While functional MRI highlights widespread functional differences between ASDf and TDf viewing human motion, analysis of the size of rare CNVs containing genes expressed in these functional MRI-identified brain regions suggests that potential impacts to striatum may be related to a sex-differential process of risk in early development. These larger ASDf CNVs support the FPE model prediction of greater genetic load in ASDf versus ASDm. Below, we discuss findings related to our major research objectives: (i) characterization of a functional MRI-based profile of ASDf (versus TDf) response to socially meaningful motion; and (ii) integration of functional MRI and genetics data.

First, we observed that the ASDf brain response during human action observation is characterized by less recruitment of parietal and posterior frontal cortex relative to TDf, particularly right somatosensory cortex, motor/premotor areas, and the putaminal region of striatum. This is distinct both from the ASD neural response associated with this paradigm in previous ASDm-predominant literature, and from trend-level TDm > ASDm results in this sample, which exhibit minimal overlap with TDf > ASDf. One prominent peak of TDf > ASDf occurred in right PMv, a region putatively associated with ‘mirroring’ properties, and which some suggest may help observers ‘fill in’ information missing from point-light human motion displays. Somatosensory regions detected in TDf > ASDf also display putative mirroring properties. Thus, greater recruitment of these regions by TDf might imply stronger engagement of such processes. PMv was not represented in BrainSpan, and was thus excluded from our Objective 2 analyses.

To contextualize our TDf > ASDf results, we also analysed differences in response between TDf and TDm, TDf and ASDm, and between ASDf and ASDm. TDf showed increased response to BIO > SCRAM relative to TDm in a variety of frontal and parietal regions. As in the sample of typically developing adults from the study by Anderson and colleagues, TDf versus TDm demonstrated greater BIO > SCRAM activation within right DFC, although other regions demonstrating typically developing child (e.g. ventromedial prefrontal cortex) or typically developing adult (e.g. amygdala) sex differences in their cohort did not replicate in our sample, possibly due to differences in the age ranges of our samples. Many of the regions that emerged from our TDf > TDm contrast overlapped with those represented in the TDf > ASDf map, including right-lateralized anterior insula, IFG, DFC, MFG, and bilateral aIPS and paracingulate. Together, these regions resemble the salience and central executive brain networks. The salience network contains bilateral fronto-insular cortex and dorsal anterior cingulate, and contributes to monitoring and detection of salient stimuli. The central executive network is correlated with right fronto-insular activity and includes DFC, supplementary motor area, and lateral parietal cortices; these systems together play a role in attention, working memory, and cognitive control. The executive and salience sites recruited more strongly by TDf could play a number of roles potentially contributory to resilience in social perception. Right anterior insula contributes to detection of novel salient stimuli and switching between the task-negative (default) and task-positive central executive network; activity in right anterior insula, IFG, and MFG/DFC can indicate renewed attention to a stimulus. These functions suggest more robust attentional reorienting among TDf to the human stimulus after a scrambled block, and/or greater attribution of salience to BIO displays by TDf than either TDm or ASDf.

In previous work examining resting state functional connectivity in our GENDAAR cohort, we found that typically developing youth demonstrated sex differences in functional connectivity of the salience but not the central executive network, while ASD youth showed the opposite pattern, with sex differences in the central executive, but not the salience network. Given our previous results, and the role of the salience network in managing switching to the central executive network, the TDf > ASDf differences we observed in response to social stimuli within nodes of these two networks could be driven by intrinsic neurotypical sex differences in the salience network that are not evident in ASD.

Unfortunately, while our present results, and those of our previous resting state work, suggest that anterior insula and aIPS might have relevance to TDf resilience in social perception, these regions were not characterized in BrainSpan, and thus could not be assessed in our Objective 2 analyses.

We did not detect significant differences between ASDf and ASDm in their functional MRI neural response to biological motion. Moreover, contrary to extant literature, ASDm did not differ from TDm on this task. In exploratory follow-up analyses, we considered whether the TDm > ASDm pattern might be similar to that of TDf > ASDf, but below our threshold for statistical detection. Under a more lenient method for statistical inference, ASDm versus TDm displayed right pSTS hypoactivation similar to that found in previous work, suggesting that modern methods of functional MRI statistical inference may reduce our power to detect this effect in exchange for greater type I error control. TDf > ASDf did not overlap with TDm > ASDm under this more lenient method. Thus, while ASDf and ASDm responses to human motion did not significantly differ, at the same time what distinguishes ASDf from TDf does not appear similar to what distinguishes ASDm from TDm.

While ASDf and ASDm functional brain response did not differ, genetic analyses demonstrated significant differences between these groups. Specifically, ASDf (versus ASDm) exhibited larger size of rare CNVs containing genes.
expressed during early development of striatum. This finding, accompanied by ASDf (versus TDf) hypoactivation of putamen (a component of the striatum) during social perception, suggests that potential impacts to striatum may be an element of developmental risk for ASD trajectories in girls. Previously, putaminal disruptions in ASD versus typically developing individuals have been documented \(^{56-61}\) albeit largely in ASDm-exclusive or ASDm-predominant samples. We interpret our findings as suggesting that striatal involvement, while not unique to ASDf, may have a particularly important role in ASDf aetiologies. The putamen, historically attributed a primarily motoric role, also appears involved in social and language functions.\(^{62}\) Among typically developing individuals, the putamen receives projections from motor/premotor (primarily terminating in dorsolateral/central putamen), and prefrontal cortex (primarily terminating in anterior putamen), and appears to serve as an interface between information about motivational value and voluntary behaviour.\(^{63,64}\) Recent work using resting state functional MRI data suggests that while TDm (females not assessed) demonstrate distinct functional segregation of putamen into anterior and posterior segments, putamen in ASDm appears as one functional unit.\(^{56}\) In the present investigation, we observed the peak coordinate of TDf > ASDf striatal response in a region of right anterior putamen characterized as having structural connectivity primarily to executive prefrontal regions (including MFG and DFC)\(^{65}\). It also may be notable that in addition to reduced ASDf response in M1C, we observed larger size of CNVs containing genes expressed in M1C in many (though not all) of our control tests. Taken together, this pattern of results could indicate disturbances to the striatomotor-cortical system more broadly and, thus, processes of linking information about motivational value to action. Differential putaminal recruitment during social perception might reflect differing organization of functional connectivity, in which the region is linked to the central executive network and, perhaps, associated protective functions for TDf but not ASDf. Genetic disruptions specifically impacting striatal cortex during development may underlie such functional atypicalities, and have greater impact via disruption of female protective mechanisms. The general lack of female characterization in the literature on ASD putaminal disruptions, however, makes it difficult to draw strong conclusions along these lines. Future work should analyse ASDf and TDf patterns of functional connectivity and gene co-expression among these regions to clarify this possibility.

When considering together our findings of robust TDf > ASDf and TDf > TDm differences in brain function, lack of ASD sex differences in brain response, and greater ASDf versus ASDm size of CNVs containing genes expressed in early striatal development, the overall picture presented is complex but not inconsistent with an FPE model. While the FPE predicts that ASDf should have greater genetic load than ASDm—a prediction supported by our findings—this does not necessarily equate to greater symptomatricity or disruption of brain function. While some ASDf may lack resilience factors typically found in TDf, other ASDf may retain aspects of female protection that make their phenotype less severe than it might otherwise have been given their greater aetiological load. Moreover, female resilience factors may also have sociocultural aspects (e.g. more emotion-oriented talk to daughters versus sons\(^{66}\)); the different socialization experiences that an ASDf might encounter could lead, by adolescence, to a brain profile that does not significantly differ from ASDm despite greater genetic load.

In sum, our findings provide new insights into ASDf brain response during social perception, reveal a potential substrate of female risk for ASD trajectories, and illuminate unique qualities of TDf response to human motion relative to TDm. In addition to the basic systems for processing social motion engaged by both sexes, TDf (unique from TDm or ASDf) recruit additional salience and central executive systems. Further, relative to TDf, ASDf show reduced recruitment of striatum during this perceptual task. Compared to ASDm, ASDf (both in our cohort and an independent sample) demonstrate larger size of rare CNVs containing genes expressed in early striatal development, suggesting that, for ASDf, potential impacts to striatum may be particularly relevant. Our results demonstrate the risk of drawing conclusions regarding ASDf based on work comprised of ASDm-predominant samples, and argue for continued attention to the unique characteristics of ASDf.

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**Competing interests**

J.C.M. consults with Blackthorn Therapeutics and has received research funding from Janssen Research and Development; he receives royalties from Guilford Press, Lambert, and Springer.

**Supplementary material**

Supplementary material is available at Brain online.
Appendix 1

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Roles and affiliations within the GENDAAR consortium, and a description of named author contributions, are described in this project’s Open Science Framework site (https://osf.io/mgzny/wiki/0.%20Authors%20%26%20Consortium%20Members/).

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