

1 **Towards robust and replicable sex differences in the intrinsic brain**
2 **function of autism**

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84 **Abstract**

85 **Background:** Marked sex differences in autism prevalence accentuate the need to understand
86 the role of biological sex-related factors in autism. Efforts to unravel sex differences in the
87 brain organization of autism have, however, been challenged by the limited availability of
88 female data. **Methods:** We addressed this gap by using a large sample of males and females
89 with autism and neurotypical (NT) control individuals (ABIDE; Autism: 362 males, 82
90 females; NT: 409 males, 166 females; 7-18 years). Discovery analyses examined main effects
91 of diagnosis, sex and their interaction across five resting-state fMRI (R-fMRI) metrics
92 (voxel-level $Z > 3.1$, cluster-level $P < 0.01$, gaussian random field corrected). Secondary
93 analyses assessed the robustness of the results to different pre-processing approaches and
94 their replicability in two independent samples: the EU-AIMS Longitudinal European Autism
95 Project (LEAP) and the Gender Explorations of Neurogenetics and Development to Advance
96 Autism Research (GENDAAR). **Results:** Discovery analyses in ABIDE revealed significant
97 main effects across the intrinsic functional connectivity (iFC) of the posterior cingulate
98 cortex, regional homogeneity and voxel-mirrored homotopic connectivity (VMHC) in several
99 cortical regions, largely converging in the default network midline. Sex-by-diagnosis
100 interactions were confined to the dorsolateral occipital cortex, with reduced VMHC in
101 females with autism. All findings were robust to different pre-processing steps. Replicability
102 in independent samples varied by R-fMRI measures and effects with the targeted sex-by-
103 diagnosis interaction being replicated in the larger of the two replication samples – EU-AIMS
104 LEAP. **Limitations:** Given the lack of *a priori* harmonization among the discovery and
105 replication datasets available to date, sample-related variation remained and may have
106 affected replicability. **Conclusions:** Atypical cross-hemispheric interactions are
107 neurobiologically relevant to autism. They likely result from the combination of sex-

108 dependent and sex-independent factors with a differential effect across functional cortical
109 networks. Systematic assessments of the factors contributing to replicability are needed and
110 necessitate coordinated large-scale data collection across studies.

111 **Background**

112

113 Autism spectrum disorder (autism) is characterized by a marked male preponderance in
114 prevalence with three times more males being diagnosed than females [1]. This pronounced
115 sex-differential prevalence implies that sex-related biological factors are likely implicated in
116 the neurobiology of autism. However, little is known about the differential underlying neural
117 expressions in males and females with autism. Such knowledge could widen our
118 understanding of potential underlying mechanisms of autism and related neurodevelopmental
119 conditions [2].

120

121 This has motivated research into the impact of biological sex on brain organization in autism
122 [2–5]. With the widely accepted view that the neurobiology of autism involves differences in
123 large-scale brain networks [6,7], resting-state functional magnetic resonance imaging (R-
124 fMRI) has proven to be a valuable complementary tool for investigating atypicalities in
125 intrinsic functional connectivity (iFC). While the exact nature of the intrinsic brain
126 organization in autism remains to be established [6], research on the impact of biological sex
127 differences in autism is just beginning to emerge.

128

129 Several R-fMRI studies have focused on autism-related sex differences in iFC [2,8,17,18,9–
130 16]. They vary on the extent of the functional networks and intrinsic properties examined.
131 Most of them examined the strength of iFC between one or more regions/networks selected *a*
132 *priori* [8–10,12,13,17,18], or via data-driven analyses [16]. A few others investigated either
133 local or homotopic iFC across the whole brain [2,11,15]. Across these different efforts, the
134 pattern of findings have also been mixed; some studies supported the predictions from the
135 ‘extreme male brain theory’ [10,11], others supported the predictions from the ‘gender-

136 incoherence' theory [8,9,12,13,16]. The extreme male brain theory model predicts that brain
137 characteristics in males and females with autism will resemble those in neurotypical males
138 (i.e., shifts towards maleness in both sexes [19]). R-fMRI results consistent with a shift
139 towards maleness in autism were reported in both Ypma et al. [10] and Kozhemiako et al.
140 [11,15]. The 'gender incoherence' model predicts that brain characteristics in females with
141 autism resemble those of neurotypical males, whereas brain characteristics in males with
142 autism resemble those of neurotypical females (i.e., androgynous patterns in the sexes [20]).
143 The 'gender incoherence' model has been supported by findings from prior R-fMRI studies
144 [8,9,12], where the results largely revealed hyper-connectivity in females with autism similar
145 to neurotypical (NT) males and hypo-connectivity in males with autism similar to NT
146 females. Such seemingly inconsistent findings of sex-related differences were in part
147 addressed by Floris et al. [2] who showed that, at least in males with autism, distinct patterns
148 of atypical sex-differentiation coexist, and vary as a function of the neural networks involved.
149 However, the intrinsic brain organization in females with autism has remained largely unclear
150 and the scarce availability of female datasets in most studies may have contributed to the
151 variability in findings in males and females [21,22].

152

153 Accordingly, to explore sex-related atypicalities in autism relative to NT controls, we used, as
154 discovery sample, a large R-fMRI datasets of both males and females of autism and NT
155 selected from the Autism Brain Imaging Data Sharing Exchange (ABIDE) [22,23]. By
156 aggregating neuroimaging datasets from multiple sources, this data sharing initiative has
157 begun to provide a means to address the challenge of underrepresentation of female datasets
158 in autism research. Examining both sexes in both autism and controls allows to directly
159 capture not only sex differences that are common across individuals (i.e., regardless of their
160 diagnosis [main effect of sex]), but also those that are specific to autism and point towards

161 atypical autism-specific sex differential patterns (i.e., sex-by-diagnosis interaction effects)
162 [4]. To do so, given prior inconsistencies in the literature and the limited insights onto the
163 brain organization of females with autism, we used a discovery approach. Unlike most prior
164 work that focused on specific networks or circuits selected *a priori*, we investigated the
165 whole-brain across multiple R-fMRI metrics. We selected R-fMRI metrics capturing unique
166 aspects of the intrinsic brain organization during typical development [24,25] and, most
167 germane to this study, being reported to be involved in typical sex differences and be affected
168 by autism. They comprised: 1) posterior cingulate cortex (PCC)-iFC – e.g., [2,10,23,26–32];
169 2) voxel-mirrored homotopic connectivity (VMHC) [33] – e.g., [11,32–35]; 3) regional
170 homogeneity (ReHo) [36] – e.g., [15,32,37,38]; 4) network degree centrality (DC) [39] – e.g.,
171 [32,39–41]; and 5) fractional amplitude of low frequency fluctuations (fALFF) [23,42] – e.g.,
172 [23,32,43].

173

174 Beside the role of small female samples, prior inconsistencies in autism-related sex
175 differences in R-fMRI can be due to other factors that can impact reproducibility. For
176 example, while there are growing concerns on the role of pre-processing strategies [44,45], a
177 recent study showed that their impact on autism-related mean group-differences is minimal
178 [46]. Additionally, while several studies have reported some degree of consistency on R-
179 fMRI findings across either independent, or partially overlapping, samples [41,47–49], results
180 from other studies have raised concerns on the replicability of group-mean diagnostic effects
181 [46,50]. However, none of these studies have explicitly examined robustness and replicability
182 of sex-by-diagnosis interaction effects, which account for a potentially relevant source of
183 variability in autism – biological sex. Thus, we conducted secondary analyses to assess the
184 extent to which the pattern of findings obtained in our discovery analyses were also observed
185 a) after applying different nuisance pre-processing steps that have been previously validated,

186 though used inconsistently in the autism literature [46], and *b*) across two independent,
187 multisite R-fMRI datasets: the EU-AIMS Longitudinal European Autism Project (LEAP)
188 [51,52] and the Gender Explorations of Neurogenetics and Development to Advance Autism
189 Research (GENDAAR) dataset [53] – i.e., *robustness* and *replicability*.

190

191 **Methods**

192

193 **Discovery sample: ABIDE I and II**

194 For discovery analyses, we examined the R-fMRI dataset with one of the largest number of
195 females and males in both the autism and the NT groups available to date, selected from the
196 Autism Brain Imaging Data Exchange (ABIDE) repositories ABIDE I and II [22,23]. The
197 final ABIDE I and II dataset of N=1,019 included N=82 females with autism, N=362 males
198 with autism, N=166 neurotypical females (NT F), and N=409 neurotypical males (NT M),
199 aggregated across 13 sites. Specific selection criteria are described in Supplementary Material
200 in the Additional file 1 and depicted as a figure in the Additional file 2. Briefly, we selected
201 cases between 7-18 years of age (the ages most represented across ABIDE sites), with MRI
202 data successfully completing brain image co-registration and transformation to standard
203 space, with FIQ between 70-148 and with mean framewise displacement (mFD) [54] within
204 three times the interquartile range (IQR) + the third quartile (Q3) of the sample (i.e., 0.39
205 mFD). Further steps included matching for mean age *across* groups as well as for mFD and
206 IQ *within* diagnostic groups. This latter step limited the number of exclusions while keeping
207 average group motion low (mFD<.2mm) and sampling biases that may result when matching
208 neurodevelopmental conditions to NT around intrinsic features such as IQ [55,56]. At each
209 step, any sites with less than three individual datasets per diagnostic/sex groups were

210 excluded. Demographics and characteristics of this sample are summarized in Table 1 and in
211 Supplementary Material in the Additional file 1.

212

213 **[TABLE 1]**

214

215 **Discovery analysis pre-processing pipeline**

216 We examined five whole-brain R-fMRI metrics previously reported to reflect typical sex
217 differences and found to be atypical in autism, including 1) PCC-iFC, 2) VMHC, 3) ReHo, 4)
218 DC and 5) fALFF (see Supplementary Material, Additional file 1). R-fMRI image pre-
219 processing steps included: slice time correction, 24 motion parameters regression [57],
220 component-based noise reduction (CompCor) [58], removal of linear and quadratic trends,
221 and band-pass filtering (0.01-0.1 Hz, for all metrics but fALFF). Functional-to-anatomical
222 co-registration was achieved by Boundary Based Registration (BBR) using FSL FLIRT [59].
223 Linear and nonlinear spatial normalization of functional echo planar images (EPIs) to
224 Montreal Neurological Institute 152 (MNI152) stereotactic space (2mm³ isotropic) was done
225 using ANTS registration (Advanced Neuroimaging Tools) [60]. Computation of voxel-
226 mirrored homotopic connectivity (VMHC) followed registration to a symmetric template. All
227 R-fMRI derivatives were smoothed by a 6mm FWHM Gaussian kernel. To account for site
228 and collection time variability across each of the data collections in ABIDE I and II data
229 repositories, site effects were removed using the ComBat function available in python [61]
230 (<https://github.com/Jfortin1/ComBatHarmonization><https://github.com/brentp/combat.py>).
231 This approach has been shown to effectively account for scanner-related variance in multi-
232 site R-fMRI data [61]. For further details see Supplementary Material in the Additional file 1.

233

234 **Discovery group-level analyses**

235 Statistical Z-maps were generated within study-specific functional volume masks including
236 all voxels in MNI space present across all subjects. Main effects of diagnosis and sex along
237 with their interaction were explored by fitting a general linear model (GLM) including
238 diagnosis or/and sex as the regressors of interest respectively, and age and mean framewise
239 displacement (mFD) [54] as nuisance covariates. In primary analyses, we did not include FIQ
240 as a covariate as this is thought to be suboptimal when comparing groups selected from
241 populations carrying intrinsic IQ differences such as autism and NT [56]. Nevertheless, to
242 provide an indication as to whether IQ may affect primary findings, in supplementary
243 analyses FIQ was also included as an additional nuisance regressor. We applied gaussian
244 random field theory correction based on strict voxel-level threshold of $Z > 3.1$ as
245 recommended by [62] and cluster level, $P < 0.01$, given the assessment of five R-fMRI metrics
246 in the same study (i.e., $P_{0.05/5 \text{ R-MRI metrics}} = 0.01$).

247

248 **Functional relevance of sex differences in autism**

249 Post-hoc analyses were conducted to functionally characterize the sex-by-diagnosis
250 interaction result(s). First, to explore the cognitive domains implicated in the cluster(s), we
251 quantified the percentage of its overlap with 12 cognitive ontology maps [63] thresholded at
252 $P = 1e-5$. We labelled these components based on the top five tasks each component recruits
253 [2]. Second, we used the Neurosynth Image Decoder (<http://neurosynth.org/decode/>) [64] to
254 visualize the terms most strongly associated with the significant cluster. After excluding
255 anatomical (e.g., occipital) and redundant terms (synonyms [e.g., saccades and eye
256 movements], plurals [e.g., object and objects] or noun/adjective/adverb equivalents [e.g.,
257 vision and visual]), we visualized the top 27 terms showing correlations with the cluster map
258 between $r = 0.64$ and $r = 0.10$. Third, to explore potential clinical relevance of the significant
259 cluster, we explored brain-behavior relationships as a function of sex within the autism

260 group. Specifically, we ran a GLM examining the interaction between biological sex and
261 available ADOS calibrated severity total score (CSS) [65], as well as social-affect (SA) and
262 restricted, repetitive behavior (RRB) subscores (see Supplementary Material, Additional file
263 1) with the dependent variable(s) being the R-fMRI metric(s) extracted from the cluster
264 mask(s) showing a statistically significant sex-by-diagnosis effect(s).

265

266 **Robustness and Replicability**

267 *Robustness.* We assessed whether patterns of results from the discovery analyses were
268 observable with two other nuisance regression analytical pipelines that include commonly
269 used data preprocessing steps. One pipeline included global signal regression (GSR) [66]
270 which has often been used in autism studies; the other included Independent Component
271 Analysis - Automatic Removal of Motion Artifacts (ICA-AROMA) [67] which is a relatively
272 novel but increasingly utilized approach [46]. Given the scope of the present study, unlike
273 prior work focusing on a wide range of individual preprocessing pipelines [46], we selected
274 GSR and ICA-AROMA as examples of previously validated approaches thought to have
275 impact on motion and physiological noise [45]. To assess robustness of the results observed
276 in discovery analyses, following the voxel-level GLM, we extracted means from the masks
277 corresponding to the same clusters that showed significant effects. These values were
278 averaged across all the voxels in the cluster mask for a given R-fMRI metric. We used them
279 to implement a full regression model including the predictors of interest (sex, diagnosis and
280 their interaction), as well as age and mFD as nuisance regressors and compute effect sizes as
281 partial eta squared (η_p^2) and their confidence intervals using the R-package 'effectsize'. For
282 visualization purposes we also used regressions (including sex, diagnosis, sex-by diagnosis,
283 age, and mFD) to obtain the residuals of these mask-averaged values.

284

285 *Replicability*. Similarly, we assessed whether the group patterns observed in significant
286 clusters identified in discovery analyses, were observed in two relatively large-scale,
287 independent datasets selected from *a)* the EU-AIMS Longitudinal European Autism Project
288 (LEAP), a large multi-site European initiative aimed at identifying biomarkers in autism
289 [51,52] and *b)* the Gender Explorations of Neurogenetics and Development to Advance
290 Autism Research (GENDAAR) dataset collected by the GENDAAR consortium and shared
291 in the National Database for Autism Research [53]. For details on autism and NT inclusion
292 and exclusion criteria for these samples, as well as our selection process, see Supplementary
293 Material in the Additional file 1 [52,53]. The resulting EU-AIMS LEAP (N=309) R-fMRI
294 datasets comprised N=133 males and N=43 females with autism as well as N=85 NT males,
295 and N=48 NT females (see Table S1, Additional file 3); resulting GENDAAR (N=196) R-
296 fMRI datasets comprised N=43 males and N=44 females with autism, as well as N=56 NT
297 males and N=53 NT females (see Table S2, Additional file 3). For a comparison of
298 demographic and clinical information between ABIDE, EU-AIMS LEAP and GENDAAR,
299 see Table S3, S4 and S5 in the Additional file 3. After applying the same ComBat and pre-
300 processing pipeline as used in the ABIDE-based discovery analyses, we extracted each of the
301 R-fMRI metrics means from the Z-maps. As for robustness, we extracted values for each R-
302 fMRI metric from the masks corresponding to the clusters showing statistically significant
303 effects in discovery analyses and computed the corresponding effect size and residuals using
304 the same methods described above.

305

306 For both robustness and replicability discovery findings were determined to be robust and/or
307 replicable (R+) based on two criteria: 1) the group mean difference(s) observed were in the
308 same direction as those identified in the findings from discovery analyses [68] and 2) their
309 effects were not negligible as defined by partial eta squared $\eta_p^2 < 0.01$ [69] (i.e., any small,

310 medium or large effects) which is also consistent with prior work [41]. Finally, for
311 consistency across analyses we also computed cluster level effect size of the discovery
312 findings using the same approach described above.

313

314 **Results**

315

316 **Discovery analyses – ABIDE**

317

318 *Main effect of diagnosis.* Analyses revealed a total of seven clusters showing a significant
319 effect of diagnosis (voxel-level $Z > 3.1$; cluster-level $P < 0.01$, corrected) for three of the five R-
320 fMRI metrics: PCC-iFC (three clusters), VMHC (two clusters) and ReHo (two clusters);
321 Figure 1, Additional file 4. These were mainly evident in anterior and posterior regions of the
322 default network (DN) across at least two or all three R-fMRI metrics. Autism-related hypo-
323 connectivity was present for: a) PCC-iFC, VMHC and ReHo within bilateral paracingulate
324 cortex and frontal pole, b) VMHC and ReHo in the bilateral PCC and precuneus, and c)
325 ReHo in right insula and central operculum (Figure 1, Additional file 4 and Additional file 5).
326 Autism-related hyper-connectivity was only evident for PCC-iFC with left superior lateral
327 occipital cortex, temporal occipital fusiform cortex and occipital fusiform gyrus (see
328 Additional file 4). These results remained essentially unchanged when additionally
329 controlling for FIQ (Additional file 6). Further, to verify that these findings were not driven
330 by particular acquisition site(s), post-hoc analyses computed group means for diagnostic
331 subgroups for the R-fMRI metrics extracted at the cluster-level masks excluding one out of
332 the 13 ABIDE sites at a time. The pattern of results was essentially unchanged (Additional
333 file 7a).

334

335 **Main effect of sex.** Analyses revealed clusters showing statistically significant main sex
336 differences (voxel-level $Z > 3.1$; cluster-level $P < 0.01$, corrected), again for three R-fMRI
337 metrics out of five in a total of 10 clusters: PCC-iFC (five clusters), VMHC (three clusters),
338 and ReHo (two clusters). Findings involved lateral and medial portions of the DN with
339 bilateral PCC and precuneus showing the highest overlap (see Figure 1 and Additional file 4).
340 Specifically, regardless of diagnosis, relative to females, males showed decreased PCC-iFC
341 with paracingulate cortex and frontal pole, right middle frontal gyrus, bilateral superior lateral
342 occipital cortex and bilateral PCC and precuneus. Males also showed decreased VMHC and
343 ReHo localized in PCC and precuneus. Decreased ReHo was also evident in the left angular
344 gyrus and lateral occipital cortex in females relative to males (Figure 1, Additional file 4, and
345 Additional file 5). These results remained essentially unchanged when additionally
346 controlling for FIQ (see Additional file 6). Post-hoc analyses assessing the consistency of
347 these findings across sites, as described above, revealed a similar pattern of results
348 (Additional file 7b).

349

350 **Sex-by-diagnosis interaction.** Statistically corrected voxel-wise analyses (voxel-level $Z > 3.1$;
351 cluster-level $P < 0.01$) revealed one cluster of significant sex-by-diagnosis interaction only for
352 VMHC which was localized in the dorsolateral occipital cortex (Figure 2a and Additional file
353 5). Post-hoc cluster-level group means showed that NT females had higher VMHC than the
354 three other groups, whereas autism females had lower VMHC than the three other groups
355 (Figure 2a). Similar to the main effects, results remained essentially unchanged when
356 additionally controlling for FIQ (Additional file 6) and analyses assessing the consistency of
357 these findings across sites, as described above, showed a similar pattern of results (Additional
358 file 8).

359

360 **Functional relevance of autism-related sex differences**

361 Post-hoc analyses to functionally characterize this VMHC sex-by-diagnosis interaction
362 indicated that the VMHC cluster in superior lateral occipital cortex overlapped with cognitive
363 maps involved in higher-order visual, oculomotor, cognitive flexibility and language-related
364 processes (Figure 3a). Further, as shown in Figure 3b, the most common terms were
365 primarily related to lower-order visual processing and higher-order visual cognition, such as
366 ‘visuospatial’ and ‘spatial attention.’ To explore potential clinical relevance of the VMHC
367 dorsolateral occipital cluster, we explored brain-behavior relationships as a function of sex,
368 within the autism group using three available ADOS scores (calibrated severity total score,
369 and non-calibrated social affect and RRB subscores; see Supplementary Material in
370 Additional file 1). Although not surviving a strict Bonferroni correction for multiple testing
371 (i.e., $0.05/3=0.02$), an interaction effect was observed for ADOS social affect scores. It
372 revealed that more severe social deficits ($F_{(1,311)}=4.44, p=0.036$) were associated with
373 decreased VMHC in females with autism ($r=-0.29$), but not in males with autism ($r=0.03$).
374 Given that ABIDE data were aggregated and released when calibrated social affect scores
375 [70] were not available to assess potential differences in language abilities and age, analyses
376 were repeated after including ADOS module (ADOS Module 2 to 4) as a nuisance covariate:
377 results remained unchanged ($F_{(1,306)}=5.0, p=0.026$) as they did also after removing the few
378 data with the less represented ADOS module 2 (see Supplementary Material, Additional file
379 1). There were no significant findings, with regard to the CSS total score and non-calibrated
380 RRB sub-score (Figure 3c), even at an exploratory statistical threshold of $P<0.05$.

381

382 **Robustness**

383 The same pattern of results identified in discovery analyses was observed in the results pre-
384 processed using GSR or ICA-AROMA, across the three R-fMRI metrics in all the clusters

385 identified in the primary analyses across main effects of diagnosis, sex and their interaction;
386 effect size ranges from small to moderate as in discovery analyses (η_p^2 range=0.01–0.07;
387 Figure 2b, Figure 4, Additional file 5, Additional file 9).

388

389 **Replicability**

390 **Main effects of diagnosis.** Main effects of diagnosis showed higher replicability (i.e., non-
391 negligible η_p^2 effects showing a similar group mean pattern as observed in discovery
392 analyses) in GENDAAR than in EU-AIMS LEAP. Specifically, across the three R-fMRI
393 metrics that showed significant diagnostic differences in discovery analyses, six of the seven
394 clusters (86%) in GENDAAR, were replicated (η_p^2 range=0.01–0.04); only two of those
395 seven (29%) were replicated in EU-AIMS LEAP (η_p^2 range=0.01–0.04). Nevertheless,
396 clusters showing decreased ReHo in ASD vs. NT across the insula and central operculum, as
397 well as in the frontal pole were replicated across all samples (Figure 4, Additional file 5,
398 Additional file 10a).

399

400 **Main effects of sex.** Across all three R-fMRI metrics, the main effects of sex observed in
401 primary discovery analyses was evident in both independent samples, for most clusters in the
402 EU-AIMS LEAP (80%; 8/10) with effects size ranging from small to moderate (η_p^2
403 range=0.01–0.06) and for half of the clusters in GENDAAR (50%; 5/10), albeit with small
404 effects (η_p^2 range=0.01–0.02); Figure 4, Additional file 5, Additional file 10b). Notably, the
405 pattern of typical sex differences localized along the default network midline (i.e., decreased
406 VMHC, ReHo and PCC-iFC) was replicated across both independent samples (Figure 4b).

407

408 **Sex-by-diagnosis interaction effect.** The pattern of autism-related VMHC sex differences
409 observed in discovery analyses in the superior lateral occipital cortex was observed in the

410 EU-AIMS LEAP dataset, ($\eta_p^2=0.01$); (Figure 2c, Figure 4, Additional file 5). In the
411 GENDAAR dataset, while group means in males with autism, NT males and NT females
412 showed a similar direction as in the ABIDE discovery findings, females with autism differed
413 in magnitude and in the direction of group differences, resulting in a negligible effect with
414 $\eta_p^2<0.01$. (Figure 2c).

415

416 **Discussion**

417 We examined autism-related sex differences for intrinsic functional brain organization across
418 multiple R-fMRI metrics in a large discovery sample of males and females with autism
419 relative to age-group matched NT selected from the ABIDE repositories [22,23]. Analyses
420 revealed significant main effects of sex and diagnosis across intrinsic functional connectivity
421 (iFC) of the posterior cingulate cortex, regional homogeneity and voxel-mirrored homotopic
422 connectivity (VMHC) in several cortical regions. Notably, main effects converged along the
423 midline of the default network. In contrast, sex-by-diagnosis interactions were limited to
424 VMHC in the superior lateral occipital cortex. Placed in the context of sex and diagnostic
425 main effects on interhemispheric homotopic connectivity in cortical regions, this result,
426 suggests that atypical interhemispheric interactions are pervasive in autism but reflect a
427 combination of sex-independent (i.e., main effect of diagnosis common across both sexes)
428 and sex-dependent (i.e., sex-by-diagnosis interaction) effects, each specific to different
429 functional cortical system. This sex-by-diagnosis interaction effect was robust to distinct pre-
430 processing strategies as those observed for main effects. Further, despite the lack of *a priori*
431 harmonization for data acquisition among the three samples, this finding was replicable in the
432 larger of the two independent samples (i.e., EU-AIMS LEAP). On the one hand, this, together
433 with largely replicable main effects of sex with variable replicability of main diagnostic
434 effects by sample, suggests that inter-sample replicability of R-fMRI can be feasible in

435 autism when sources of variability in diagnostic groups are accounted for in samples sized
436 properly to address such variability. On the other hand, our results highlight the urgent need
437 to obtain multiple harmonized datasets properly powered to systematically address and
438 understand sources of heterogeneity, including and beyond the role of biological sex.

439

440 **Sex-dependent and independent atypical interhemispheric interactions in autism**

441 VMHC reflects inter-hemispheric homotopic relations. Its strength has been suggested to
442 index coordinated cross-hemispheric processing: *stronger* VMHC index weaker hemispheric
443 specialization and vice versa [33,71]. Several lines of evidence support the notion that the
444 neurobiology of autism is related to atypical hemispheric interactions, including homotopic
445 connectivity and hemispheric lateralization [35,72,81,73–80]. VMHC and functional
446 hemispheric lateralization have also been shown to be sex-differential in NT [33,82,83]. The
447 dorsolateral occipital association cortex identified in our discovery analyses is known to serve
448 hemispherically specialized processes, such as visuospatial coordination [84]. Thus, our
449 findings of NT males' VMHC in dorsolateral lateral occipital cortex being lower than NT
450 females are consistent with the notion of increased hemispheric lateralization in this cortical
451 region in NT males relative to NT females. In our data, females with autism instead showed
452 even lower VMHC than NT males, while males with autism showed slightly higher VMHC
453 than NT males. This pattern is indicative of 'gender-incoherence' [20] as males and females
454 with autism display the opposite pattern expected in NT per their biological sex. Findings of
455 'gender incoherence' have been reported in earlier neuroimaging studies of autism using
456 different modalities [3,85,86]. Among them, several R-fMRI studies explicitly focusing on
457 detecting diagnosis-by-sex interactions (i.e., the regression model included a sex-by-
458 diagnosis interaction term) [3,9] yielded a pattern of results consistent with 'gender
459 incoherence.' In contrast, other studies [10–12] reported a pattern consistent with the

460 ‘extreme male brain theory’ [19] – i.e., a shift towards maleness in both females and males
461 with autism. While the seemingly diverging conclusions of these two sets of studies may be
462 attributed to methodological differences, such as the extent of brain networks explored and
463 the statistical modelling employed, findings from our prior work suggest that both shifts
464 towards either maleness or femaleness co-occur in the intrinsic brain of males with autism, in
465 a network-specific manner [2]. However, such prior work did not include female data. Thus,
466 by not directly assessing sex-by-diagnosis interactions, unlike the present study, results could
467 not point to patterns affecting diagnostic differences between the sexes vs. those that are
468 common to autism across both sexes [4]. This is relevant for efforts focusing on identifying
469 underlying mechanisms. Findings resulting from sex-by-diagnosis interactions may shed light
470 on sex differential mechanisms that go awry in autism and may reflect sex-specific
471 susceptibility mechanisms. On the other hand, diagnostic atypicalities common for both sexes
472 may reflect factors central to the emergence of autism, regardless of whether they overlap
473 with patterns known to be differential between sexes [87]. Interestingly, a recent study based
474 on a sample selected from GENDAAR [14] revealed that the iFC between the nucleus
475 accumbens (selected *a priori*) and a region of the dorsolateral occipital cortex partially
476 overlapping with that identified by our VMHC analyses, was differentially modulated by the
477 aggregate number of oxytocin receptor risk alleles in females with autism vs. NT females and
478 vs. males with autism. Although VMHC was not directly tested in that earlier study [14], its
479 result in dorsolateral occipital cortex is consistent with our observation of atypical sexual
480 differentiation of this visual network region and, together, suggest the need for future whole-
481 brain studies of oxytocin effects in autism.

482

483 Along with the sex-dependent autism patterns, our analyses found statistically significant
484 main effects of diagnosis in inter-hemispheric interactions indexed by VMHC in distinct

485 cortical circuits. These were localized along the midline of the DN (paracingulate/frontal
486 cortex consistently and PCC/precuneus) where main effects of PCC-iFC and ReHo also
487 converged. Our results are consistent with prior reports of atypical intrinsic organization of
488 the DN in autism [10,23,26,88–90]. Together they support the role for a common, sex-
489 independent DN role in autism. This is also supported by a recent autism neurosubtyping
490 study that identified three latent iFC factors, all sharing DN atypicalities along with their
491 neurosubtype-specific patterns [91]. Building on this evidence to disentangle the specific role
492 of each of the factors affecting autism in sex-independent and sex-dependent ways, a
493 necessary next step is to engage in novel large-scale data collection efforts including more
494 female data.

495

496 **Robustness, replicability and sources of variability**

497 The growing awareness of the replication crisis in neuroscience [92–94] motivated our
498 analyses examining robustness and replicability of findings. While a comprehensive and
499 systematic reproducibility assessment is beyond the scope of the study, here we focused on
500 examining whether the findings observed in the discovery analyses were also seen after using
501 different preprocessing pipelines – *robustness* – as well as in fully independent, albeit of
502 convenience samples (i.e., not harmonized *a priori* with each other) – *replicability*. To this
503 end, given the lack of consensus on quantitative metrics of replicability, we opted to use
504 measures of effect size. These are considered complementary to null hypothesis significance
505 testing [95]. In the context of this study, given the use of convenience samples of different
506 sizes, their selection was considered an advantageous and practical means to provide
507 information on the magnitude of group differences in diagnosis and sex, as well as their
508 interaction. Here, we considered findings to be robust and/or replicable for any non-
509 negligible effects (i.e., $\eta_p^2 \geq 0.01$; [69]). We reasoned that given their distributed and

510 heterogenous nature [6], atypicalities in the autism connectome can stem from a combination
511 of differently sized non-negligible effects, as shown for autism in other biological domains
512 such as genetics [96,97].

513

514 With this in mind, across the two preprocessing methods examined here, the patterns of
515 findings were consistent with those observed in discovery analyses across all R-fMRI metrics
516 and effects. These robustness results are consistent with a prior study by He and colleagues
517 [46] reporting that differences in a wider range of pre-processing pipelines have marginal
518 effects on variation in diagnostic group average comparisons. Our study confirms and builds
519 on this earlier report by extending findings of robustness to sex group mean differences and
520 their interactions with diagnosis.

521

522 A more nuanced picture emerged from the inter-sample analyses as replicability varied by
523 sample, across the effects and R-fMRI metrics examined. Specifically, while inter-sample
524 main effects of sex were moderately to largely replicable across R-fMRI metrics on both
525 independent samples (~50 to 80% of the clusters in GENDAAR and EU-AIMS-LEAP,
526 respectively), replicability of diagnostic effects significantly varied by sample (86% to 29%)
527 across R-FMRI metrics. This is at least in part consistent with findings by King et al. [50]
528 also showing that, depending on the R-fMRI feature examined, diagnostic group differences
529 varied across samples. Even in this scenario, King et al. also reported that findings of
530 decreased homotopic connectivity in autism were relatively more stable than other R-fMRI
531 metrics [50]. This observation, combined with replicability of our VMHC sex-by-diagnosis
532 interaction findings in the larger of the two independent samples (EU-AIMS LEAP), suggests
533 that measures of homotopic connectivity may have a relevant biological relevance for autism.

534 It is also possible that given its moderate to high test-retest reliability, VMHC is more
535 suitable in efforts assessing replicability [98,99].

536

537 The striking clinical and biological heterogeneity in autism should be considered as a major
538 contributor to discrepancies in findings of studies focusing on the main effects of diagnostic
539 group means contrasts/interactions [100–103]. Against this background, we interpret our
540 replicability findings on diagnostic effects and, in turn, diagnosis-by-sex interactions. Inter-
541 sample differences may have contributed to the more variable results of replicability on the
542 diagnosis main effects. These may include autism symptom level, age, and IQ, albeit
543 secondary analyses suggested that the examined IQ range did not substantially affect the
544 pattern of discovery results. For example, the EU-AIMS LEAP sample was on-average older,
545 had lower VIQ and most notably, lower symptom severity across all subscales of the ADOS
546 and ADI-R than the ABIDE sample. On the other hand, the GENDAAR sample (which has
547 greater number of replicable diagnostic mean group patterns) did not differ from ABIDE in
548 these variables, except for mean age. Furthermore, a fact that is often neglected, is that the
549 NT groups may also present with considerable sample heterogeneity between studies
550 [100,104]. For instance, our NT controls in the EU-AIMS LEAP sample had lower VIQ than
551 both ABIDE and GENDAAR NT controls. This has potentially influenced the low
552 replicability of diagnosis main effects in EU-AIMS LEAP.

553

554 In contrast, sex-by-diagnosis effect on VMHC in the dorsolateral occipital cortex was
555 replicable in the larger samples, the EU-AIMS LEAP, but not in GENDAAR. Small samples
556 introduce larger epistemic variability (i.e., greater variation related to known and unknown
557 confounds) [105]. Increasing the number of subjects/data allows mitigating epistemic
558 variability and, thus, capturing the underlying variability of interest. Thus, although the rate

559 of EU-AIMS LEAP replicability for the main effect of diagnosis was limited, accounting for
560 biological sex, a known key source variability in autism, may have contributed to a replicable
561 sex-by-diagnosis pattern in this larger sample. In line with sample size concerns, using four
562 datasets sized between 36 and 44 individuals selected from the ABIDE repository, He et al.
563 [46] found low similarity rates of diagnostic group-level differences on the strength of iFC
564 edges in contrast with the largely similar pattern of results across pipelines. Of note, unlike
565 prior efforts [46,50], we controlled for site effects within each of the samples (i.e., ABIDE,
566 GENDAAR and EU-AIMS LEAP), using ComBat. Future large-scale harmonized data
567 collections are needed to control and assess the impact of inter-sample variability. Taken
568 together, these findings highlight that sample differences can impact replicability.

569

570 Beyond clinical and biological sources of variation, samples may differ in MRI acquisition
571 methods, as well as in approaches used to mitigate head motion during data collection and its
572 impact on findings [106]. Adequately controlling for head motion remains a key challenge for
573 future studies assessing inter-sample replicability. For the present study, we excluded
574 individuals with high motion, retained relatively large samples with group average low
575 motion (mean \pm standard deviation range mFD range=0.09-0.16 \pm 0.06-0.10 mm), as well as
576 included mFD at the second-level analyses as a nuisance covariate. Overall, the extent to
577 which each sample-related factor affects replicability needs to be systematically examined in
578 future well-powered studies. Only this type of studies will allow for emerging subtyping
579 approaches to dissect heterogeneity by brain imaging features using a range of data-driven
580 methods [107,108], including normative modelling [109–111].

581

582 Inter-sample differences and methodological differences, beyond nuisance regression, may
583 have contributed to some differences in findings between the present and earlier studies,

584 conducted with independent or partially overlapping samples [9,23,41]. For example, Alaerts
585 et al. [9] also examined sex-by-diagnosis interaction in PCC-iFC in a dataset selected from
586 ABIDE I only. Although their pattern of results was consistent with the ‘gender incoherence’
587 model, the resulting circuit(s) did not involve the dorsolateral occipital cortex as identified
588 with VMHC in the present study. Along with differences in samples selected from the same
589 data repositories, other ABIDE-based methodological choices that may affect results. For
590 example, prior studies differed with the present one in the inclusion of sex-by-diagnosis
591 interaction [15], the extent of the whole-brain voxel-based analyses [13,14], or the statistical
592 threshold utilized [23]. Nevertheless, it is remarkable that even in light of these differences,
593 consistent results have emerged including the role of the ‘gender incoherence’ model for
594 females with autism, atypical inter-hemispheric interactions in autism, and sex-dependent and
595 sex-independent atypical intrinsic brain function across distinct functional networks.

596

597 **Limitations**

598 Along with the inter-sample differences resulting from the lack of sufficiently available
599 harmonized inter-site replication datasets in the field, other limitations of this study should be
600 addressed in future efforts. One regards the lack of measures differentiating the effects of sex
601 vs. that of gender so as to disentangle their relative roles (e.g., gender-identity and gender-
602 expression) in the intrinsic brain properties [112]. Further, in-depth cognitive measures to
603 directly characterize the role of VMHC findings were not available. Additional behavioral
604 measures are needed to establish whether our result in VMHC of the dorsolateral occipital
605 cortex mainly applies to low-level (bottom-up) visual processing differences or higher-level
606 (top-down) attentional/controlled processes in males and females with autism. As a
607 neurodevelopmental disorder, autism shows striking inter-individual differences in clinical
608 and developmental trajectories, as well as outcomes. Thus, age may influence symptom

609 presentation [113,114] and neurobiology [11,110,115]. Despite the considerable size of the
610 samples available for this study, it is still difficult to sufficiently cover a broad age range
611 across both males and females and diagnostic groups across contributing sites and to evaluate
612 age effects appropriately. Even larger cross-sectional samples are needed to derive
613 meaningful age-related information that ultimately require confirmation in longitudinal study
614 designs. Such longitudinal studies would allow to examine the potential impact of puberty
615 and related surge of sex steroids reported in NT boys and girls [116], in autism specifically.
616 Here, we have no direct measure of puberty other than age, but future studies should aim to
617 include such measures. Further, the value of a large-scale and publicly available multi-site
618 resource such as ABIDE also comes with unavoidable differences in site differences which
619 must be considered in data selection, analyses and interpretation of results. Although residual
620 site-related effects may have remained in findings even after using the novel Bayesian
621 approach for correcting for batch-effects, replicability in independent samples suggest that
622 effects are not simply driven by site variability. These results are consistent with earlier
623 reports of reproducible imaging biomarkers even when accounting for inter-site differences in
624 multisite datasets such as the ABIDE I repository [47]. Finally, despite the advantages of
625 effect sizes over p-value when comparing independently collected samples of different sizes
626 and potentially different variances, it is important to acknowledge they are not without
627 limitations [117] and should be interpreted with caution. Similar to p-values, they are
628 dependent on sample sizes and have the equivalent risks of p-hacking. Finally, effect sizes
629 standard errors can be large - a concern we addressed through including of confidence
630 intervals.

631

632 **Conclusions**

633 The present work revealed sex differences in the intrinsic brain of autism, particularly in
634 dorsolateral occipital interhemispheric interactions, which were robust to pre-processing
635 pipeline decisions and replicable in the larger of the two independent samples. While
636 differences in nuisance regression pipelines have little influence on the consistency of
637 findings, sample heterogeneity represents a challenge for replicability of findings. Lateralized
638 cognitive functions and cross-hemispheric interactions should be further explored in relation
639 to sex differences in autism while addressing this challenge with future harmonized data
640 acquisition efforts with even larger samples.

641

642 **List of abbreviations**

643 ABIDE: Autism Brain Imaging Data Exchange

644 ASD: Autism spectrum disorder

645 DC: Degree centrality

646 DN: Default network

647 EPI: Echo-planar image

648 LEAP: Longitudinal European Autism Project

649 fALFF: Fractional amplitude of low frequency fluctuations

650 FIQ: Full-scale IQ

651 GENDAAR: Gender Explorations of Neurogenetics and Development to Advance Autism
652 Research

653 GSR: Global Signal Regression

654 ICA-AROMA: Independent Component Analysis - Automatic Removal of Motion Artifacts

655 iFC: Intrinsic functional connectivity

656 mFD: Mean framewise displacement

657 NDAR: National Database for Autism Research

658 NT: Neurotypical

659 PCC: Posterior cingulate cortex

660 ReHo: Regional Homogeneity

661 R-fMRI: Resting-state functional magnetic resonance imaging

662 VMHC: Voxel-mirrored homotopic connectivity

663

664 **Ethics approval and consent to participate**

665 ABIDE I and II: All contributions were based on studies approved by the local Institutional
666 Review Boards, and data were fully anonymized (removing all 18 HIPAA (Health Insurance
667 Portability and Accountability)-protected health information identifiers, and face information
668 from structural images).

669 EU-AIMS LEAP: Ethical approval was obtained through ethics committees at each site.

670 GENDAAR: Informed assent and consent were obtained from all participants and their legal
671 guardians, and the experimental protocol was approved by the Institutional Review Board at
672 each participating site.

673

674 **Consent for publication**

675 Not applicable

676

677 **Availability of data and materials**

678 *ABIDE I and II*: Data are freely accessible in the publicly available Autism Brain Imaging
679 Data Exchange repository (http://fcon_1000.projects.nitrc.org/indi/abide).

680 *EU-AIMS LEAP*: Data are currently only available for sites involved in data collection, but
681 data (starting with the first wave) will be made available on request.

682 *GENDAAR*: Anonymized data are publicly available through the National Database for
683 Autism Research (NDAR) and access can be requested via <https://nda.nih.gov/about.html>.

684

685 **Competing interests**

686 ADM receives royalties from the publication of the Italian version of the Social
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689 and Servier. He is not an employee of any of these companies and not a stock shareholder of
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701

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722

723 **Authors' contributions (in alphabetical order by contribution)**

724 ADM and DLF have contributed to the study's conception; ADM, DLF, JOAF, M-CL, MPM
725 have contributed to distinct aspects of the study design and interpretation of all findings; DLF
726 and JOAF conducted the analyses; SG has provided support in all data analyses requiring
727 CPAC; ADM, DLF, JOAF have generated figures and tables; ADM and DLF drafted the
728 manuscript; ADM, DLF, JOAF, M-CL, and MPM have revised and edited multiple versions
729 of the manuscript; CFB, MM, MO, have organized the EU-AIMS LEAP data and edited
730 latest manuscript versions and its revisions; CFB, CE, CM, DGMM, EL, FDA, GD, JKB, JT,
731 SB-C, TC, TB, SD have contributed to the coordination, data acquisition and coordination of

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734

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1057 **Figure Captions**

1058 **Fig 1. Overlap across R-fMRI metrics for main effects of diagnosis and sex**

1059 Upper panel: the surface inflated maps depict the extent of overlap across clusters showing
1060 significant main effects of diagnosis (left) and sex (right) across any of three resting state
1061 fMRI (R-fMRI) metrics showing statistically significant effects ($Z > 3.1$, $P < 0.01$). Purple
1062 clusters represent areas of significant group differences emerging for only one of any of the
1063 three R-fMRI measures, orange and yellow clusters indicate measures with overlap among 2
1064 and 3 R-fMRI measures (see Additional file 5 for statistical maps of main effects for each R-
1065 fMRI metric). Cluster masks are overlaid on inflated brain maps generated by BrainNet
1066 Viewer. Lower panel: for each of the yellow and orange clusters in panel A, the table lists the
1067 cluster's anatomical label based on the Harvard Oxford atlas, the specific R-fMRI metrics
1068 involved, and the group difference direction (ASD<NT or M<F in blue, ASD>NT or M>F in
1069 red). Abbreviations: L= Left hemisphere; R= Right hemisphere, PCG/FP=Paracingulate
1070 cortex/frontal pole, ACC=Anterior cingulate cortex, PCC/Prec=Posterior cingulate
1071 cortex/precuneus, ASD=autism spectrum disorder, NT=neurotypical, M=Males, F=Females).

1072 **Fig 2. Sex-by-diagnosis interaction effect, its robustness and replicability**

1073 a) On the right, surface maps show the cluster with a significant ($Z > 3.1$, $P < 0.01$) sex-by-
1074 diagnosis interaction for voxel-mirrored homotopic connectivity (VMHC) resulting from
1075 discovery analyses in the ABIDE sample using the component-based noise reduction
1076 (CompCor) pipeline. The statistical Z maps are overlaid on inflated brain maps generated by
1077 BrainNet Viewer. b) The upper panels show the pattern of VMHC group means in males and
1078 females by each diagnostic group (ASD and NT) extracted from the same cluster in data pre-
1079 processed following two alternative denoising pipelines, Global Signal Regression (GSR,

1080 left) and Independent Component Analysis – Automatic Removal of Motion Artifacts (ICA-
1081 AROMA, right). Results show a pattern similar to the those observed in discovery analyses
1082 with small to moderate effect sizes (η_p^2 range=0.01–0.07). c) The lower graph shows
1083 replicability in two independent samples: the Gender Explorations of Neurogenetics and
1084 Development to Advance Autism Research (GENDAAR) and the EU-AIMS Longitudinal
1085 European Autism Project (LEAP). The pattern of results was replicable in the EU-AIMS
1086 LEAP (N=309) with a small effect size ($\eta_p^2 = 0.01$) and had a negligible effect size in
1087 GENDAAR sample (N=196; $\eta_p^2 < 0.01$). For all graphs VMHC data are shown as residuals
1088 obtained after regressing out mean framewise displacement and age effects. Abbreviations:
1089 L=left, R=right, A=anterior, P=posterior.

1090 **Fig 3. Functional relevance of sex-by-diagnosis interaction in VMHC**

1091 a) The radar plot shows the percentage (0-80%) of overlap between the voxels in the
1092 dorsolateral occipital cluster showing a significant VMHC sex-by-diagnosis interaction in
1093 discovery analyses and the 12 Yeo cognitive ontology probability maps [63] (probability
1094 threshold at $P = 1e-5$) for cognitive components C1-C12. As in Floris et al. [2], we labelled
1095 each component based on the top five tasks reported to be most likely recruited by a given
1096 component. b) Word cloud based on the top 27 terms showing correlations between $r=0.64$ to
1097 $r=0.10$ associated with the same VMHC cluster based on the Neurosynth Image Decoder. c)
1098 Sex-differential association between each individual's VMHC at the cluster showing a
1099 significant sex-by-diagnosis interaction in primary analyses and available ADOS social-affect
1100 uncalibrated sub-scores in males and females with ASD. VMHC data are shown as residuals
1101 obtained after regressing out mean framewise displacement and age effects. While males
1102 showed no significant associations at corrected and uncorrected thresholds, females with

1103 lower dorsolateral occipital VMHC showed more severe social-affect symptoms at
1104 uncorrected statistical threshold ($F_{(1,311)}=4.44, p=0.036$).

1105 **Fig 4. Robustness and replicability summary**

1106 a) The histogram summarizes the percentage of clusters showing a robust and replicable
1107 pattern of results as that observed in discovery analyses in the ABIDE sample for main
1108 effects of diagnosis (Dx; green; N=7 clusters), sex (yellow; N=10 clusters) and their
1109 interaction (blue; N=1 cluster) across three R-fMRI metrics. All findings were robust to
1110 different preprocessing pipelines. Across R-fMRI metrics, main sex effects were moderately
1111 (50%) to largely (80%) replicable across independent samples: Gender Explorations of
1112 Neurogenetics and Development to Advance Autism Research (GENDAAR) and the EU-
1113 AIMS Longitudinal European Autism Project (LEAP), respectively. Replicability for main
1114 effects of diagnosis was largely replicable in GENDAAR (86%) and minimally replicable in
1115 EU-AIMS LEAP (29%). The VMHC pattern observed for sex-by-diagnosis interaction in
1116 primary discovery analyses was replicated in EU-AIMS LEAP only. b) Surface conjunction
1117 maps show the clusters replicated in GENDAAR only (G, purple), EU-AIMS LEAP only (E,
1118 blue) and in both samples (G&E, red) for each effect separately. Cluster masks are overlaid
1119 on inflated brain maps generated by BrainNet Viewer.

1120 **Additional files**

1121 **Additional file 1**

1122 **Title:** Supplementary Material

1123 **File format:** docx

1124 **Description:** Supplementary Methods

1125 **Additional file 2**

1126 **Title:** Selection flowchart for the ABIDE sample

1127 **File format:** tif

1128 **Description:** The flowchart illustrates the selection process resulting in the final ABIDE I

1129 and II combined sample of 1019 subjects. At each flowchart step, the numbers outside the

1130 parentheses represent the total number of datasets across both ABIDE I and ABIDE II; in

1131 parenthesis are the number of datasets derived from ABIDE I (the resulting difference

1132 between these numbers would be the numbers for dataset stemming from ABIDE II). The

1133 rationale for each selection step is detailed in Supplementary Material. Abbreviations:

1134 ASD=autism spectrum disorder, NT=neurotypical, A I=ABIDE I.

1135 **Additional file 3**

1136 **Title:** Supplementary Tables

1137 **File format:** docx

1138 **Description:** Characterization of EU-AIMS LEAP and GENDAAR samples, comparison

1139 between samples and summary table of main effects.

1140 **Additional file 4**

1141 **Title:** Main effects of diagnosis and sex in the ABIDE discovery sample

1142 **File format:** tif

1143 **Description:** Significant results ($Z > 3.1$, $P < 0.01$, corrected) of voxel-wise discovery analyses

1144 conducted in the ABIDE dataset for main effects (ME) of diagnosis (left) and sex (right) for

1145 seed-based intrinsic functional connectivity of the posterior cingulate cortex- (PCC), voxel

1146 mirror homotopic connectivity (VMHC), and Regional Homogeneity (ReHo). Significant

1147 clusters are overlaid on inflated brain maps generated by BrainNet Viewer. No significant

1148 effects were detected for degree centrality or fractional amplitude of low frequency

1149 fluctuations. ME Diagnosis: PCC-iFC: bilateral paracingulate cortex and frontal pole

1150 (PCG/FP), superior lateral occipital cortex (sLOC), temporal occipital fusiform cortex and
1151 occipital fusiform gyrus (TOFC/OFC); VMHC: bilateral posterior cingulate gyrus and
1152 precuneus (PCC/Prec), PCG/FP; ReHo: PCG/FP, central operculum and insula (CO/Ins). ME
1153 Sex: PCC-iFC: bilateral sLOC, middle frontal gyrus (MFG), bilateral PCC/Prec, bilateral
1154 PCG/FP; VMHC: bilateral PCC/Prec, bilateral anterior cingulate cortex (ACC); ReHo:
1155 bilateral PCC, angular gyrus and lateral occipital cortex (AnG/LOC). See Additional file 3:
1156 Table S6 for details on each cluster sizes. *Due to processing failure of two subjects for
1157 VMHC, the sample size comprised 1017 subjects instead of 1019.

1158 **Additional file 5**

1159 **Title:** Characteristics of the clusters with significant effect and effect size across analyses

1160 **File format:** pdf

1161 **Description:** Additional file 5 summarizes cluster' anatomical labels, center of gravity
1162 coordinates and statistics derived from discovery analyses, as well as effect size and their
1163 confidence of interval for each of these clusters across all analyses In green are the effect
1164 found to be robust/replicable based on our criteria (*i.e.*, the group mean difference(s) in the
1165 same direction as those identified in discovery analyses and effect sizes not negligible as
1166 defined by partial eta squared $\eta_p^2 < 0.01$) and in yellow those that did not. PCC-iFC: posterior
1167 cingulate cortex intrinsic functional connectivity, VMHC: voxel-mirrored homotopic
1168 connectivity, ReHo: regional homogeneity, TOFC/OFG: temporal occipital fusiform
1169 cortex/occipital fusiform gyrus, sLOC: superior lateral occipital cortex, PCG/FP:
1170 paracingulate cortex/frontal pole, PCC/Prec: posterior cingulate gyrus/precuneus, CO/Ins:
1171 central operculum/insula, MFG: middle frontal gyrus, ACC: anterior cingulate cortex, SMG:
1172 supramarginal gyrus, AnG/LOC: angular gyrus/lateral occipital cortex. *Due to processing

1173 failure of two subjects for VMHC, the sample size comprised 1017 subjects instead of 1019
1174 for ABIDE and 307 instead of 309 for EU-AIMS.

1175 **Additional file 6**

1176 **Title:** Main effects of diagnosis and sex in the ABIDE discovery sample when additionally
1177 covarying for FIQ

1178 **File format:** tif

1179 **Description:** Including full-scale IQ (FIQ) as a nuisance regressor in addition to age and
1180 mean FD in the voxel-wise model yielded significant ($Z > 3.1$, $P < 0.01$, corrected) findings
1181 highly similar to those observed in discovery analyses across main effects (ME) of diagnosis
1182 (left) and sex (right), sex and their interaction. As in the discovery approach, analyses were
1183 conducted for seed-based intrinsic functional connectivity of the posterior cingulate cortex-
1184 (iFC-PCC), voxel mirror homotopic connectivity (VMHC), and Regional Homogeneity
1185 (ReHo). Significant clusters are overlaid on inflated brain maps generated by BrainNet
1186 Viewer. No significant effects were detected for degree centrality or fractional amplitude of
1187 low frequency fluctuations. ME Diagnosis: PCC-iFC: bilateral paracingulate cortex and
1188 frontal pole (PCG/FP), superior lateral occipital cortex (sLOC), temporal occipital fusiform
1189 cortex and occipital fusiform gyrus (TOFC/OFC); VMHC: bilateral posterior cingulate gyrus
1190 and precuneus (PCC/Prec), PCG/FP; ReHo: PCG/FP, central operculum and insula (CO/Ins).
1191 ME Sex: PCC-iFC: bilateral sLOC, middle frontal gyrus (MFG), bilateral PCC/Prec, bilateral
1192 PCG/FP; VMHC: bilateral PCC/Prec, bilateral anterior cingulate cortex (ACC); ReHo:
1193 bilateral PCC, angular gyrus and lateral occipital cortex (AnG/LOC). Sex-by-diagnosis:
1194 VMHC: bilateral dorsolateral occipital cortex. *Due to processing failure of two subjects for
1195 VMHC, the sample size comprised 1017 subjects instead of 1019.

1196 **Additional file 7**

1197 **Title:** Stability of main effects

1198 **File format:** tif

1199 **Description:** Inter-site stability was assessed after extracting group means at masks

1200 corresponding to the clusters showing significant main effects of diagnosis (7a) and sex (7b)
1201 in the discovery analyses and then deriving the group mean when leaving one acquisition site
1202 out at the time. The pattern of results was unchanged. Different ABIDE sites are color-coded
1203 on legend on the side. Due to processing failure of two subjects for VMHC, the sample size
1204 comprised 1017 subjects. Abbreviations: ASD=autism spectrum disorder, NT=neurotypical,
1205 PCC-iFC=posterior cingulate cortex intrinsic functional connectivity (x=0, y=-53, z=26),
1206 VMHC=voxel-mirrored homotopic connectivity, ReHo=regional homogeneity, L=left,
1207 R=right. Different sites in ABIDE are color-coded on the top left. Data are shown as residuals
1208 obtained after regressing out mean framewise displacement and age effects.

1209 **Additional file 8**

1210 **Title:** Stability of sex-by-diagnosis interaction effect

1211 **File format:** tif

1212 **Description:** Inter-site stability of the sex-by-diagnosis interaction pattern was assessed after
1213 extracting group means at the mask corresponding to the clusters showing a significant
1214 interaction in the discovery analyses and then deriving the group mean when leaving one
1215 acquisition site out at the time. The pattern of results was unchanged. Different sites in
1216 ABIDE are color-coded in the legend on the right. Due to processing failure of two subjects
1217 for VMHC, the sample size comprised 1017 subjects. Abbreviations: ASD=autism spectrum
1218 disorder, NT=neurotypical, PCC-iFC=posterior cingulate cortex intrinsic functional
1219 connectivity (x=0, y=-53, z=26), VMHC=voxel-mirrored homotopic connectivity,

1220 ReHo=regional homogeneity, L=left, R=right. Different sites in ABIDE are color-coded on
1221 the top left.

1222 **Additional file 9**

1223 **Title:** Robustness to nuisance corrections of main effects of diagnosis and sex

1224 **File format:** tif

1225 **Description:** Cluster-level replication of the results emerging from the voxel-wise discovery
1226 analyses in the ABIDE dataset preprocessed using CompCor for the statistically significant
1227 main effects of diagnosis (9a) and sex (9b) after preprocessing with GSR and with ICA-
1228 AROMA. The second column on the left shows the clusters ($Z > 3.1$, $P < 0.01$, corrected) with
1229 significant diagnostic and sex effects for posterior cingulate cortex intrinsic functional
1230 connectivity (PCC-iFC), voxel-mirrored homotopic connectivity (VMHC), and regional
1231 homogeneity (ReHo). Results are overlaid on inflated brain maps generated by BrainNet
1232 Viewer. The bar plots represent the residual means resulting from regressing out diagnosis or
1233 sex effects depending on the desired main effect from the cluster means. 9a) The ABIDE
1234 GSR and ABIDE ICA-AROMA columns illustrate, for each of these R-fMRI indices, the
1235 diagnostic group mean pattern across clusters with a diagnostic effect size of $\eta_p^2 \geq 0.01$. Color
1236 codes: Red=ASD; Green=NT. PCC-iFC: bilateral paracingulate cortex and frontal pole
1237 (PCG/FP), superior lateral occipital cortex (sLOC), temporal occipital fusiform cortex and
1238 occipital fusiform gyrus (TOFC/OFC); VMHC: bilateral posterior cingulate gyrus and
1239 precuneus (PCC/Prec), PCG/FP; ReHo: PCG/FP, central operculum and insula (CO/Ins).
1240 ABIDE GSR: 7 out of 7 main effects of diagnosis replicated (100%). ABIDE ICA-AROMA:
1241 7 out of 7 main effects of diagnosis replicated (100%) 9b) The ABIDE GSR and ABIDE
1242 ICA-AROMA columns illustrate, for each of these R-fMRI indices, the sex group mean
1243 pattern across clusters with an effect size of $\eta_p^2 \geq 0.01$. Color codes: Blue=males;
1244 Pink=females. PCC-iFC: bilateral sLOC, middle frontal gyrus (MFG), bilateral PCC/Prec,

1245 bilateral PCG/FP; VMHC: bilateral PCC/Prec, bilateral anterior cingulate cortex (ACC);
1246 ReHo: bilateral PCC, angular gyrus and lateral occipital cortex (AnG/LOC). ABIDE GSR: 10
1247 out of 10 main effects of diagnosis replicated (100%). ABIDE ICA-AROMA: 10 out of 10
1248 main effects of diagnosis replicated (100%). Due to processing failure of two subjects for
1249 VMHC, the sample size comprised 1017 subjects instead of 1019. VMHC data are shown as
1250 residuals obtained after regressing out mean framewise displacement and age effects.
1251 Abbreviations: ASD=autism spectrum disorder, NT=neurotypical, M=males, F=females,
1252 CompCor=component base noise reduction, GSR=Global Signal Regression, ICA-
1253 AROMA=independent component analysis – automatic removal of motion artifacts, PCC-
1254 iFC=posterior cingulate cortex intrinsic functional connectivity (x=0, y=-53, z=26),
1255 VMHC=voxel-mirrored homotopic connectivity, ReHo=regional homogeneity, L=left,
1256 R=right, R+=replication based on same direction of results and $\eta_p^2 \geq 0.01$, R-=non-replication
1257 of results (displayed in gray plots).

1258 **Additional file 10**

1259 **Title:** Replicability of main effects of diagnosis and sex

1260 **File format:** tif

1261 **Description:** Cluster-level replication of the results emerging from the voxel-wise analyses
1262 in the ABIDE dataset for main effects of diagnosis (10a) and sex (10b) in the Gender
1263 Explorations of Neurogenetics and Development to Advance Autism Research (GENDAAR)
1264 and the EU-AIMS Longitudinal European Autism Project (LEAP) samples. The ABIDE
1265 column on the left shows the clusters ($Z > 3.1$, $P < 0.01$, corrected) with significant diagnostic
1266 and sex effects for posterior cingulate cortex intrinsic functional connectivity (PCC-iFC),
1267 voxel-mirrored homotopic connectivity (VMHC), and regional homogeneity (ReHo). No
1268 significant effects were detected for degree centrality and fractional amplitude of low
1269 frequency fluctuations. Results are overlaid on inflated brain maps generated by BrainNet

1270 Viewer. The bar plots represent the residual means resulting from the linear Gaussian
1271 regression for each group. 10a) The GENDAAR and EU-AIMS LEAP columns illustrate, for
1272 each R-fMRI index, the diagnostic group mean pattern across clusters with a diagnostic effect
1273 size of $\eta_p^2 \geq 0.01$. Color codes: Red=ASD; Green=NT. PCC-iFC: bilateral paracingulate
1274 cortex and frontal pole (PCG/FP), superior lateral occipital cortex (sLOC), temporal occipital
1275 fusiform cortex and occipital fusiform gyrus (TOFC/OFC); VMHC: bilateral posterior
1276 cingulate gyrus and precuneus (PCC/Prec), PCG/FP; ReHo: PCG/FP, central operculum and
1277 insula (CO/Ins). GENDAAR: 6 out of 7 main effects of diagnosis replicated (86%); EU-
1278 AIMS LEAP: 2 out of 7 main effects of diagnosis replicated (29%). 10b) The GENDAAR
1279 and EU-AIMS LEAP columns illustrate, for each of these R-fMRI index, the sex group mean
1280 pattern across clusters with an effect size of $\eta_p^2 \geq 0.01$. Color codes: Blue=males,
1281 Pink=females. PCC-iFC: bilateral sLOC, middle frontal gyrus (MFG), bilateral PCC/Prec,
1282 bilateral PCG/FP; VMHC: bilateral PCC/Prec, bilateral anterior cingulate cortex (ACC);
1283 ReHo: bilateral PCC, angular gyrus and lateral occipital cortex (AnG/LOC). GENDAAR: 5
1284 out of 10 main effects of sex replicated (50%); EU-AIMS LEAP: 8 out of 10 main effects of
1285 sex replicated (80%). *Due to processing failure of two subjects for VMHC, the sample size
1286 comprised 1017 subjects instead of 1019. Abbreviations: ASD=autism spectrum disorder,
1287 NT=neurotypical, M=males, F=females, PCC-iFC=posterior cingulate cortex intrinsic
1288 functional connectivity (x=0, y=-53, z=26), VMHC=voxel-mirrored homotopic connectivity,
1289 ReHo=regional homogeneity, L=left, R=right, R+=replication based on same direction of
1290 results and $\eta_p^2 \geq 0.01$, R-=non-replication of results.

1291 **Table 1. Characterization of sample merged across ABIDE I and II**

ABIDE I + II	Sites ^a	ASD M (N=362)	ASD F (N=82)	NT M (N=409)	NT F (N=166)		
	N	Mean (SD) [Range]	Mean (SD) [Range]	Mean (SD) [Range]	Mean (SD) [Range]	Statistics	Post-hoc
Age	13	11.8 (2.6) [7-17.9]	11.7 (2.7) [7-18]	11.8 (2.6) [7.1-18.2]	11.4 (2.3) [7.8-17.4]	$F_{(3)}=1.18$, $p=0.32$	
Full-Scale IQ^b	13	106 (16.6) [72-148]	104 (16.3) [73-147]	112 (12.7) [73-148]	114 (12.7) [80-144]	$F_{(3)}=19.24$, $p<0.001$	(ASD M=ASD F) < (NT M=NT F)
Verbal IQ^c	12	107 (17.9) [57-180]	105 (17.3) [62-145]	114 (13.5) [73-147]	114 (14.4) [83-146]	$F_{(3)}=16.78$, $p<0.001$	(ASD M=ASD F) < (NT M=NT F)
Performance IQ^d	12	106 (17.0) [59-157]	104 (17.1) [67-148]	109 (14.2) [62-147]	109 (13.2) [79-145]	$F_{(3)}=3.1$, $p=0.03$	(ASD M=ASD F) < (NT M=NT F)
Mean FD	13	0.11 (0.07) [0.02-0.39]	0.13 (0.09) [0.02-0.39]	0.09 (0.06) [0.02-0.39]	0.09 (0.06) [0.02-0.38]	$H_{(3)}=29.6$, $p<0.001$	(ASD M=ASD F) < (NT M=NT F)
ADI-R							
Social^e	11	19.7 (5.2) [4-30]	19.6 (5.5) [7-30]	-	-	$t_{(93)}=0.14$, $p=0.89$	
Communication^f	11	15.6 (4.5) [2-25]	15.2 (5.0) [4-24]	-	-	$t_{(92)}=0.61$, $p=0.54$	
RRB^f	11	6.0 (2.4) [0-13]	5.8 (2.5) [0-12]	-	-	$t_{(96)}=0.51$, $p=0.61$	
ADOS-2							
Social-Affect^g	11	9.1 (3.7) [1-20]	8.7 (3.2) [4-18]	-	-	$t_{(87)}=0.97$, $p=0.33$	
RRB^h	11	3.2 (1.8) [0-8]	2.8 (1.5) [0-5]	-	-	$t_{(93)}=1.79$, $p=0.08$	
CSS totalⁱ	11	6.9 (2.1) [1-10]	6.8 (1.8) [2-10]	-	-	$t_{(93)}=0.11$, $p=0.32$	
		N	N			Statistics	Post-hoc

Comorbidity	5	99 ^j	25 ^h	-	-	$\chi^2_{(1)}=0.2,$ $p=0.66$
Psychoactive	10	112	26	-	-	$\chi^2_{(1)}<0.01,$
Meds						$p=0.99$

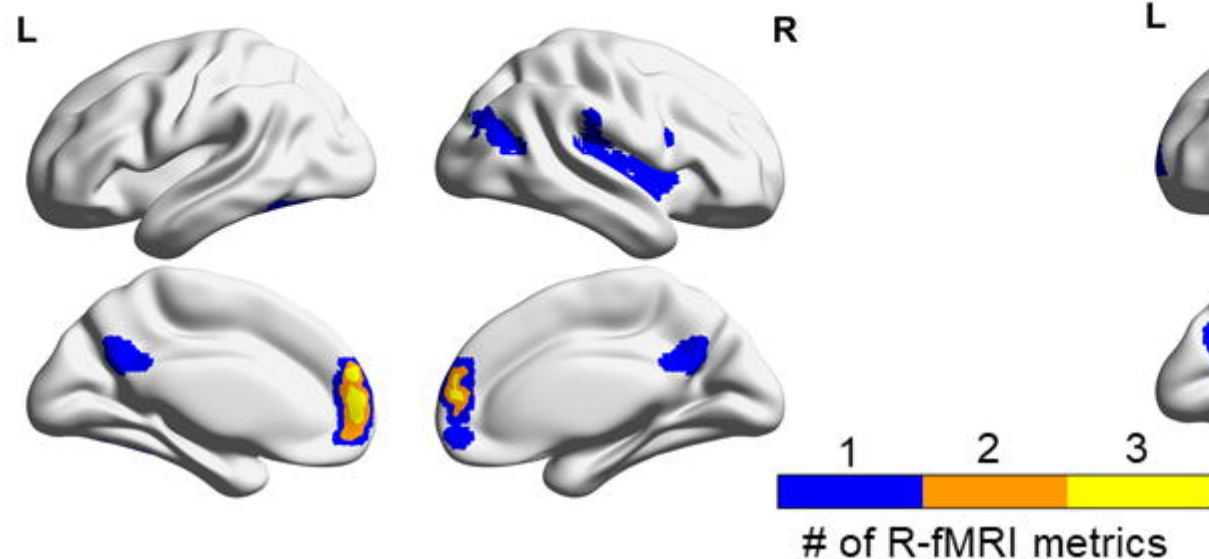
1292

1293 **Abbreviations:** *ABIDE*: Autism Brain Imaging data exchange; *ADI-R* = Autism Diagnostic Interview-Revised;
1294 *ADOS-2* = Autism Diagnostic Observation Schedule-2; *ASD* = Autism Spectrum Disorder; *CSS* = Calibrated
1295 Severity Score; *F* = females; *IQ* = intellectual quotient; *M* = males; *Mean FD* = mean framewise displacement [54];
1296 *NT* = neurotypical; *RRB*= restricted repetitive behaviors. **Notes:** ^aABIDE I data collections: KKI, Leuven_2, NYU,
1297 OHSU, Pitt, SDSU, Stanford, UCLA_1, UM_1, and Yale. ABIDE II data collections: ABIDEII-GU_1, ABIDEII-
1298 KKI_1, ABIDEII-KKI_2, ABIDEII-NYU_1, ABIDEII-OHSU_1, ABIDEII-SDSU_1, ABIDEII-UCD_1 and
1299 ABIDEII-UCLA_1. KKI and ABIDEII-KKI_1, NYU and ABIDEII-NYU_1, SDSU and ABIDEII-SDSU_1, OHSU
1300 and ABIDEII-OHSU_1 and UCLA_1 and ABIDEII-UCLA_1 were merged into one site across ABIDE I and
1301 ABIDE II collections. ^bFIQ was available for 362 males with ASD (2 missing from UM_1, ABIDEII-SDSU_1), 81
1302 females with ASD (1 missing from ABIDEII-GU_1), 407 neurotypical males (NT M) (3 missing from ABIDEII-
1303 GU_1 (N=1), UM_1 (N=2)) and all 166 NT females (NT F). ^cVIQ was available for 315 males with ASD (47
1304 missing; KKI (N=14), ABIDEII-OHSU_1 (N=22), OHSU (N=9), ABIDEII-SDSU_1 (N=1); ABIDEII-UCLA_1
1305 (N=1), 70 females with ASD (12 missing, ABIDEII-GU_1 (N=1), KKI (N=4), ABIDEII-OHSU_1 (N=7)), 351 NT
1306 M (59 missing; ABIDEII-GU_1 (N=1), KKI (N=23), OHSU (N=15), ABIDEII-OHSU_1 (N=20)), and 139 NT F
1307 (27 missing; KKI (N=8), ABIDEII-OHSU_1 (N=19)). ^dPIQ was available for 306 males with ASD (56 missing;
1308 ABIDEII-GU_1 (N=9), KKI (N=14), OHSU (N=9), ABIDEII-OHSU_1 (N=22), ABIDEII-UCLA_1 (N=1), UM_1,
1309 (N=1)), 67 females with ASD (15 missing; ABIDEII-GU_1 (N=4), KKI (N=4), ABIDEII-OHSU_1 (N=7)), 349 NT
1310 M (61 missing; ABIDEII-GU_1 (N=1), KKI (N=23), OHSU (N=15), ABIDEII-OHSU_1 (N=20), UM_1, (N=2)),
1311 139 NT F (27 missing; KKI (N=8), ABIDEII-OHSU_1 (N=19)). ^eADI-R Social scores were available for 317 males
1312 with ASD (45 missing; ABIDEII-GU_1 (N=1), Leuven_2 (N=10), NYU (N=3), ABIDEII-NYU_1 (N=1), SDSU
1313 (N=2), ABIDEII-UCD_1 (N=11), ABIDEII-UCLA_1 (N=14), UM_1, (N=2), Yale (N=3)) and 68 females with
1314 ASD (14 missing; ABIDEII-GU_1 (N=1), ABIDEII-KKI_1 (N=2), Leuven_2 (N=3), NYU (N=1), Pitt (N=1),
1315 ABIDEII-UCD_1 (N=3), ABIDEII-UCLA_1 (N=1), Yale (N=2)). ^fADI-R Communication and RRB scores were

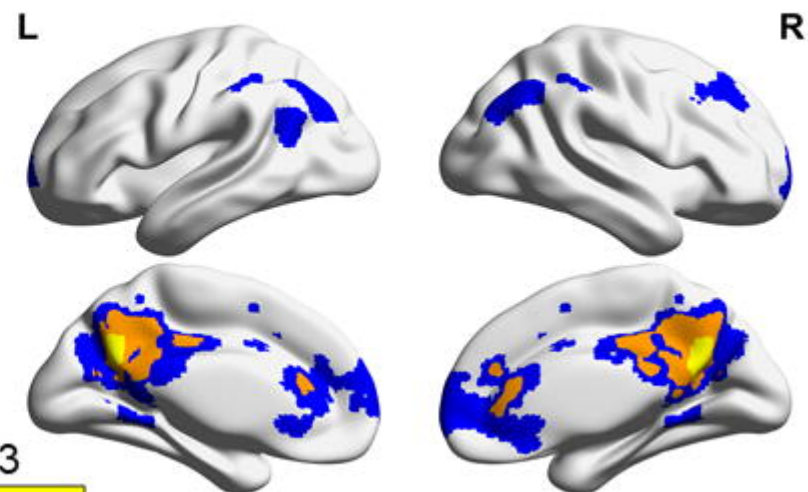
1316 available for 318 males with ASD (45 missing; ABIDEII-GU_1 (N=1), Leuven_2 (N=10), NYU (N=2), ABIDEII-
1317 NYU_1 (N=1), SDSU (N=2), ABIDEII-UCD_1 (N=11), ABIDEII-UCLA_1 (N=14), UM_1, (N=2), Yale (N=3))
1318 and 68 females with ASD (14 missing; ABIDEII-GU_1 (N=1), ABIDEII-KKI_1 (N=2), Leuven_2 (N=3), NYU
1319 (N=1), Pitt (N=1), ABIDEII-UCD_1 (N=3), ABIDEII-UCLA_1 (N=1), Yale (N=2)). ^gADOS-Gotham Social-Affect
1320 was available for 261 males with ASD (101 missing; ABIDEII-GU_1 (N=27), ABIDEII-KKI_1 (N=13), Leuven_2
1321 (N=10), NYU (N=7), OHSU (N=11), Pitt (N=8), SDSU (N=8), Stanford (N=6), ABIDEII-UCLA_1 (N=5), UM_1
1322 (N=6), Yale (N=1)) and 55 females with ASD (27 missing; ABIDEII-GU_1 (N=6), ABIDEII-KKI_1 (N=7),
1323 Leuven_2 (N=3), ABIDEII-OHSU_1 (N=1), Pitt (N=4), Stanford (N=1), ABIDEII-UCD_1 (N=1), UCLA_1 (N=1),
1324 UM_1 (N=3)). ^hADOS-Gotham RRB was available for 264 males with ASD (98 missing; ABIDEII-GU_1 (N=27),
1325 ABIDEII-KKI_1 (N=13), Leuven_2 (N=10), NYU (N=7), OHSU (N=11), Pitt (N=8), SDSU (N=8), Stanford (N=3),
1326 ABIDEII-UCLA_1 (N=5), UM_1 (N=6), Yale (N=1)) and 56 females with ASD (26 missing; ABIDEII-GU_1
1327 (N=6), ABIDEII-KKI_1 (N=7), Leuven_2 (N=3), ABIDEII-OHSU_1 (N=1), Pitt (N=4), ABIDEII-UCD_1 (N=1),
1328 UCLA_1 (N=1), UM_1 (N=3)). ⁱADOS-Gotham calibrated severity scores [65] were available for 347 males with
1329 ASD (15 missing) and 77 females with ASD (5 missing). ^jAttention Deficit Hyperactivity Disorder (ADHD; N=63);
1330 anxiety disorder (N=22); Oppositional Defiant Disorder (ODD; N=17); mood disorder (N=11); Tourettes/Tics
1331 (N=6); Obsessive-Compulsive Disorder (OCD; N=6); enuresis (N=8); encopresis (N=4); developmental articulation
1332 disorder (N=1); developmental dyslexia (N=1); sensory integration disorder (N=1). ^hADHD (N=17); anxiety
1333 disorder (N=7); ODD (N=10); mood disorder (N=2); OCD (N=2); enuresis (N=2); encopresis (N=1). The three
1334 group means were compared with ANOVA tests (or Kruskal-Wallis test in the case of non-parametric mean FD)
1335 followed by post-hoc pairwise t-test comparisons (or Mann-Whitney U-tests in the case of non-parametric mean FD)
1336 when statistically significant (significance cut-off set at $p < 0.05$).

1337

Main Effects of Diagnosis



Main Effects of Sex



Main effect	Region with overlap	R-fMRI metric		
		PCC-iFC	VMHC	ReHo
DX	Bilateral PCG/FP			
Sex	Bilateral ACC			
	Bilateral PCC/Prec			

ASD<NT
M<F

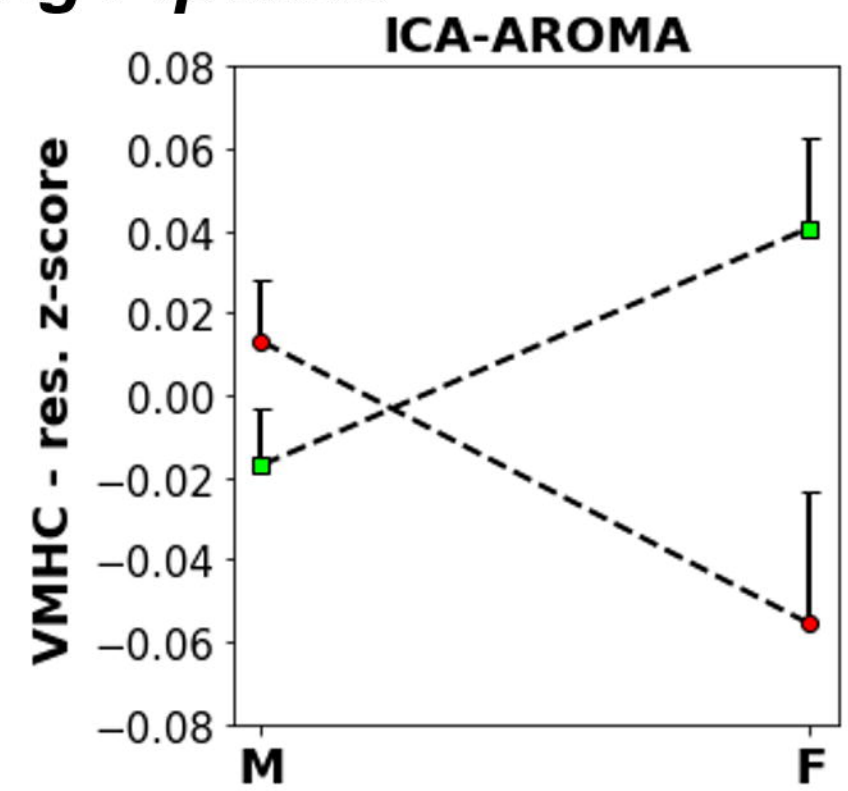
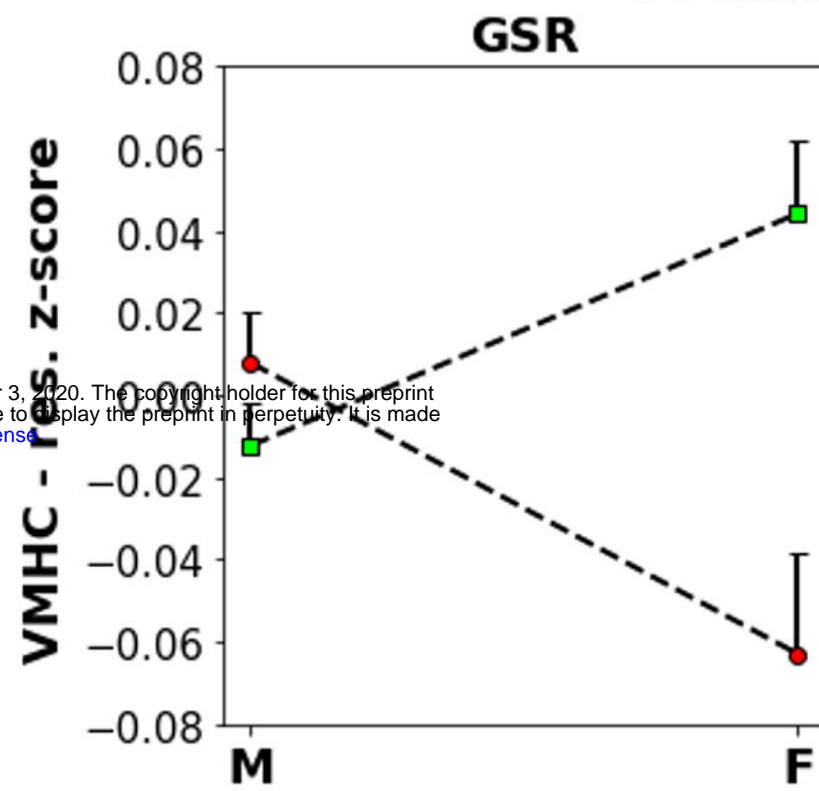
ASD>NT
M>F

Processing Pipeline

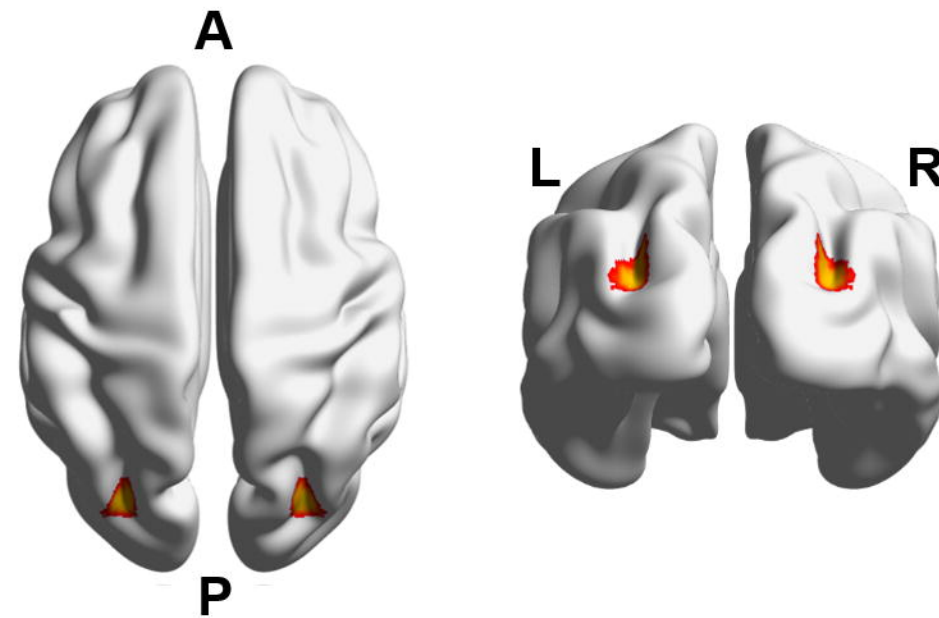
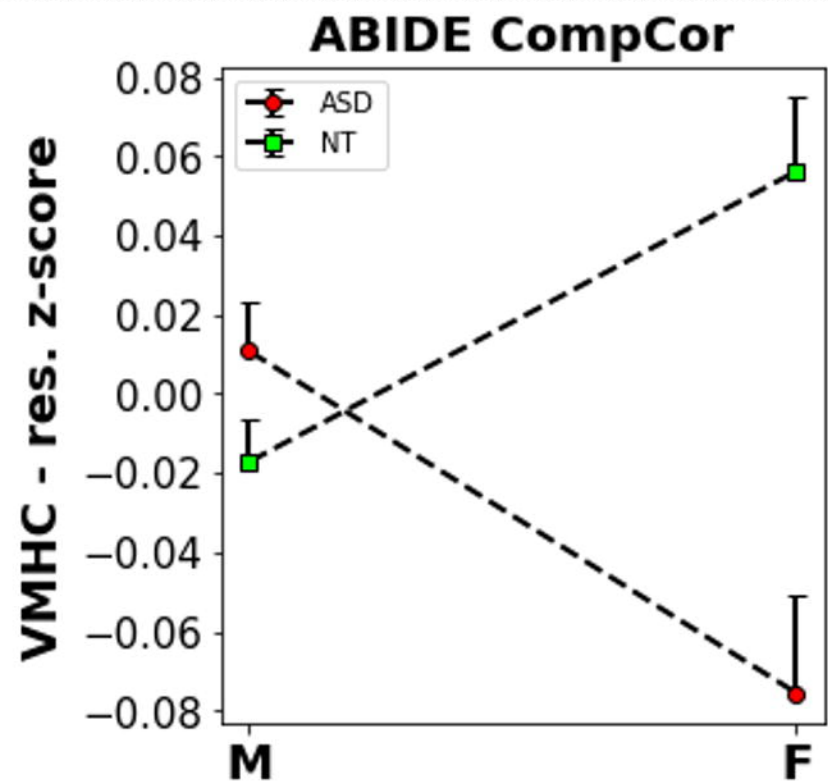
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2b

Robustness

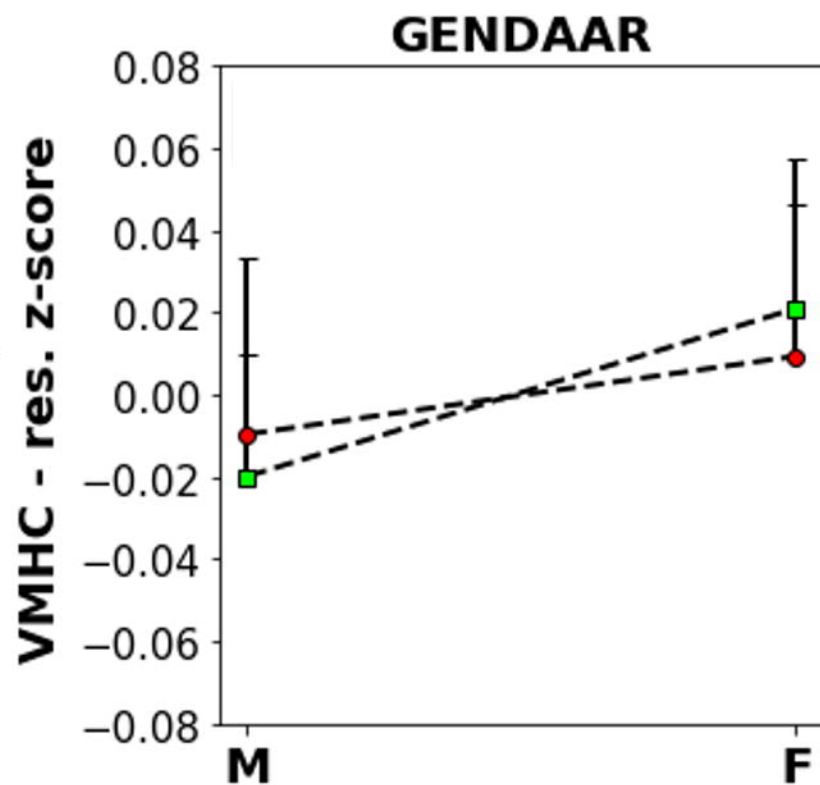


2a

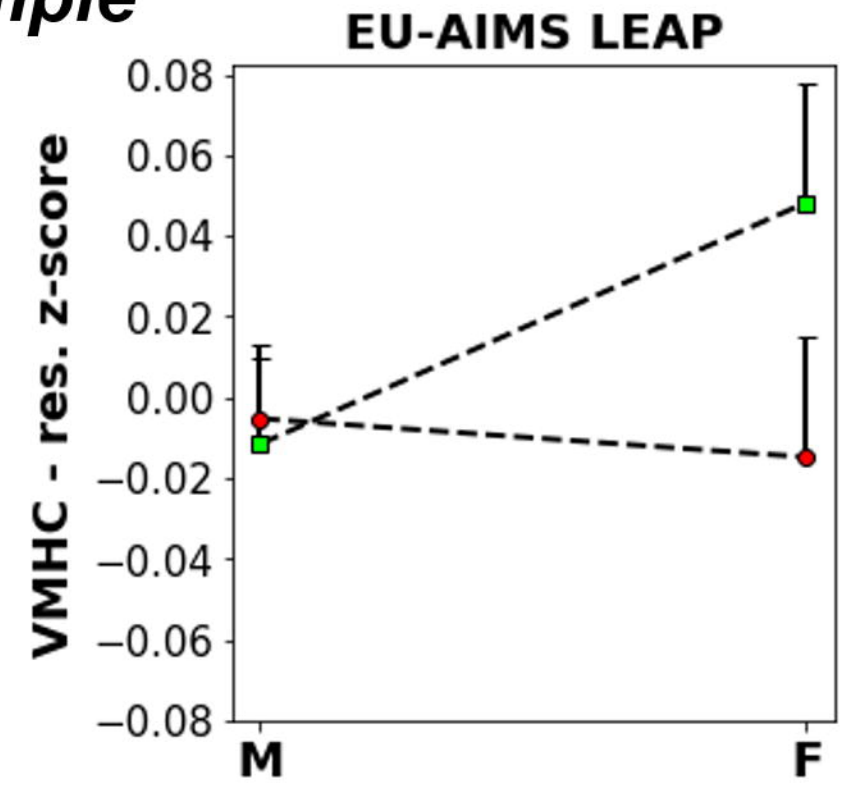


2c

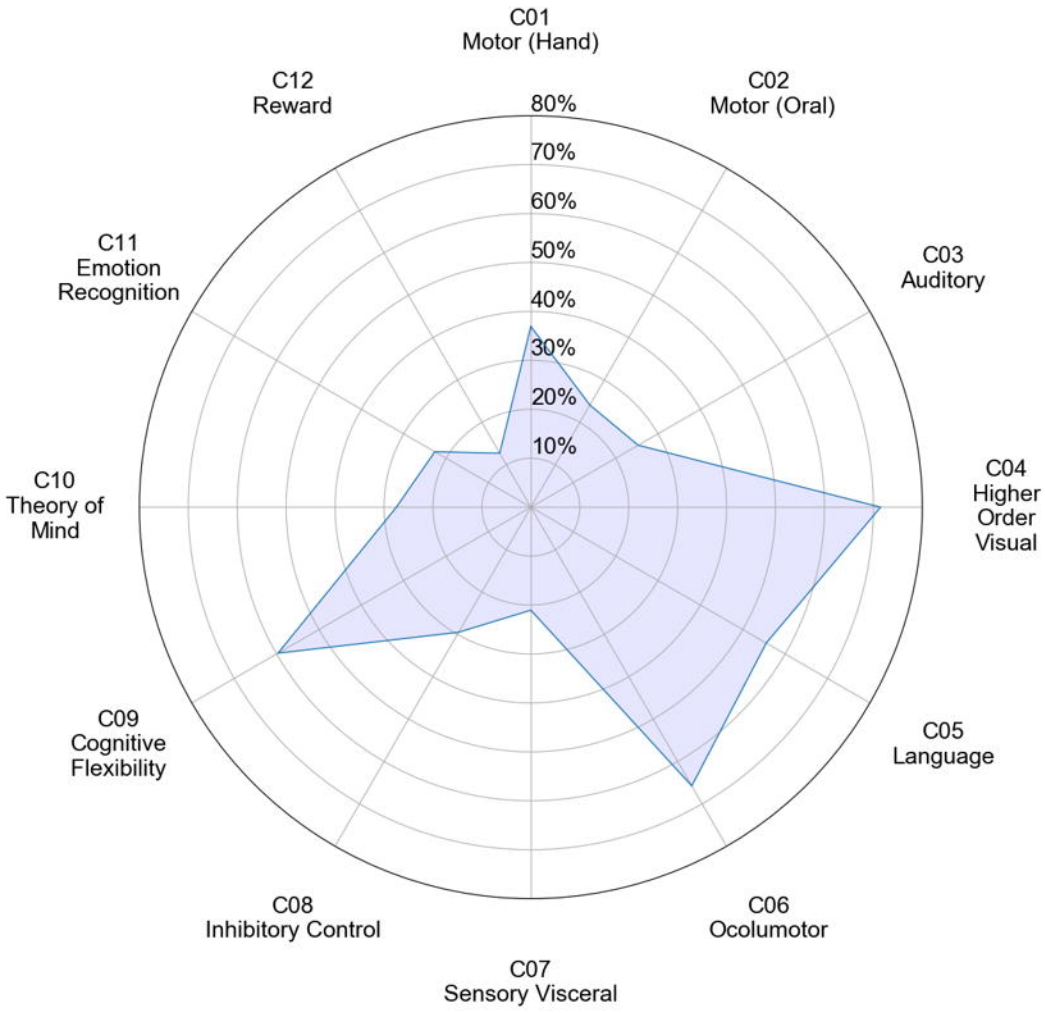
Replicability



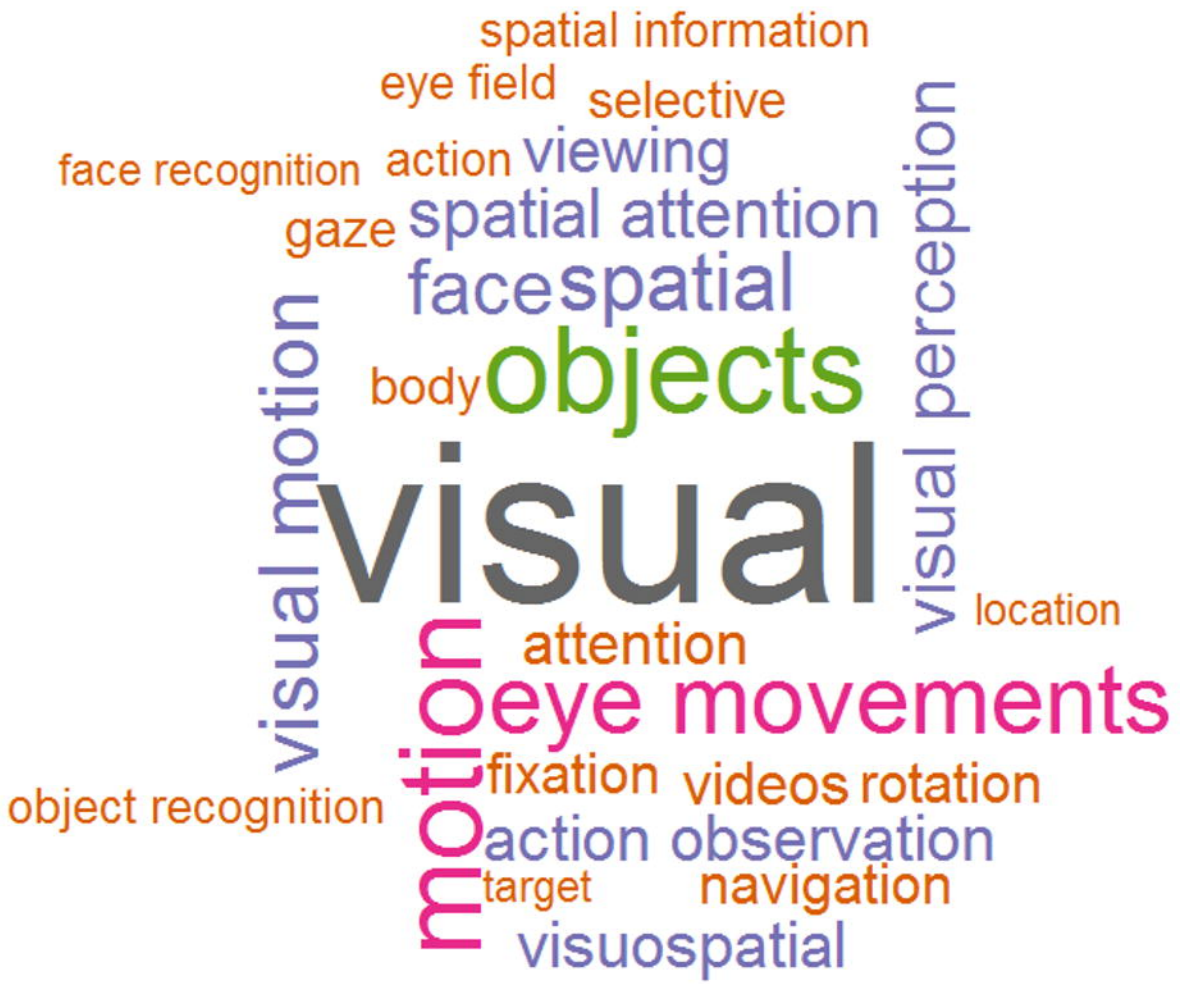
Sample



a) Overlap of VMHC cluster with cognitive ontology maps



b) Neurosynth terms correlation with VMHC cluster



c) Correlation between social-affect ADOS scores and VMHC cluster

