1 Full article title: Neuropsychiatric Phenotypes and a Distinct Constellation of ASD Features in
2 3q29 Deletion Syndrome: Results from the 3q29 Registry

3 Authors: Rebecca M Pollak[1], Melissa M Murphy[2], Michael P Epstein[2], Michael E Zwick[2,3],
4 Cheryl Klaiman[3,4], Celine A Saulnier[3], the Emory 3q29 Project, Jennifer G Mulle[2,5]
5 Affiliations: [1]Genetics and Molecular Biology, Laney Graduate School, Emory University,
6 [2]Department of Human Genetics, School of Medicine, Emory University, [3]Department of
7 Pediatrics, School of Medicine, Emory University, [4]Marcus Autism Center, Children's
8 Healthcare of Atlanta and Emory University School of Medicine, [5]Department of Epidemiology,
9 Rollins School of Public Health, Emory University
10 Corresponding author information: Jennifer Gladys Mulle, MHS, PhD, Whitehead 305M, 615
11 Michael Street, Atlanta, GA 30322; (404) 727-3042; jmulle@emory.edu
12 Author email addresses: Rebecca M Pollak, rebecca.pollak@emory.edu; Melissa M Murphy,
13 melissa.murphy@emory.edu; Michael P Epstein, mpepste@emory.edu; Michael E Zwick,
14 mzwick@emory.edu; Cheryl Klaiman, cheryl.klaiman@emroy.edu; Celine A Saulnier,
15 celine.saulnier@emory.edu; Jennifer G Mulle, jmulle@emory.edu
16
17
18
19
20
21
22
23

ABSTRACT

Background

The 1.6 Mb 3q29 deletion is associated with neurodevelopmental and psychiatric phenotypes, including increased risk for autism spectrum disorder (ASD) and a 20-40-fold increased risk for schizophrenia. However, the phenotypic spectrum of the deletion, particularly with respect to ASD, remains poorly described.

Methods

We ascertained individuals with 3q29 deletion syndrome (3q29Del, "cases", n=93, 58.1% male) and typically developing controls (n=64, 51.6% male) through the 3q29 registry (https://3q29deletion.patientcrossroads.org). Self-report of neuropsychiatric illness was evaluated for 93 cases. Subsets of participants were evaluated with the Social Responsiveness Scale (SRS, n=48 cases, 56 controls), Social Communication Questionnaire (SCQ, n=33 cases, 46 controls), Autism Spectrum Screening Questionnaire (ASSQ, n=24 cases, 35 controls), and Achenbach Behavior Checklists (n=48 cases, 57 controls).

Results

3q29Del cases report a higher prevalence of autism diagnoses versus the general population (29.0% vs. 1.47%, p<2.2E-16). Notably, 3q29 deletion confers a greater influence on risk for ASD in females (OR=41.8, p=4.78E-05) than in males (OR=24.6, p=6.06E-09); this is aligned with the reduced male:female bias from 4:1 in the general population to 2:1 in our study sample. Although 71% of cases do not report a diagnosis of ASD, there is evidence of significant social disability (3q29Del SRS *T-score*=71.8, control SRS *T-score*=45.9, p=2.16E-13). Cases also report increased frequency of generalized anxiety disorder compared to controls (28.0% vs. 6.2%, p=0.001), which is mirrored by elevated mean scores on the Achenbach DSM-oriented

sub-scales ($p<0.001$). Finally, cases show a distinct constellation of ASD features on the SRS as compared to idiopathic ASD, with substantially elevated Restricted Interests and Repetitive Behaviors, but only mild impairment in Social Motivation.

Conclusions

Our sample of 3q29Del is significantly enriched for ASD diagnosis, especially among females, and features of autism may be present even when an ASD diagnosis is not reported. Further, the constellation of ASD features in this population is distinct from idiopathic ASD, with substantially less impaired social motivation. Our study implies that ASD evaluation should be the standard of care for individuals with 3q29Del. From a research perspective, the distinct ASD subtype present in 3q29Del is an ideal entry point for expanding understanding of ASD.

Keywords

Autism, copy number variants, 3q29 deletion, psychiatric genetics, SRS, developmental delay, genomic disorder

93

94 **BACKGROUND**

95 3q29 deletion syndrome (3q29Del) is a rare (~1 in 30,000) [1, 2] genomic disorder
96 characterized by a 1.6 Mb typically *de novo* deletion on chromosome 3 [3-5]. The interval
97 contains 21 distinct protein-coding genes, 3 antisense transcripts, 1 long noncoding RNA, and 1
98 microRNA. Our understanding of the syndrome phenotype continues to evolve. Initial reports
99 found developmental delay/intellectual disability universal among 3q29 deletion carriers, though
100 some case reports have since identified individuals without cognitive impairment [6]. The 3q29
101 deletion is associated with a 20-40-fold increased risk for schizophrenia (SZ), with multiple
102 replication studies supporting this association [7-11]. Case reports also indicate other
103 neuropsychiatric phenotypes may exist, including attention deficit/hyperactivity disorder
104 (ADHD) and bipolar disorder [3, 4, 12-16]. Previous work by our team examining self-report
105 data from 44 individuals with 3q29Del revealed a high prevalence (~20%) of generalized anxiety
106 disorder [5]. Further, case reports have long suggested an association with autism spectrum
107 disorder (ASD), and studies with large sample sizes indicate that the 3q29 deletion may confer a
108 19-fold increased risk for ASD ($p = 0.001$) [17, 18].

109 The range of neuropsychiatric manifestations in 3q29Del is consistent with other
110 genomic disorders. For example, the 22q11.2 deletion has a well-known association with
111 schizophrenia but is also associated with intellectual disability (ID), ASD, anxiety, mood
112 disorders, and ADHD [19, 20]. A similar constellation of phenotypes, including ASD, ADHD,
113 ID, SZ, and anxiety, has been identified in 16p11.2 deletion and duplication syndromes [21, 22],
114 7q11.23 duplication syndrome [23], and 1q21.1 deletion syndrome [24]. Thus, risk for multiple

115 neuropsychiatric phenotypes appears to be a feature common to many genomic disorders,
116 including 3q29 deletion syndrome.

117 The present study aims to improve the current understanding of 3q29 deletion-associated
118 neuropsychiatric and neurodevelopmental phenotypes, and ASD in particular, by examining data
119 from comprehensive, standardized questionnaires in the largest cohort of individuals with
120 3q29Del ever assembled. Developing a clearer and more comprehensive picture of 3q29
121 deletion-associated phenotypes will aid in management of the syndrome for both families and
122 clinicians, which may in turn improve long-term outcomes. Additionally, a careful description of
123 the phenotypic spectrum of 3q29Del provides a basis for cross-disorder comparison between
124 genomic disorders, which may ultimately create inroads for identifying common mechanisms
125 underlying 3q29Del and similar CNV disorders.

126 **METHODS AND MATERIALS**

127 **Sample**

128 Individuals with 3q29Del were ascertained through the internet-based 3q29 deletion
129 registry (https://3q29deletion.patientcrossroads.org) as previously reported [5]. Briefly,
130 information about the registry was emailed to health care providers, medical geneticists, genetic
131 counselors, and support organizations; the registry is also advertised via Google AdWords,
132 where specific keywords were chosen to target the registry website in internet searches.
133 Participant recruitment, informed consent and assent, and data collection are all performed
134 through the registry website. Data were securely downloaded and de-identified for analysis.
135 After data cleaning of the electronic records (removing spam accounts, duplicate records, and
136 related individuals), 93 3q29Del registrants (58.1% male) were included in the present study,
137 ranging in age from 0.1-41.0 years (mean = 10.0±8.6 years). Clinical diagnosis of 3q29 deletion

138 syndrome was confirmed in 58% of our study subjects via review of clinical genetics reports
139 and/or medical records. To confirm that adaptation of standardized questionnaires to an online
140 format did not skew results, 64 typically developing controls (51.6% male) were included,
141 ranging in age from 1.0-41.0 years (mean = 9.9±7.2 years). Controls were recruited via emails
142 sent to intramural CDC and Emory listservs and invited to fill out surveys in an identical fashion
143 to cases. Controls reporting a clinical diagnosis of any neurodevelopmental disorder were
144 excluded (n = 1). Description of the study sample can be found in Table 1. This study was
145 approved by Emory University's Institutional Review Board (IRB00064133).

146 **Table 1: Characteristics of study participants with 3q29Del and controls.**

	3q29 Deletion Syndrome	Control	P value
Age, years (mean ± SD)	10.0 ± 8.6	9.9 ± 7.2	0.945
Sex (n, %)			0.521
Male	54 (58.1%)	33 (51.6%)	
Female	39 (41.9%)	31 (48.4%)	
Race (n, %)			0.0003
White	81 (87.1%)	41 (64.1%)	
Black/African American	2 (2.2%)	12 (18.8%)	
Other	10 (10.8%)	9 (14.1%)	
Blank	0 (0%)	2 (3.1%)	
Heart Defect (n, %)			2.37E-07
Yes	27 (29.0%)	2 (3.1%)	
No	54 (58.1%)	61 (95.3%)	
Blank	12 (12.9%)	1 (1.6%)	
Age at Walking (n, %)			2.16E-09
Normal	42 (45.7%)	60 (93.8%)	
Delayed	23 (25.0%)	1 (1.6%)	
Extremely Delayed	12 (13.0%)	1 (1.6%)	
Unsure	10 (10.9%)	2 (3.1%)	
Not applicable	5 (5.4%)	0 (0%)	

147 Demographic data collected from the custom Medical & Demographic Questionnaire completed
148 by participants upon enrollment in the online 3q29 Registry. P values were calculated with

149 Student's t-test (age), Fisher's exact test (race, heart defect, age at walking), or Pearson's chi
150 square test (sex).

151 **Questionnaires**

152 Upon registration, the participant or his/her parent completed a custom medical and
153 demographic questionnaire. This questionnaire includes questions on the sex, birthdate, race, and
154 ethnicity of the participant, as well as a detailed medical history, including developmental
155 milestones and prior clinical diagnoses of any neuropsychiatric or neurodevelopmental disorders
156 [5].

157 Four standardized questionnaires were used to assess ASD-related symptomology and
158 general behavioral problems in the participants. The Social Responsiveness Scale (SRS;
159 preschool, school-age, and adult forms; n = 48 3q29Del, 56 controls) is a 65-item, 4 point Likert-
160 scaled questionnaire designed to assess ASD-related symptoms along a normative continuum
161 [25]. The Social Communication Questionnaire (SCQ, n = 33 3q29Del, 46 controls) is a 40-item,
162 yes/no questionnaire designed to assess ASD-related symptoms keyed to DSM criteria [26]. The
163 Autism Spectrum Screening Questionnaire (ASSQ, n = 24 3q29Del, 35 controls) is a 27-item,
164 yes/somewhat/no questionnaire designed to assess ASD-related symptoms in high-functioning
165 individuals with no to mild ID [27]. The Child Behavior Checklist (CBCL) and Adult Behavior
166 Checklist (ABCL) are 100-, 113-, or 126-item (CBCL preschool, CBCL school-age, and ABCL,
167 respectively; n = 48 3q29Del, 57 controls), 3 point Likert-scaled questionnaires designed to
168 assess behavioral or developmental problems [28, 29]. Data from the CBCL and ABCL were
169 pooled for analysis. All standardized questionnaires were adapted for the online 3q29 deletion
170 registry and were completed by the participant or parent/guardian of the participant upon
171 registration. Some participants were not eligible to complete the standardized questionnaires

172 because the proband was too young. Demographic characteristics of the respondents for each
173 questionnaire can be found in Table S1, demonstrating that the average age and sex distribution
174 of participants who completed the medical and demographic questionnaire was not different
175 from the average age and sex distribution of participants who completed each standardized form.

176 **Analysis**

177 Data from standardized questionnaires were imported into R [30] and were recoded and
178 scored according to the publisher's guidelines. Features of interest from the medical history
179 questionnaire (heart defects, age at walking, ASD diagnosis, global developmental delay/mental
180 retardation (GDD/MR) diagnosis) were recoded for analysis as follows: heart defects, yes/no;
181 age at walking, binned as normal (≤18 months), delayed (19-24 months), and extremely delayed
182 (>24 months); ASD diagnosis, yes/no; GDD/MR diagnosis yes (reported diagnosis of global
183 developmental delay and/or mental retardation)/no. To compare responses between 3q29Del
184 cases and controls, linear models and logistic regression models were implemented using the
185 stats R package [30]. To perform case-only analysis within 3q29Del cases, linear models and
186 logistic regression models were implemented using the stats R package [30] and cumulative link
187 proportional-odds models were implemented using the ordinal R package [31]. All statistical
188 models included age, race, and sex as covariates. To compare rates of self-reported diagnoses
189 and demographic parameters between 3q29Del cases and controls, Fisher's exact test was
190 implemented using the stats R package [30]. To compare rates of self-reported diagnoses in
191 3q29Del cases to population prevalence values, one-sample proportion tests with Yates'
192 continuity correction were implemented using the stats R package [30]. To compare sex
193 distribution between 3q29Del participants and controls, Pearson's chi square test was
194 implemented using the stats R package [30]. To compare age distribution in 3q29Del participants

195 and controls, two sample t-test was implemented using the stats R package [30]. To compare
196 scores in 3q29Del participants to mean values for children with idiopathic ASD, one sample t-
197 test was implemented using the stats R package [30]. Odds ratios and p values were calculated
198 using the fmsb R package [32]. Figures were generated using the plotly and ggplot2 R packages
199 [33][34].

200 *Sensitivity Analysis*

201 The questionnaires for 90 participants with 3q29Del (96.8%) were completed by a parent
202 or guardian ("parent-registered"), while 3 participants with 3q29Del (3.2%) completed all
203 questionnaires themselves ("self-registered"). All control participants were parent-registered. To
204 assess whether responses from the self-registered 3q29Del participants were influencing the
205 results, self-registrants were removed and the data were re-analyzed. Self-registrants were not
206 found to have a significant effect on the analyses (Tables S2 and S3). All results include both
207 parent- and self-registrants.

208 **RESULTS**

209 **Self-report of neuropsychiatric diagnosis in 3q29Del**

210 Self-report of neuropsychiatric diagnoses in our 3q29Del study subjects (Table 2)
211 revealed a higher prevalence of neuropsychiatric disorder diagnoses compared to controls,
212 including anxiety (28.0%), and compared to general population frequencies, including ASD
213 (29.0%, Figure 2A) and GDD/MR (59.1%) (Table 2), confirming prior work by our group [5].
214 Reported rates of conduct disorder (1.1% vs. 3.5%) and oppositional defiant disorder (3.2% vs.
215 3.5%) were similar to those observed in the general population. While a small proportion of
216 participants reported diagnoses of bipolar/manic depression (4.3%), depression (6.5%), and
217 schizophrenia (4.3%), we focused on ASD due to the young age (mean = 10.0 years) of our study

218 population, since many study participants have not reached the age of risk for schizophrenia and
219 other adult-onset disorders. Despite this young age, the self-reported rate of SZ diagnoses in our
220 adult study subjects (age > 18 years, n = 13) was 15-30 times higher than expected (15.4%
221 compared to an expected 0.5-1% in the general population; n = 2) [35-39] and the frequency of
222 bipolar disorder was ~1.8 times higher than expected [40]. A summary of neuropsychiatric
223 diagnoses can be found in Table 2.
224 [Table 2 here]

225 **SRS, SCQ, ASSQ, and CBCL/ABCL scores**

226 In 3q29 deletion study subjects, the mean SRS score was in the moderate range (*T-score*
227 = 71.8), the mean ASSQ score was in the clinical range (mean = 22.2), and the mean
228 CBCL/ABCL score was in the borderline range (*T-score* = 62.5). The mean SCQ score in 3q29
229 deletion carriers was at the extremely high end of the normal range (mean = 13.9, clinical cutoff
230 = 15) and elevated as compared to controls (mean = 3.5). Mean scores for typically developing
231 controls were all in the normal range (SRS *T-score* = 45.9, ASSQ mean = 2.2, CBCL/ABCL *T-*
232 *score* = 41.8, SCQ mean = 3.5) (Figure 1). Participants with 3q29Del scored significantly higher
233 than typically developing controls on all four scales ($p < 3.0E-12$, Table S4).

234 **Standardized scores stratified by ASD diagnosis**

235 Next, we examined the relationship between SRS scores and reported ASD diagnosis, to
236 determine whether the score inflation we observed in our study population as a whole was
237 largely due to the increased prevalence of ASD. As expected, we observed that individuals with
238 3q29Del and an ASD diagnosis scored significantly higher than both controls and individuals
239 with 3q29Del without an ASD diagnosis (3q29Del with ASD n = 17, *T-score* = 82.41; 3q29Del
240 without ASD n = 31, *T-score* = 65.90; control n = 56, *T-score* = 45.90; $p < 3.0E-13$; Figure 2B).

We were interested to observe that individuals with 3q29Del without an ASD diagnosis also scored significantly higher than controls (3q29Del without ASD n = 31, *T-score* = 65.90; control n = 56, *T-score* = 45.90; p = 2.16E-13; Figure 2B), indicating that increased SRS scores in individuals with 3q29Del are not driven by ASD diagnostic status alone (Table S5). Similar features were observed in the contribution of ASD diagnosis status to SCQ scores (Figure S1, Table S6).

Standardized scores stratified by sex

Both males and females with 3q29Del reported a significantly increased frequency of ASD diagnoses, with a substantially greater burden for ASD on females with 3q29Del. Males with 3q29Del are at 16-fold increased risk for ASD as compared to the general population (37.0% vs. 2.34%, OR = 24.6, p = 6.06E-09) and females are at 34-fold risk compared to the general population (17.9% vs. 0.52%, OR = 41.8, p = 4.78E-05) (figure 2A) [41], resulting in a male:female ratio in our study population of 2:1, as compared to the general population ratio of 4:1. Taken together, this indicates that the 3q29 deletion elevates the risk for ASD in females more substantially than in males.

Based on the sex differences in ASD risk for individuals with 3q29Del, we also examined possible sex differences in scores. We found that both males and females with 3q29Del scored significantly higher than controls (3q29Del male n = 26, *T-score* = 74.31; control male n = 30, *T-score* = 45.80; p = 7.70E-11; 3q29Del female n = 22, *T-score* = 68.73; control female n = 26, *T-score* = 46.04; p = 7.42E-09); while 3q29Del males have higher scores than females, the differences are not statistically significant (3q29Del male n = 26, *T-score* = 74.31; 3q29Del female n = 22, *T-score* = 68.73; p > 0.05; Figure 2C). After stratifying our study population further by sex and ASD diagnosis status, we determined that both male and female 3q29Del

264 participants without an ASD diagnosis had significantly higher scores than controls (3q29Del
265 male without ASD n = 14, *T-score* = 66.29; control male n = 30, *T-score* = 45.80; p = 1.20E-06;
266 3q29Del female without ASD n = 17, *T-score* = 65.69; control female n = 26, *T-score* = 46.04; p
267 = 5.04E-07; Figure 2D). Taken together, this suggests that increased SRS scores in individuals
268 with 3q29Del are not driven by sex alone or by sex and ASD diagnosis status in combination
269 (Table S5); rather, the presence of the deletion itself confers a greater risk for social disability.
270 Furthermore, these data show an enrichment for female ASD in our study population, based on
271 the reduction in male bias and the highly similar scores between males and females with
272 3q29Del, irrespective of ASD diagnosis status. Similar features were observed in the
273 contribution of sex to SCQ scores (Figure S1, Table S6).

274 **ASD presentation of 3q29Del**

275 While total scores on the SRS, SCQ, ASSQ, and CBCL/ABCL can give an indication of
276 the overall level of impairment of individuals, sub-scores can reveal nuanced deficits in specific
277 behavioral domains. To this end, we analyzed all SRS sub-scales (Social Awareness, Social
278 Cognition, Social Communication, Social Motivation, Restricted Interests and Repetitive
279 Behaviors, and Social Communication and Interaction) to better understand the extent of social
280 disability in our study population; our goal was to determine whether our observed total score
281 inflation was due to a specific severe deficit in a few domains, or if individuals with 3q29Del
282 showed high scores across all sub-scales. The mean score for the Restricted Interests and
283 Repetitive Behaviors sub-scale was in the severe range (*T-score* = 77.3). Mean scores for Social
284 Awareness (*T-score* = 67.3), Social Cognition (*T-score* = 69.1), Social Communication (*T-score*
285 = 69.7), and Social Communication and Interaction (*T-score* = 69.5) were all in the moderate
286 range. Notably, the mean score for Social Motivation was in the mild range (*T-score* = 62.1,

Figure 3A, Table 3). This sub-score profile is strikingly different from that reported in studies of idiopathic ASD, where children tend to score equally high on all sub-scales (3q29Del Social Motivation *T-score* = 62.1, idiopathic ASD Social Motivation *T-score* = 78.4, p = 7.66E-11) [42]. This atypical behavioral profile is supported by clinical data; direct assessment of individuals with 3q29Del by clinicians affiliated with the Emory 3q29 Project (http://genome.emory.edu/3q29/, [43]) show less impaired social motivation as compared to children with idiopathic ASD.

ASD presentation stratified by sex

To determine whether this unusual SRS sub-score profile was influenced by sex, we examined profiles of male and female 3q29 deletion carriers separately. We found that the shape of the profiles were identical, with males scoring on average 5 points higher than females on every sub-scale (n = 26 male, 22 female; $p > 0.05$; Figure 3B; Table 3), demonstrating that the social disability in 3q29Del is not qualitatively different between males and females.

ASD presentation stratified by ASD diagnosis

We then stratified our study subjects according to reported ASD diagnosis status and examined subscale scores separately for 3q29Del individuals reporting a diagnosis of ASD and those not reporting a diagnosis of ASD. We observed that the shape of the profile is shared between 3q29Del individuals reporting a diagnosis of ASD and those not reporting a diagnosis of ASD, with individuals reporting a diagnosis of ASD scoring on average 10-15 points higher on every sub-scale (Figure 3C). As expected, 3q29Del participants with ASD scored significantly higher on all sub-scales than 3q29Del participants without ASD (n = 17 with ASD, 31 without ASD; $p < 0.005$; Table 3); however, 3q29Del participants without ASD still scored significantly higher than controls on all sub-scales (n = 31 without ASD, 56 control; $p < 5.0E-05$; Table 3).

310 [Table 3 here]

311 **Additional neuropsychiatric phenotypes in 3q29Del**

312 To further assess behavioral features associated with the 3q29 deletion, we examined the
313 DSM-oriented Attention Deficit/Hyperactivity Problems, Anxiety Problems, and Depressive
314 Problems sub-scales from the CBCL and ABCL. These DSM-oriented sub-scales align with
315 neuropsychiatric diagnoses reported by individuals with 3q29Del [5]. Individuals with 3q29Del
316 scored significantly higher than typically developing controls on all three scales (3q29Del
317 Attention Deficit/Hyperactivity Problems *T-score* = 61.0, control Attention Deficit/Hyperactivity
318 Problems *T-score* = 51.3, 3q29Del Anxiety Problems *T-score* = 60.9, control Anxiety Problems
319 *T-score* = 52.9, 3q29Del Depressive Problems *T-score* = 62.7, control Depressive Problems *T-*
320 *score* = 52.3, all $p < 0.001$, Figure 3D, Table S7), supporting previous reports of increased risk
321 for neuropsychiatric phenotypes associated with the 3q29 deletion [5].

322 **Confounding due to heart defects and/or ID-related phenotypes**

323 A previous study of 3q29Del by our group showed that approximately 25% of individuals
324 with 3q29Del reported a congenital heart defect [5]. Early hypoxic insult due to a heart defect
325 has been hypothesized to contribute to later neuropsychiatric and neurodevelopmental outcomes
326 [44-49]. To determine if the high frequency of heart defects in our study population was driving
327 adverse neurodevelopmental outcomes within 3q29Del cases, we implemented generalized linear
328 and cumulative link models to assess the relationship between congenital heart defects and
329 clinical ASD diagnosis, GDD/MR diagnosis, and age at walking, which has been reported to be a
330 suitable proxy for ID in the absence of available IQ and adaptive behavior measures [50] .
331 Congenital heart defects were not associated with self-reported ASD or GDD/MR diagnoses or
332 age at walking ($p > 0.05$, Table S8). Individuals with 3q29Del are also commonly diagnosed

333 with mild to moderate ID [5]. To ask whether ASD phenotypes or ASD features were
334 disproportionately overrepresented in individuals with more pronounced ID-related phenotypes
335 and/or heart defects, we stratified the data according to these phenotypes. Within our 3q29Del
336 study population, congenital heart defects were associated with significantly increased scores on
337 the SCQ and CBCL/ABCL ($p < 0.05$); however, reported GDD/MR diagnosis and age at
338 walking were not significantly associated with scores on the SRS, SCQ, ASSQ, or CBCL/ABCL
339 ($p > 0.05$, Table S9). These data indicate that ID-related phenotypes were not driving the
340 increased scores in our study population.

341 **DISCUSSION**

342 Previous studies have found enrichment of the 3q29 deletion in large samples ascertained
343 based on clinical ASD diagnosis [17, 18]. We have approached the association of 3q29Del with
344 ASD from a different angle; by ascertaining subjects with 3q29Del and investigating the
345 prevalence of reported ASD diagnosis and ASD-related phenotypes, the current study
346 complements the existing literature, providing additional evidence for the 3q29 deletion as a
347 genetic risk factor for ASD. Notably, the male:female ratio of self-reported ASD diagnosis in our
348 study population is 2:1. This is a reduction from the 4:1 male bias observed in idiopathic ASD in
349 the general population. A substantial reduction in male bias in ASD prevalence has been
350 observed in studies of other CNVs and single-gene mutations; a recent study has shown that as
351 the severity of a mutation increases, the sex ratio in ASD prevalence approaches 1:1 [51]. Taken
352 together, this suggests that the 3q29 deletion is approaching the severe end of the spectrum of
353 ASD-associated mutations.

354 We have shown that compared to typically developing children, our 3q29Del sample is
355 significantly enriched for ASD features and other behavioral problems, irrespective of a clinical

356 ASD diagnosis. This finding is particularly concerning; while individuals with 3q29Del who
357 have an ASD diagnosis tend to score higher on symptomology scales overall, 3q29Del
358 individuals without an ASD diagnosis still score significantly higher than typically developing
359 children. This indicates several possible explanations: a) an enrichment for ASD features or
360 social disability that falls short of diagnostic criteria, b) possible undiagnosed ASD in our study
361 population, or c) non-specificity of the SRS, and potentially SCQ, for phenotypes other than
362 ASD, such as anxiety. The possibility of undiagnosed ASD in our study population is aligned
363 with anecdotal reports from parents of our study participants, where they have reported concerns
364 about atypical social development that do not appear to have been addressed using gold-standard
365 ASD evaluations. Based on the elevated symptomology scores in our study population, the
366 substantially increased risk for ASD associated with the 3q29 deletion, and the apparent severity
367 of the 3q29 deletion, our data suggest that gold-standard ASD evaluations should be the
368 recommended standard of care for individuals diagnosed with 3q29Del. If implemented, this
369 practice would enable patients to gain access to early interventions, treatments, and therapeutic
370 programs that are known to improve later outcomes.

371 Based on the SRS sub-scales, participants with 3q29Del display a strikingly different
372 behavioral profile as compared to a study of children with idiopathic ASD [42]. Male and female
373 3q29Del individuals show substantially less impaired social motivation in the context of an
374 otherwise typical ASD profile, with the most severe deficits in the Restricted Interests and
375 Repetitive Behaviors domain. This profile is also observed when dividing scores for 3q29Del
376 participants based on reported ASD diagnosis. This qualitative difference from idiopathic ASD
377 may serve as an inroad to therapeutic interventions in 3q29Del, as well as an investigative inroad
378 to a distinct subtype of ASD. Because social motivation appears to be relatively well-preserved

379 in 3q29Del, this suggests that therapies such as cognitive-behavioral therapy to teach social skills
380 and effective strategies for social interaction may be particularly successful in this patient
381 population.

382 Some facets of the difference in ASD features between 3q29Del and idiopathic ASD are
383 recapitulated by the scores on the Withdrawn sub-scale of the CBCL and ABCL. Previous
384 studies utilizing the CBCL in idiopathic ASD have found that mean scores for participants with
385 ASD are in the borderline range, with over 50% of subjects scoring in the borderline or clinical
386 range [52, 53]. While 3q29Del participants generally, as well as males and females separately,
387 score significantly higher than controls, their mean score is still in the normal range (Figure S2A
388 and B). However, 60% of 3q29Del participants reporting an ASD diagnosis score in the
389 borderline or clinical range (Figure S2C, Table S10), which is in line with what is expected
390 based on studies of idiopathic ASD [52, 53]. This is in conflict with the relatively well-preserved
391 social motivation in 3q29Del individuals with ASD identified in our analysis of the SRS sub-
392 scales and suggests that a more refined analysis is merited to identify the true degree of social
393 disability in this population.

394 We tested the hypothesis that the score inflation observed in our 3q29Del study subjects
395 may be due to the high prevalence of developmental delay or congenital heart defects [5]. Our
396 available data do not support this hypothesis, and instead reveal that social disability is equally
397 distributed in our study population. Lack of direct measures of intellectual disability, and errors
398 or missing data in self-report measures, may obscure this relationship; however, numerous
399 studies of the relationship between ID and ASD in genomic disorders suggests that when the
400 population is stratified by the presence of a specific genetic variant, the association between
401 these two phenotypes diminishes. A large study of several genetic disorders showed that the

402 prediction of genetic diagnosis based on ADI-R scores was not confounded by IQ [54]; a study
403 of 7q11.23 duplication found that IQ was not significantly associated with ASD status [55]; and
404 multiple studies of 22q11.2 deletion have shown that IQ is not significantly associated with SRS
405 score, ASD severity, and ASD status [56-58]. A question ripe for future investigation is the
406 potential role for microcephaly in the ASD-related phenotypes observed in 3q29Del.
407 Microcephaly, ASD, and ID are associated with the 16p11.2 duplication [21]; microcephaly has
408 been shown to be associated with ASD and ID in probands with pathogenic CNVs [59]; and
409 children with "complex autism", defined as ASD with microcephaly and/or dysmorphology,
410 have significantly lower cognitive function than children with "essential autism" [60]. Reports
411 have shown a high prevalence of microcephaly in 3q29Del [3, 4, 12]; however, this question was
412 not probed in the current study due to the high rate (>50%) of 3q29Del participants responding
413 "Unsure" to the medical history questionnaire regarding their child's head circumference at birth,
414 rendering this data unreliable. Ongoing studies with direct evaluation of study subjects [43] will
415 address these questions.

416 While this study is the most comprehensive study of behavioral phenotypes in 3q29Del to
417 date, it is not without limitations. All of the data used in the present study were collected from
418 questionnaires completed by the parents and guardians of individuals with 3q29Del, which
419 introduces several potential sources of bias. Some studies have questioned the validity and
420 reliability of parent-report data [61]; however, a recent study in Williams syndrome patients has
421 shown that parents are more accurate in predicting their child's social behaviors than the child
422 themselves [62]. The responses to the medical and demographic questionnaire are more likely to
423 include error due to the fact that the data is retrospective. By limiting our study to only a few key
424 points in the medical history (heart defects, age at walking, and ID/ASD diagnosis) we aimed to

425 reduce recall errors; however, we only had proxies for ID, rather than direct evaluation of
426 cognitive ability. Further, the sample sizes for our stratified analyses were small, rendering them
427 underpowered; while the differences between males and females were not statistically
428 significant, males do score higher than females on all measures. Studies with larger sample size
429 will be better able to assess the importance of and estimate the true effect size of any difference
430 between males and females. Additionally, there is likely ascertainment bias within our sample.
431 First, our sample of 93 individuals with 3q29Del is 87.1% white, indicating that we are not
432 adequately reaching minority populations. Second, parents that register their children and
433 complete in-depth questionnaires are likely to be highly motivated, possibly because their
434 children experience significant morbidity – a potential indication that we are sampling from the
435 extreme of the phenotypic distribution of 3q29Del. Thus, scores on the standardized
436 questionnaires, as well as rates of heart defects and clinical neuropsychiatric diagnoses, may be
437 higher in our study sample than in the general 3q29Del population. Additionally, the odds ratios
438 calculated for the increased risk for ASD associated with the 3q29 deletion may also be
439 overestimated, due to the combined effects of self-report data and ascertainment bias; however,
440 if this increased risk is replicated using gold-standard diagnostic measures, it could provide
441 valuable insight into possible sex-specific effects of the deletion. Finally, the lack of observed
442 association between congenital heart defects and neurodevelopmental outcomes may be obscured
443 by the high rate of patent ductus arteriosus in 3q29 deletion syndrome [5], which is a relatively
444 mild heart defect; however, the low number of participants with different types of heart defects
445 rendered analyses to assess their associations with neurodevelopment underpowered (Table S11).
446 Ongoing studies by the Emory 3q29 Project (http://genome.emory.edu/3q29/), including direct

447 in-person patient evaluations [43] aim to address some of the weaknesses of the present work by
448 performing comprehensive gold-standard evaluations by expert clinicians.

449 While direct in-person evaluations are the ideal method to corroborate the findings of this
450 study, the low population frequency of the 3q29 deletion and geographic dispersal of our study
451 population (Figure S3) renders this approach infeasible for a large number of study subjects.
452 However, a small number of 3q29 deletion study subjects have been directly assessed as part of
453 the Emory 3q29 Project (http://genome.emory.edu/3q29/). We confirm high concordance
454 between registry-leveraged data and gold-standard direct evaluation, as all participants
455 qualifying for an ASD diagnosis based on gold-standard evaluation have clinically significant
456 scores on the SRS and all participants reporting an ASD diagnosis qualified for an ASD
457 diagnosis after gold-standard assessment by the Emory 3q29 Project team (Table S12). Notably,
458 one participant that did not report a prior diagnosis of ASD received an ASD diagnosis after
459 assessment by our team, supporting our hypothesis that ASD may be underdiagnosed in the
460 3q29Del population. Five additional participants with a clinically significant SRS score did not
461 qualify for an ASD diagnosis, suggesting that the SRS is not selectively identifying children with
462 ASD in participants with 3q29Del, possibly due to the high rates of reported anxiety in our study
463 population. However, this comparison does suggest that our analysis, though based on self-report
464 data, reveals valid conclusions about behavioral phenotypes in 3q29 deletion syndrome. For
465 genetic syndromes with low population frequencies, data collection through remote means such
466 as online patient registries remains a valuable phenotyping tool.

467 While the current understanding of the 3q29 deletion is still evolving, there are more
468 well-understood CNV disorders that can be used as a comparison point to determine whether the
469 social disability phenotypes described in this study are distinct to 3q29Del. These include

470 Williams Syndrome (WS, or the 7q11.23 deletion), the reciprocal 7q11.23 duplication, 16p11.2
471 deletion and duplication, Smith-Magenis Syndrome (SMS), and 22q11.2 deletion. WS is
472 typically associated with hyper-sociability [63], and patients with WS show more problems with
473 social cognition than with pro-social behaviors [64], similar to what we have observed in our
474 population of individuals with 3q29Del. However, the prevalence of restricted interests and
475 repetitive behaviors appears to be lower in WS as compared to 3q29Del [64], and the mean SRS
476 sub-scale Social Motivation score indicates enhanced social motivation in WS as compared to
477 3q29Del (WS mean *T-score* = 55.24, 3q29Del mean *T-score* = 62.1, p = 0.0005) [65]. Studies of
478 the reciprocal 7q11.23 duplication showed that parent-reported ASD symptomology via
479 standardized questionnaires was higher than ASD features as assessed by gold-standard
480 instruments; that some probands had been diagnosed with ASD based on delayed speech and
481 social anxiety but did not qualify for ASD via gold-standard measures; that substantially more
482 males than females qualified for an ASD diagnosis; and that 7q11.23 duplication probands were
483 indistinguishable from children with idiopathic ASD on measures of ASD severity and diagnosis
484 status [55, 66, 67]. This is qualitatively different from our 3q29Del population; all of the
485 participants with a prior ASD diagnosis who were later assessed by the Emory 3q29 Project team
486 had their diagnosis confirmed using gold-standard measures (Table S12), the male:female ratio
487 in our sample is 2:1, and we see significant differences between 3q29Del cases and idiopathic
488 ASD [42] on the SRS Social Motivation sub-scale.

489 Similar to 7q11.23 duplication, ASD probands with 16p11.2 deletion or duplication were
490 indistinguishable from idiopathic ASD probands [67]; probands with 16p11.2 deletion also have
491 a significantly higher mean SRS score as compared to 3q29Del (16p11.2 mean *T-score* = 77.8,
492 3q29Del mean *T-score* = 71.8, p = 0.003) [22], and males with 16p11.2 deletion are at increased

risk for ASD compared to females and are overrepresented when cases are ascertained based on neurodevelopmental disorders [68, 69], indicating a different sex-based ASD risk as compared to 3q29Del. A study of 16p11.2 duplication probands found that scores on the SRS Social Motivation sub-scale were not significantly different from controls and that ASD cases had specific impairments in social cognition and communication [70]; 3q29Del cases score significantly higher than controls on the SRS Social Motivation sub-scale, and do not have substantially higher scores on the Social Cognition or Social Communication sub-scales relative to the other SRS sub-scales.

A recent study of SMS showed that female probands scored higher than males on SRS sub-scales and the sex ratio of ASD was reversed, with more females than males qualifying for a diagnosis [71], which we do not observe in our 3q29Del study population. Finally, studies of 22q11.2 deletion show some similarities with 3q29Del, including SRS total scores that are not significantly different, high levels of ASD features in the absence of ASD diagnosis, and a male:female ASD ratio of approximately 1:1 [19, 57, 58, 72]; however, 22q11.2 deletion probands have a significantly lower mean ASSQ score as compared to 3q29Del (22q11.2 mean = 11, 3q29Del mean = 22.2, $p = 0.00004$), and 3q29Del cases have significantly higher scores on several CBCL/ABCL sub-scales (Table S13) [73, 74]. Taken together, this evidence suggests that while the ASD features in 3q29Del reported in this study share some characteristics with other CNV disorders, the complete constellation of symptoms is discrete from previously described genomic syndromes.

There are significant strengths of this study as compared to previous studies of 3q29Del. First, this is the largest cohort of individuals with 3q29Del ever assembled. This is a critical step in capturing the true phenotypic spectrum associated with the 3q29 deletion. Our use of

516 standardized questionnaires allowed for comparison between ASD features present in 3q29Del
517 and those reported in idiopathic ASD and ASD in other CNV disorders. Additionally, our online
518 patient registry allows for remote data collection, which has enabled us to expand our sample
519 size. This study has shown that high-quality, comprehensive medical history and symptomology
520 data can be collected through an online patient registry, effectively reducing the patient-
521 ascertainment burden associated with studying rare disorders. Taken together, these attributes
522 make the present study an excellent complement to previously published case reports on
523 individuals with 3q29Del; by capturing a larger patient base with systematic assessments, we are
524 able to more accurately measure the presence of a variety of neuropsychiatric and
525 neurodevelopmental phenotypes associated with the 3q29 deletion. The findings reported here
526 indicate that comprehensive neuropsychiatric and neurodevelopmental assessments with gold
527 standard tools are merited for individuals diagnosed with 3q29Del, and that such assessments
528 should be the standard of care for this patient population.

529 **CONCLUSIONS**

530 The present study confirms previous reports of phenotypes in 3q29Del, as well as
531 expanding the spectrum of behavioral phenotypes associated with the deletion. We found that
532 individuals with 3q29Del report a significantly higher prevalence of ASD diagnosis than the
533 general population, and significantly elevated scores on the SRS, SCQ, ASSQ, and CBCL/ABCL
534 irrespective of ASD diagnosis indicate significant social disability overall in our study
535 population. Further, 3q29Del participants showed a distinct profile of ASD-related phenotypes
536 on the SRS sub-scales, marked by less impaired scores on the Social Motivation sub-scale and
537 extremely high scores on the Restricted Interests and Repetitive Behaviors sub-scale. This score
538 profile is consistent between 3q29Del males and females and between 3q29Del participants with

539 and without ASD, suggesting that it may be a hallmark behavioral feature of the syndrome and
540 providing a potential therapeutic inroad for the treatment of individuals with 3q29Del. Finally,
541 we identify a high degree of social disability in female 3q29Del participants; the 3q29 deletion
542 elevates the risk ASD in females (OR=41.8, p=4.78E-05) more substantially than in males
543 (OR=24.6, p=6.06E-09). These results demonstrate that there is a benefit to studying rare CNVs
544 such as 3q29Del; studying a single genomic variant with large effect allows us to control for
545 genetic etiology and unmask the mechanisms underlying the development of neuropsychiatric
546 and neurodevelopmental disorders.
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561

562

563

564 **Abbreviations**

565 **ASD**: autism spectrum disorder; **3q29Del**: 3q29 deletion syndrome; **SRS**: Social Responsiveness
566 Scale; **SCQ**: Social Communication Questionnaire; **ASSQ**: Autism Spectrum Screening
567 Questionnaire; **CBCL**: Child Behavior Checklist; **ABCL**: Adult Behavior Checklist; **DSM**:
568 Diagnostic and Statistical Manual; **SZ**: schizophrenia; **ADHD**: attention deficit/hyperactivity
569 disorder; **CNV**: copy number variation; **ID**: intellectual disability; **GDD**: global developmental
570 delay; **MR**: mental retardation; **WS**: Williams Syndrome; **SMS**: Smith-Magenis Syndrome

571

572

573

574

575

576

577

578

579

580

581

582

583

584

585

586

587 **DECLARATIONS**

588 **Ethics approval and consent to participate**

589 This study was approved by Emory University's Institutional Review Board (IRB00064133). All
590 study subjects gave informed consent prior to participating in this study.

591 **Consent for publication**

592 Not applicable.

593 **Availability of data and material**

594 The datasets used and analyzed during the current study are available from the corresponding
595 author on reasonable request.

596 **Competing interests**

597 CAS reports receiving royalties from Pearson Clinical for the Vineland-3.
598 The remaining authors have no competing interests to disclose.

599 **Funding**

600 Funded by NIH 1R01MH110701-01A1 (Mulle), the Treasure Your Exceptions Project (Zwick),
601 and NIH T32 GM0008490 (Pollak).

602 **Authors' contributions**

603 RMP performed the statistical analysis, produced all figures and tables, and wrote the
604 manuscript. MMM collected the data. MPE helped with statistical analyses and interpretation.
605 MMM, CK, and CAS helped with data interpretation. MEZ and JGM edited the manuscript and
606 provided guidance on analyzing and interpreting data. JGM was the principle investigator

607 responsible for study direction. All authors participated in commenting on the drafts and have
608 read and approved the final manuscript.

609 **Acknowledgements**

610 We gratefully acknowledge our study population, the 3q29 deletion community, for their
611 participation and commitment to research. This manuscript is currently available on the bioRxiv
612 preprint server (https://doi.org/10.1101/386243).
613 We also acknowledge the contributions of the members of the Emory 3q29 Project: Hallie
614 Averbach, Emily Black, Gary J Bassell, T Lindsey Burrell, Grace Carlock, Tamara Caspary,
615 Joseph F Cubells, David Cutler, Paul A Dawson, Roberto Espana, Michael J Gambello, Katrina
616 Goines, Henry R Johnston, Sookyong Koh, Elizabeth J Leslie, Longchuan Li, Bryan Mak,
617 Tamika Malone, Trenell Mosley, Derek Novacek, Ryan Purcell, Timothy Rutkowski, Rossana
618 Sanchez, Jason Schroeder, Esra Sefik, Brittney Sholar, Sarah Shultz, Nikisha Sisodiya, Steven
619 Sloan, Elaine F Walker, Stephen T Warren, David Weinshenker, Zhexing Wen, and Mike
620 Zinsmeister.

621

622

623

624

625

626

627

628

629

630

631

632

633

634 **REFERENCES**

635 1. Stefansson H, Meyer-Lindenberg A, Steinberg S, Magnusdottir B, Morgen K,
636 Arnarsdottir S, Bjornsdottir G, Walters GB, Jonsdottir GA, Doyle OM, et al: **CNVs**
637 **conferring risk of autism or schizophrenia affect cognition in controls.** *Nature* 2014,
638 **505:**361-366.

639 2. Kendall KM, Rees E, Escott-Price V, Einon M, Thomas R, Hewitt J, O'Donovan MC,
640 Owen MJ, Walters JTR, Kirov G: **Cognitive Performance Among Carriers of**
641 **Pathogenic Copy Number Variants: Analysis of 152,000 UK Biobank Subjects.** *Biol*
642 *Psychiatry* 2017, **82:**103-110.

643 3. Willatt L, Cox J, Barber J, Cabanas ED, Collins A, Donnai D, FitzPatrick DR, Maher E,
644 Martin H, Parnau J, et al: **3q29 microdeletion syndrome: clinical and molecular**
645 **characterization of a new syndrome.** *American Journal of Human Genetics* 2005,
646 **77:**154-160.

647 4. Ballif BC, Theisen A, Coppinger J, Gowans GC, Hersh JH, Madan-Khetarpal S, Schmidt
648 KR, Tervo R, Escobar LF, Friedrich CA, et al: **Expanding the clinical phenotype of the**
649 **3q29 microdeletion syndrome and characterization of the reciprocal**
650 **microduplication.** *Molecular Cytogenetics* 2008, **1:**8.

651 5. Glassford MR, Rosenfeld JA, Freedman AA, Zwick ME, Mulle JG, Unique Rare
652 Chromosome Disorder Support G: **Novel features of 3q29 deletion syndrome: Results**

653 **from the 3q29 registry.** *American Journal of Medical Genetics Part A* 2016, **170A:**999-
654 1006.
655 6. Cobb W, Anderson A, Turner C, Hoffman RD, Schonberg S, Levin SW: **1.3 Mb de novo**
656 **deletion in chromosome band 3q29 associated with normal intelligence in a child.**
657 *European Journal of Medical Genetics* 2010, **53:**415-418.
658 7. Mulle JG: **The 3q29 deletion confers >40-fold increase in risk for schizophrenia.**
659 *Molecular Psychiatry* 2015, **20:**1028-1029.
660 8. Mulle JG, Dodd AF, McGrath JA, Wolyniec PS, Mitchell AA, Shetty AC, Sobreira NL,
661 Valle D, Rudd MK, Satten G, et al: **Microdeletions of 3q29 confer high risk for**
662 **schizophrenia.** *American Journal of Human Genetics* 2010, **87:**229-236.
663 9. Marshall CR, Howrigan DP, Merico D, Thiruvahindrapuram B, Wu W, Greer DS, Antaki
664 D, Shetty A, Holmans PA, Pinto D, et al: **Contribution of copy number variants to**
665 **schizophrenia from a genome-wide study of 41,321 subjects.** *Nature Genetics* 2017,
666 **49:**27-35.
667 10. Kirov G, Pocklington AJ, Holmans P, Ivanov D, Ikeda M, Ruderfer D, Moran J,
668 Chambert K, Toncheva D, Georgieva L, et al: **De novo CNV analysis implicates**
669 **specific abnormalities of postsynaptic signalling complexes in the pathogenesis of**
670 **schizophrenia.** *Mol Psychiatry* 2012, **17:**142-153.
671 11. Szatkiewicz JP, O'Dushlaine C, Chen G, Chambert K, Moran JL, Neale BM, Fromer M,
672 Ruderfer D, Akterin S, Bergen SE, et al: **Copy number variation in schizophrenia in**
673 **Sweden.** *Molecular Psychiatry* 2014, **19:**762.
674 12. Cox DM, Butler MG: **A clinical case report and literature review of the 3q29**
675 **microdeletion syndrome.** *Clinical dysmorphology* 2015, **24:**89-94.

676 13. Città S, Buono S, Greco D, Barone C, Alfei E, Bulgheroni S, Usilla A, Pantaleoni C,
677 Romano C: **3q29 microdeletion syndrome: Cognitive and behavioral phenotype in**
678 **four patients.** *American Journal of Medical Genetics Part A* 2013, **161A:**3018-3022.
679 14. Sagar A, Bishop JR, Tessman DC, Guter S, Martin CL, Cook EH: **Co-occurrence of**
680 **autism, childhood psychosis, and intellectual disability associated with a de novo**
681 **3q29 microdeletion.** *American Journal of Medical Genetics Part A* 2013, **161A:**845-
682 849.
683 15. Quintero-Rivera F, Sharifi-Hannauer P, Martinez-Agosto JA: **Autistic and psychiatric**
684 **findings associated with the 3q29 microdeletion syndrome: case report and review.**
685 *American Journal of Medical Genetics Part A* 2010, **152A:**2459-2467.
686 16. Biamino E, Di Gregorio E, Belligni EF, Keller R, Riberi E, Gandione M, Calcia A,
687 Mancini C, Giorgio E, Cavalieri S, et al: **A novel 3q29 deletion associated with autism,**
688 **intellectual disability, psychiatric disorders, and obesity.** *American Journal of*
689 *Medical Genetics Part B, Neuropsychiatric Genetics* 2016, **171B:**290-299.
690 17. Itsara A, Cooper GM, Baker C, Girirajan S, Li J, Absher D, Krauss RM, Myers RM,
691 Ridker PM, Chasman DI, et al: **Population analysis of large copy number variants and**
692 **hotspots of human genetic disease.** *American Journal of Human Genetics* 2009,
693 **84:**148-161.
694 18. Sanders SJ, He X, Willsey AJ, Ercan-Sencicek AG, Samocha KE, Cicek AE, Murtha
695 MT, Bal VH, Bishop SL, Dong S, et al: **Insights into Autism Spectrum Disorder**
696 **Genomic Architecture and Biology from 71 Risk Loci.** *Neuron* 2015, **87:**1215-1233.
697 19. Schneider M, Debbané M, Bassett AS, Chow EWC, Fung WLA, van den Bree M, Owen
698 M, Murphy KC, Niarchou M, Kates WR, et al: **Psychiatric disorders from childhood to**

699 **adulthood in 22q11.2 deletion syndrome: results from the International Consortium**
700 **on Brain and Behavior in 22q11.2 Deletion Syndrome.** *The American Journal of*
701 *Psychiatry* 2014, **171:**627-639.
702 20. McDonald-McGinn DM, Sullivan KE, Marino B, Philip N, Swillen A, Vorstman JA,
703 Zackai EH, Emanuel BS, Vermeesch JR, Morrow BE, et al: **22q11.2 deletion syndrome.**
704 *Nat Rev Dis Primers* 2015, **1:**15071.
705 21. D'Angelo D, Lebon S, Chen Q, Martin-Brevet S, Snyder LG, Hippolyte L, Hanson E,
706 Maillard AM, Faucett WA, Mace A, et al: **Defining the Effect of the 16p11.2**
707 **Duplication on Cognition, Behavior, and Medical Comorbidities.** *JAMA Psychiatry*
708 2016, **73:**20-30.
709 22. Hanson E, Bernier R, Porche K, Jackson FI, Goin-Kochel RP, Snyder LG, Snow AV,
710 Wallace AS, Campe KL, Zhang Y, et al: **The cognitive and behavioral phenotype of**
711 **the 16p11.2 deletion in a clinically ascertained population.** *Biological Psychiatry*
712 2015, **77:**785-793.
713 23. Mervis CB, Klein-Tasman BP, Huffman MJ, Velleman SL, Pitts CH, Henderson DR,
714 Woodruff-Borden J, Morris CA, Osborne LR: **Children with 7q11.23 duplication**
715 **syndrome: psychological characteristics.** *American Journal of Medical Genetics Part A*
716 2015, **167:**1436-1450.
717 24. Brunetti-Pierri N, Berg JS, Scaglia F, Belmont J, Bacino CA, Sahoo T, Lalani SR,
718 Graham B, Lee B, Shinawi M, et al: **Recurrent reciprocal 1q21.1 deletions and**
719 **duplications associated with microcephaly or macrocephaly and developmental and**
720 **behavioral abnormalities.** *Nature Genetics* 2008, **40:**1466-1471.

25. Constantino JN, Todd RD: *The social responsiveness scale manual.* 2 edn. Los Angeles: Western Psychological Services; 2012.

26. Rutter M, Bailey A, Lord C: *The Social Communication Questionnaire - Manual.* Los Angeles: Western Psychological Services; 2003.

27. Ehlers S, Gillberg C, Wing L: **A screening questionnaire for Asperger syndrome and other high-functioning Autism Spectrum Disorders in school age children.** *Journal of Autism and Developmental Disorders* 1999, **29**.

28. Achenbach TM, Rescorla LA: *Manual for the ASEBA School-Age Forms & Profiles.* Burlington, VT: University of Vermont, Research Center for Children, Youth and Families; 2001.

29. Achenbach TM, Rescorla LA: *Manual for the ASEBA Adult Forms & Profiles.* Burlington, VT: University of Vermont, Research Center for Children, Youth and Families; 2003.

30. R Core Team: **R: A language and environment for statistical computing.** *R Foundation for Statistical Computing, Vienna, Austria* 2008.

31. Christensen RHB: **ordinal - Regression Models for Ordinal Data.** *R package version 20156-28* 2015.

32. Nakazawa M: **fmsb: Functions for medical statistics book with some demographic data.** *R package version 063* 2018.

33. Sievert C, Parmer C, Hocking T, Chamberlain S, Ram K, Corvellec M, Despouy P: **plotly: Create Interactive Web Graphics via 'plotly.js'.** *R package version 460* 2017.

34. Wickham H: *ggplot2: Elegant Graphics for Data Analysis.* New York: Springer-Verlag; 2009.

744 35. Kessler RC, Birnbaum H, Demler O, Falloon IR, Gagnon E, Guyer M, Howes MJ,
745 Kendler KS, Shi L, Walters E, Wu EQ: **The prevalence and correlates of nonaffective**
746 **psychosis in the National Comorbidity Survey Replication (NCS-R).** *Biol Psychiatry*
747 2005, **58:**668-676.
748 36. Wu EQ, Shi L, Birnbaum H, Hudson T, Kessler R: **Annual prevalence of diagnosed**
749 **schizophrenia in the USA: a claims data analysis approach.** *Psychol Med* 2006,
750 **36:**1535-1540.
751 37. Desai PR, Lawson KA, Barner JC, Rascati KL: **Estimating the direct and indirect costs**
752 **for community-dwelling patients with schizophrenia.** *Journal of Pharmaceutical*
753 *Health Services Research* 2013, **4:**187-194.
754 38. Saha S, Chant D, Welham J, McGrath J: **A systematic review of the prevalence of**
755 **schizophrenia.** *PLoS Med* 2005, **2:**e141.
756 39. Moreno-Kustner B, Martin C, Pastor L: **Prevalence of psychotic disorders and its**
757 **association with methodological issues. A systematic review and meta-analyses.**
758 *PLoS One* 2018, **13:**e0195687.
759 40. Merikangas KR, He JP, Burstein M, Swanson SA, Avenevoli S, Cui L, Benjet C,
760 Georgiades K, Swendsen J: **Lifetime prevalence of mental disorders in U.S.**
761 **adolescents: results from the National Comorbidity Survey Replication--Adolescent**
762 **Supplement (NCS-A).** *J Am Acad Child Adolesc Psychiatry* 2010, **49:**980-989.
763 41. Christensen DL, Braun KVN, Baio J, Bilder D, Charles J, Constantino JN, Daniels J,
764 Durkin MS, Fitzgerald RT, Kurzius-Spencer M, et al: **Prevalence and Characteristics**
765 **of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and**

766 **Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012.**
767 *MMWR Surveill Summ* 2018, **65:**1-23.
768 42. Torske T, Naerland T, Oie MG, Stenberg N, Andreassen OA: **Metacognitive Aspects of**
769 **Executive Function Are Highly Associated with Social Functioning on Parent-Rated**
770 **Measures in Children with Autism Spectrum Disorder.** *Front Behav Neurosci* 2017,
771 **11:**258.
772 43. Murphy MM, Lindsey Burrell T, Cubells JF, Espana RA, Gambello MJ, Goines KCB,
773 Klaiman C, Li L, Novacek DM, Papetti A, et al: **Study protocol for The Emory 3q29**
774 **Project: evaluation of neurodevelopmental, psychiatric, and medical symptoms in**
775 **3q29 deletion syndrome.** *BMC Psychiatry* 2018, **18:**183.
776 44. Rogers BT, Msali ME, Buck GM, Lyon NR, Norris MK, Roland JMA, Gingell RL,
777 Cleveland DC, Pieroni DR: **Neurodevelopmental outcome of infants with hypoplastic**
778 **left heart syndrome.** *The Journal of Pediatrics* 1995**:**496-498.
779 45. Forbess JM, Visconti KJ, Hancock-Friesen C, Howe RC, Bellinger DC, Jonas RA:
780 **Neurodevelopmental outcome after congenital heart surgery: Results from an**
781 **institutional registry.** *Circulation* 2002, **106:**I-95-I-102.
782 46. Wernovsky G, Shillingford AJ, Gaynor JW: **Central nervous system outcomes in**
783 **children with complex congenital heart disease.** *Current Opinions in Cardiology*
784 2005**:**94-99.
785 47. Karsdorp PA, Everaerd W, Kindt M, Mulder BJ: **Psychological and cognitive**
786 **functioning in children and adolescents with congenital heart disease: a meta-**
787 **analysis.** *J Pediatr Psychol* 2007, **32:**527-541.

788 48. Shillingford AJ, Glanzman MM, Ittenbach RF, Clancy RR, Gaynor JW, Wernovsky G:
789 **Inattention, hyperactivity, and school performance in a population of school-age**
790 **children with complex congenital heart disease.** *Pediatrics* 2008, **121:**e759-767.
791 49. Kovacs AH, Saidi AS, Kuhl EA, Sears SF, Silversides C, Harrison JL, Ong L, Colman J,
792 Oechslin E, Nolan RP: **Depression and anxiety in adult congenital heart disease:**
793 **predictors and prevalence.** *Int J Cardiol* 2009, **137:**158-164.
794 50. Bishop SL, Thurm A, Farmer C, Lord C: **Autism Spectrum Disorder, Intellectual**
795 **Disability, and Delayed Walking.** *Pediatrics* 2016, **137:**e20152959.
796 51. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Cicek AE, Kou Y, Liu L,
797 Fromer M, Walker S, et al: **Synaptic, transcriptional and chromatin genes disrupted**
798 **in autism.** *Nature* 2014, **515:**209-215.
799 52. Noterdaeme M, Minow F, Amorosa H: **[Applicability of the Child Behavior Checklist**
800 **in developmentally delayed children].** *Z Kinder Jugendpsychiatr Psychother* 1999,
801 **27:**183-188.
802 53. Mazefsky CA, Anderson R, Conner CM, Minshew N: **Child Behavior Checklist Scores**
803 **for School-Aged Children with Autism: Preliminary Evidence of Patterns**
804 **Suggesting the Need for Referral.** *Journal of psychopathology and behavioral*
805 *assessment* 2011, **33:**31-37.
806 54. Bruining H, Eijkemans MJ, Kas MJ, Curran SR, Vorstman JA, Bolton PF: **Behavioral**
807 **signatures related to genetic disorders in autism.** *Mol Autism* 2014, **5:**11.
808 55. Klein-Tasman BP, Mervis CB: **Autism Spectrum Symptomatology Among Children**
809 **with Duplication 7q11.23 Syndrome.** *J Autism Dev Disord* 2018, **48:**1982-1994.

810 56. Hidding E, Swaab H, de Sonneville LM, van Engeland H, Sijmens-Morcus ME, Klaassen
811 PW, Duijff SN, Vorstman JA: **Intellectual functioning in relation to autism and**
812 **ADHD symptomatology in children and adolescents with 22q11.2 deletion**
813 **syndrome.** *J Intellect Disabil Res* 2015, **59:**803-815.
814 57. Vorstman JA, Breetvelt EJ, Thode KI, Chow EW, Bassett AS: **Expression of autism**
815 **spectrum and schizophrenia in patients with a 22q11.2 deletion.** *Schizophr Res* 2013,
816 **143:**55-59.
817 58. Vorstman JA, Morcus ME, Duijff SN, Klaassen PW, Heineman-de Boer JA, Beemer FA,
818 Swaab H, Kahn RS, van Engeland H: **The 22q11.2 deletion in children: high rate of**
819 **autistic disorders and early onset of psychotic symptoms.** *J Am Acad Child Adolesc*
820 *Psychiatry* 2006, **45:**1104-1113.
821 59. Qiao Y, Riendeau N, Koochek M, Liu X, Harvard C, Hildebrand MJ, Holden JJ, Rajcan-
822 Separovic E, Lewis ME: **Phenomic determinants of genomic variation in autism**
823 **spectrum disorders.** *J Med Genet* 2009, **46:**680-688.
824 60. Flor J, Bellando J, Lopez M, Shui A: **Developmental functioning and medical Co-**
825 **morbidity profile of children with complex and essential autism.** *Autism Res* 2017,
826 **10:**1344-1352.
827 61. Finlay WML, Lyons E: **Methodological issues in interviewing and using self-report**
828 **questionnaires with people with mental retardation.** *Psychological Assessment* 2001,
829 **13:**319-335.
830 62. Fisher MH, Mello MP, Dykens EM: **Who reports it best? A comparison between**
831 **parent-report, self-report, and the real life social behaviors of adults with Williams**
832 **syndrome.** *Research in Developmental Disabilities* 2014, **35:**3276-3284.

833 63. Lincoln AJ, Searcy YM, Jones W, Lord C: **Social interaction behaviors discriminate**
834 **young children with autism and Williams syndrome.** *J Am Acad Child Adolesc*
835 *Psychiatry* 2007, **46:**323-331.
836 64. Riby DM, Hanley M, Kirk H, Clark F, Little K, Fleck R, Janes E, Kelso L, O'Kane F,
837 Cole-Fletcher R, et al: **The interplay between anxiety and social functioning in**
838 **Williams syndrome.** *J Autism Dev Disord* 2014, **44:**1220-1229.
839 65. van der Fluit F, Gaffrey MS, Klein-Tasman BP: **Social Cognition in Williams**
840 **Syndrome: Relations between Performance on the Social Attribution Task and**
841 **Cognitive and Behavioral Characteristics.** *Front Psychol* 2012, **3:**197.
842 66. Morris CA, Mervis CB, Paciorkowski AP, Abdul-Rahman O, Dugan SL, Rope AF, Bader
843 P, Hendon LG, Velleman SL, Klein-Tasman BP, Osborne LR: **7q11.23 Duplication**
844 **syndrome: Physical characteristics and natural history.** *Am J Med Genet A* 2015,
845 **167a:**2916-2935.
846 67. Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, Chu
847 SH, Moreau MP, Gupta AR, Thomson SA, et al: **Multiple recurrent de novo CNVs,**
848 **including duplications of the 7q11.23 Williams syndrome region, are strongly**
849 **associated with autism.** *Neuron* 2011, **70:**863-885.
850 68. Niarchou M, Chawner S, Doherty JL, Maillard AM, Jacquemont S, Chung WK, Green-
851 Snyder L, Bernier RA, Goin-Kochel RP, Hanson E, et al: **Psychiatric disorders in**
852 **children with 16p11.2 deletion and duplication.** *Transl Psychiatry* 2019, **9:**8.
853 69. Zufferey F, Sherr EH, Beckmann ND, Hanson E, Maillard AM, Hippolyte L, Macé A,
854 Ferrari C, Kutalik Z, Andrieux J, et al: **A 600 kb deletion syndrome at 16p11.2 leads to**

855 **energy imbalance and neuropsychiatric disorders.** *Journal of Medical Genetics* 2012,
856 **49:**660-668.
857 70. Green Snyder L, D'Angelo D, Chen Q, Bernier R, Goin-Kochel RP, Wallace AS, Gerdts
858 J, Kanne S, Berry L, Blaskey L, et al: **Autism Spectrum Disorder, Developmental and**
859 **Psychiatric Features in 16p11.2 Duplication.** *J Autism Dev Disord* 2016, **46:**2734-
860 2748.
861 71. Nag HE, Nordgren A, Anderlid BM, Nærland T: **Reversed gender ratio of autism**
862 **spectrum disorder in Smith-Magenis syndrome.** *Mol Autism* 2018, **9:**1.
863 72. Schreiner MJ, Karlsgodt KH, Uddin LQ, Chow C, Congdon E, Jalbrzikowski M, Bearden
864 CE: **Default mode network connectivity and reciprocal social behavior in 22q11.2**
865 **deletion syndrome.** *Soc Cogn Affect Neurosci* 2014, **9:**1261-1267.
866 73. Niklasson L, Rasmussen P, Oskarsdottir S, Gillberg C: **Autism, ADHD, mental**
867 **retardation and behavior problems in 100 individuals with 22q11 deletion**
868 **syndrome.** *Res Dev Disabil* 2009, **30:**763-773.
869 74. Sobin C, Kiley-Brabeck K, Monk SH, Khuri J, Karayiorgou M: **Sex differences in the**
870 **behavior of children with the 22q11 deletion syndrome.** *Psychiatry Res* 2009, **166:**24-
871 34.
872 75. Zablotsky B, Black LI, Blumberg SJ: **Estimated Prevalence of Children With**
873 **Diagnosed Developmental Disabilities in the United States, 2014-2016.** *NCHS Data*
874 *Brief* 2017**:**1-8.
875
876
877

Additional Files

File name: Supplemental Information

File format: Microsoft Word document (.docx)

Title of data: Supplementary figures and tables

Description of data: Supplementary figures are (S1) SCQ scores split by ASD status, sex, and ASD status/sex, (S2) CBCL/ABCL Withdrawn sub-scale scores split by genotype, sex, and ASD status, and (S3) geographic distribution of study participants with 3q29Del. Supplementary tables are (S1) questionnaire demographics for the medical questionnaire, SRS, SCQ, ASSQ, and CBCL/ABCL; (S2 and S3) sensitivity analysis description and results for effect of 3q29Del self-registrants; (S4) comparison of scores on all four scales for 3q29Del versus control; (S5) SRS score comparison stratified by ASD status and sex; (S6) SCQ score comparison stratified by ASD status and sex; (S7) CBCL/ABCL DSM-oriented sub-scale score comparison; (S8) contribution of congenital heart defects to phenotypes of interest; (S9) test for confounding factors contributing to symptomology questionnaire scores; (S10) CBCL/ABCL Withdrawn sub-scale score comparison; (S11) heart defects present in study sample; (S12) comparison of 3q29 registry-leveraged and gold-standard phenotyping measures; and (S13) comparison of CBC/ABCL sub-scale scores between 3q29Del and 22q11.2 deletion.

901 **Table 2: Self-reported neuropsychiatric diagnoses.**

		3q29 Deletion Syndrome			Control			P value; 3q29Del vs. Control
		Total	Male	Female	Total	Male	Female	
GDD/MR (n, %)								<2.20E-16
	Yes	55 (59.1%)	31 (57.4%)	24 (61.5%)	1.14%*	1.48%*	0.90%*	
	No	38 (40.9%)	23 (42.6%)	15 (38.5%)	64 (100%)	33 (100%)	31 (100%)	
ASD (n, %)								<2.20E-16
	Yes	27 (29.0%)	20 (37.0%)	7 (17.9%)	1.47%*	2.34%*	0.52%*	
	No	66 (71.0%)	34 (63.0%)	32 (82.1%)	64 (100%)	33 (100%)	31 (100%)	
Anxiety (n, %)								0.001
	Yes	26 (28.0%)	15 (27.8%)	11 (28.2%)	4 (6.2%)	2 (6.1%)	2 (6.5%)	
	No	67 (72.0%)	39 (72.2%)	28 (71.8%)	60 (93.8%)	31 (93.9%)	29 (93.5%)	
Bipolar/Manic Depression (n. %)								0.146
	Yes	4 (4.3%)	2 (3.7%)	2 (5.1%)	0 (0%)	0 (0%)	0 (0%)	
	No	89 (95.7%)	52 (96.3%)	37 (94.9%)	64 (100%)	33 (100%)	31 (100%)	
Conduct Disorder (n, %)								1.00
	Yes	1 (1.1%)	1 (1.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
	No	92 (98.9%)	53 (98.1%)	39 (100%)	64 (100%)	33 (100%)	31 (100%)	
Depression (n, %)								1.00
	Yes	6 (6.5%)	2 (3.7%)	4 (10.3%)	4 (6.2%)	4 (12.1%)	0 (0%)	
	No	87 (93.5%)	52 (96.3%)	35 (89.7%)	60 (93.8%)	29 (87.9%)	31 (100%)	
Oppositional Defiant Disorder (n, %)								0.271
	Yes	3 (3.2%)	2 (3.7%)	1 (2.6%)	0 (0%)	0 (0%)	0 (0%)	
	No	90 (96.8%)	52 (96.3%)	38 (97.4%)	64 (100%)	33 (100%)	31 (100%)	
Panic Attacks (n, %)								0.045
	Yes	12 (12.9%)	9 (16.7%)	4 (10.3%)	2 (3.2%)	0 (0%)	2 (6.5%)	
	No	81 (87.1%)	45 (83.3%)	35 (89.7%)	62 (96.8%)	33 (100%)	29 (93.5%)	
Schizophrenia (n, %)								0.146

Yes	4 (4.3%)	1 (1.9%)	3 (7.7%)	0 (0%)	0 (0%)	0 (0%)
No	89 (95.7%)	53 (98.1%)	36 (92.3%)	64 (100%)	33 (100%)	31 (100%)

902 Characteristics of self-reported neuropsychiatric diagnoses in study participants with 3q29Del and controls. Asterisks indicate where
903 3q29Del was compared to general population prevalence values [41, 75]. P values were calculated with one-sample proportion test
904 with Yates' continuity correction when comparing to population prevalence and Fisher's exact test when comparing to controls.
905
906
907
908
909
910
911
912
913
914
915
916
917

Table 3: SRS sub-scale score comparison stratified by genotype, ASD status, and sex.

	Social Awareness		Social Cognition		Social Communication		Social Motivation		RRB		SCI	
	Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value
Genotype												
Control	47.04 ± 8.88	-	45.27 ± 7.64	-	45.88 ± 8.14	-	46.13 ± 7.66	-	47.66 ± 8.51	-	45.50 ± 7.74	-
3q29Del	67.33 ± 13.28	1.45E-13	69.06 ± 15.51	1.20E-15	69.69 ± 13.92	<2.00E-16	62.10 ± 13.52	1.62E-10	77.31 ± 14.25	<2.00E-16	69.52 ± 14.63	<2.00E-16
Sex												
Male control	45.97 ± 9.15	-	45.23 ± 7.42	-	45.73 ± 6.34	-	46.57 ± 6.58	-	47.63 ± 5.59	-	45.37 ± 6.60	-
Male 3q29Del	69.92 ± 13.92	7.61E-09	71.08 ± 17.65	1.07E-08	72.12 ± 15.47	1.74E-10	63.92 ± 15.23	3.39E-06	79.92 ± 14.82	2.84E-13	72.00 ± 16.34	7.12E-10
Female control	48.27 ± 8.56	-	45.31 ± 8.03	-	46.04 ± 9.95	-	45.62 ± 8.85	-	47.69 ± 11.08	-	45.65 ± 9.01	-
Female 3q29Del	64.27 ± 12.08	4.52E-06	66.68 ± 12.51	1.29E-08	66.82 ± 11.52	2.99E-08	59.95 ± 11.14	1.36E-05	74.23 ± 13.22	1.30E-09	66.59 ± 12.02	1.87E-08
ASD Status												
Control	47.04 ± 8.88	-	45.27 ± 7.64	-	45.88 ± 8.14	-	46.13 ± 7.66	-	47.66 ± 8.51	-	45.50 ± 7.74	-
No ASD diagnosis 3q29Del	62.61 ± 13.20	5.17E-09	64.10 ± 15.80	5.77E-11	64.61 ± 13.54	1.20E-12	58.00 ± 13.15	1.42E-06	70.58 ± 11.50	<2.00E-16	64.13 ± 14.39	5.19E-12
ASD diagnosis 3q29Del	75.94 ± 8.33	2.43E-15	78.12 ± 10.18	<2.00E-16	78.94 ± 9.18	<2.00E-16	69.59 ± 10.99	5.12E-12	89.59 ± 10.04	<2.00E-16	79.35 ± 9.02	<2.00E-16

919 Comparison of mean scores on the SRS sub-scales between study participants with 3q29Del and controls. 3q29Del participants were
920 stratified by ASD status and sex for further analysis. P values were calculated using simple linear regression, adjusting for age, race,
921 and sex.
922
923
924
925
926
927
928
929
930
931
932
933
934
935

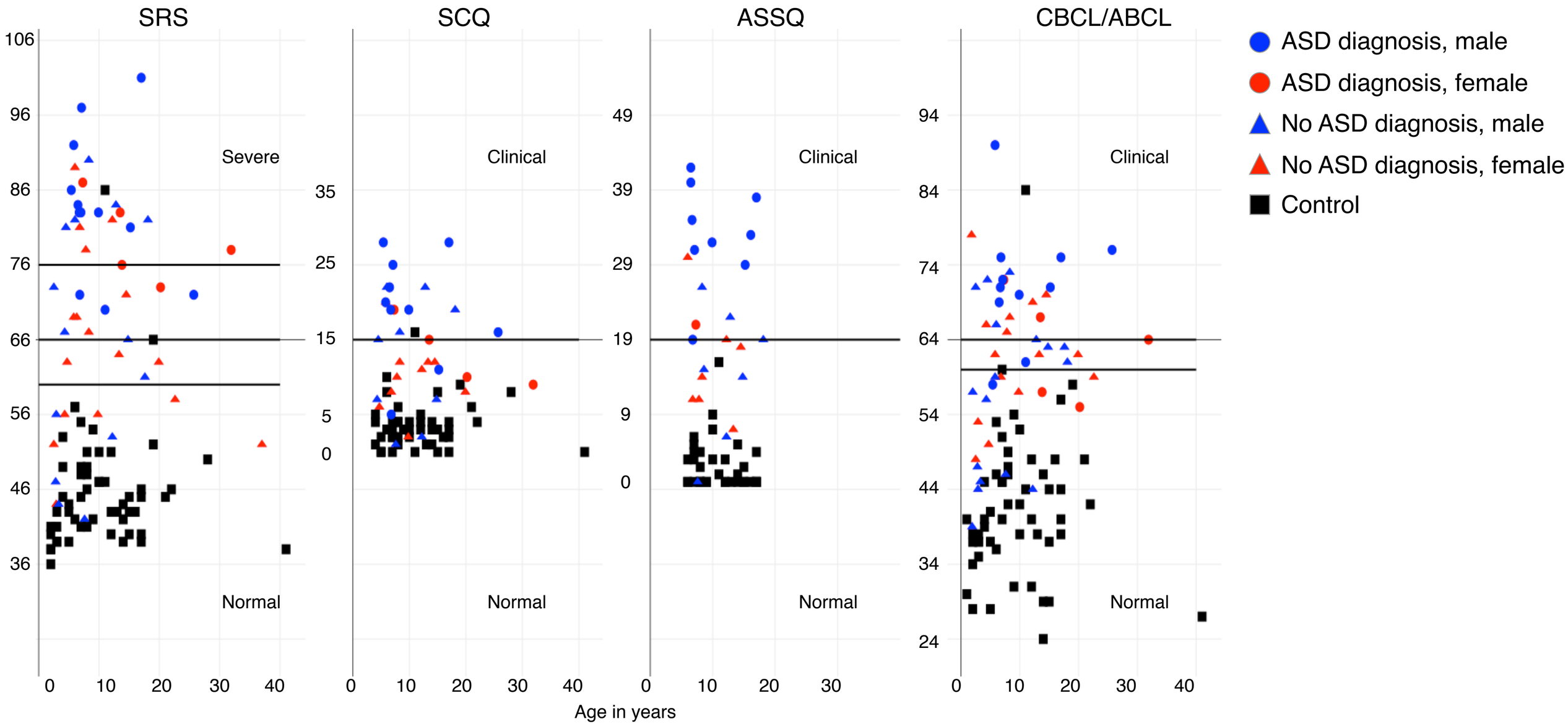
936 **Figure Titles and Legends**

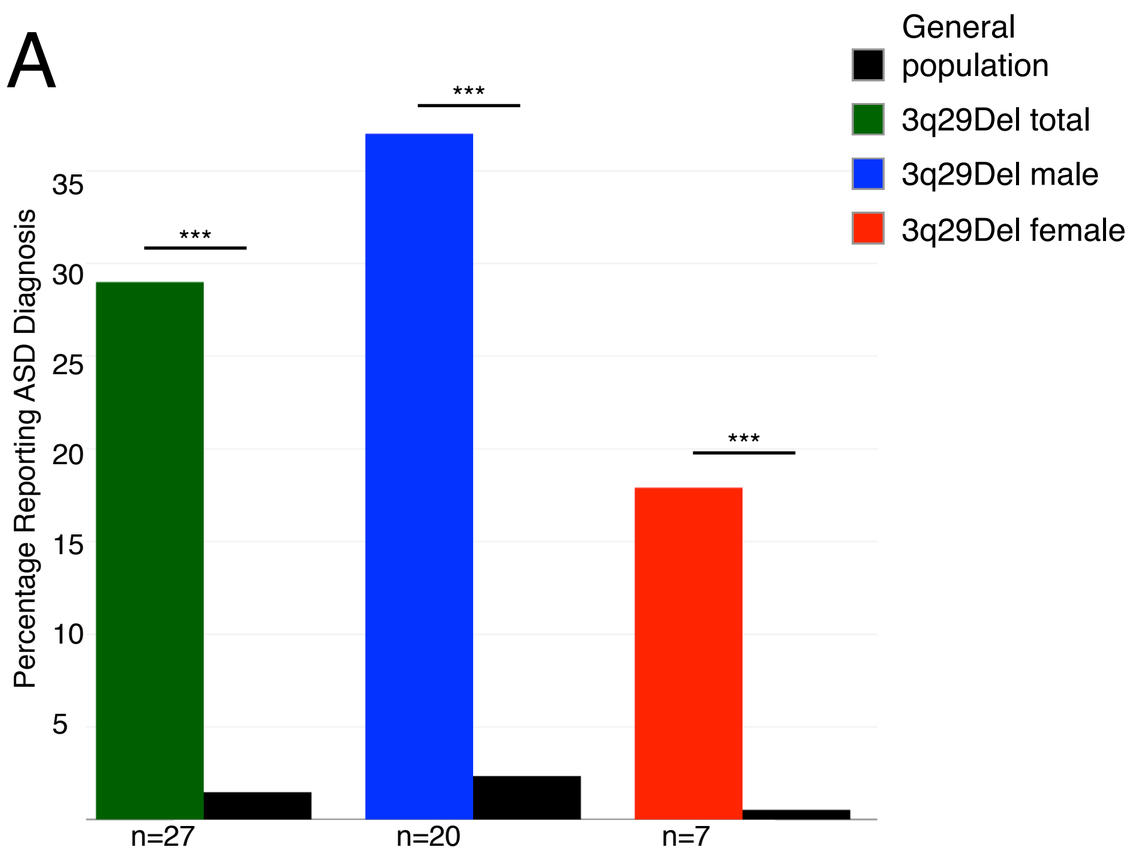
937 **Figure 1. Score distribution for 3q29Del and controls on the SRS, SCQ, ASSQ, and**
938 **CBCL/ABCL.** Total scores on the SRS (n=48 3q29Del, 56 control), SCQ (n=33 3q29Del, 46
939 control), ASSQ (n=24 3q29Del, 35 control), and CBCL/ABCL (n=48 3q29Del, 57 control) for
940 registry participants. Self-reported diagnosis of ASD is denoted by shape (circle/ASD,
941 triangle/no ASD), and sex of participant is denoted by color (red/female, blue/male). Controls are
942 shown in black.

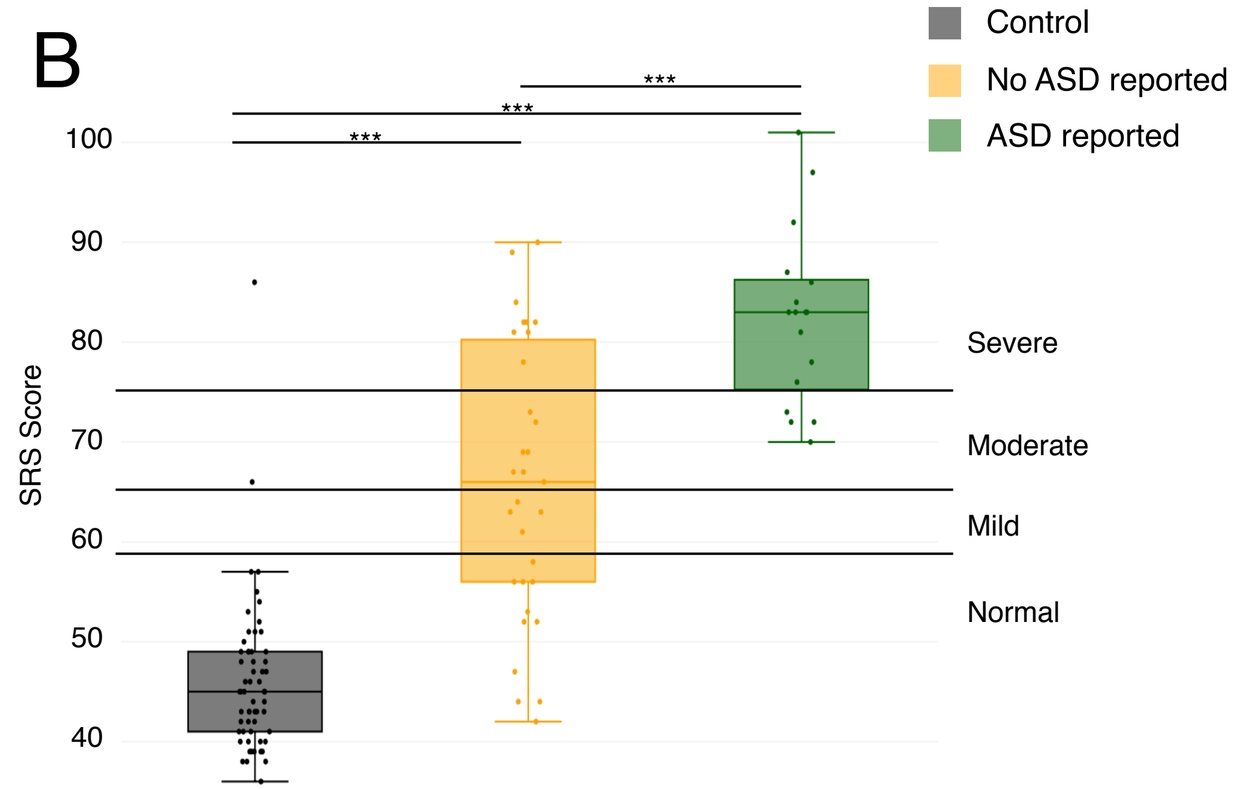
943 **Figure 2. Comparison of ASD prevalence and SRS scores between 3q29Del and controls. A)**
944 Proportion of participants with 3q29Del self-reporting a diagnosis of ASD (not all respondents
945 completed symptom questionnaires); 27 cases report an ASD diagnosis (green), comprised of 20
946 males (blue) and 7 females (red). Compared to general population frequencies (black), cases
947 report significantly higher incidence of ASD. **B)** SRS scores split by control (n=59), 3q29Del not
948 reporting an ASD diagnosis (n=31), and 3q29Del reporting an ASD diagnosis (n=17), showing a
949 significant association between self-reported diagnostic status and SRS score. **C)** SRS scores
950 split by sex, with control (n=59), 3q29Del female (n=22), and 3q29Del male (n=26), showing a
951 lack of sex bias in scores for 3q29Del participants. **D)** SRS scores split by sex and self-reported
952 diagnostic status, with control (n=59), 3q29Del female reporting ASD (n=5), 3q29Del female not
953 reporting ASD (n=17), 3q29Del male reporting ASD (n=12), and 3q29Del male not reporting
954 ASD (n=14), showing inflated scores for 3q29Del participants irrespective of sex or diagnostic
955 status. ***, $p<0.001$

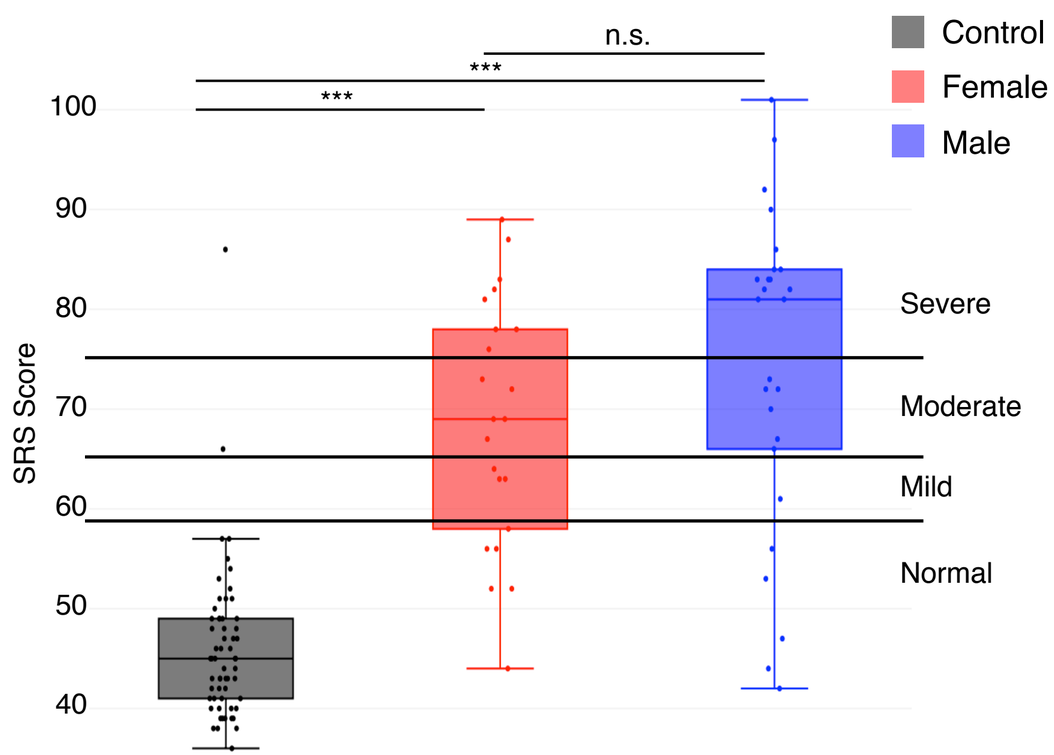
956 **Figure 3. Comparison of SRS sub-scales and CBCL/ABCL DSM-oriented sub-scales**
957 **between 3q29Del and controls. A)** Profile of individuals with 3q29Del (n=48) and controls
958 (n=59) across SRS sub-scales, showing moderate to severe impairment of 3q29Del participants

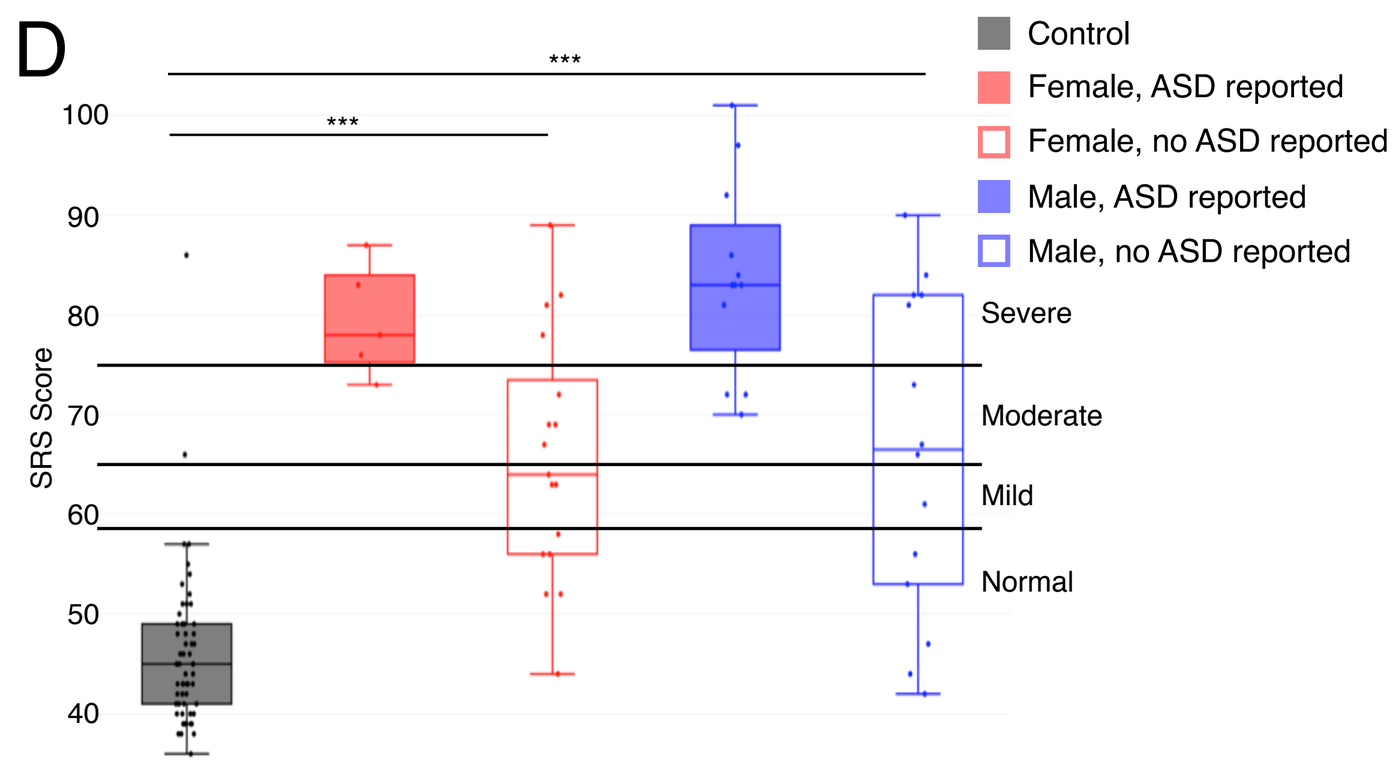
959 in all domains except Social Motivation (RRB, Restricted Interests and Repetitive Behaviors;
960 SCI, Social Communication and Interaction). **B)** Profile of 3q29Del males (n=26) and females
961 (n=22) and controls (n=59) across SRS sub-scales, showing that 3q29Del males and females
962 both score significantly higher than controls and that there are no significant differences in score
963 between males and females. **C)** Profile of 3q29Del participants reporting an ASD diagnosis
964 (n=17) and participants not reporting an ASD diagnosis (n=31) and controls (n=59) across SRS
965 sub-scales, showing that 3q29Del participants score significantly higher than controls
966 irrespective of ASD status, with 3q29Del participants reporting an ASD diagnosis scoring
967 significantly higher than those not reporting an ASD diagnosis. **D)** Profile of 3q29Del
968 participants (n=48) and controls (n=57) across 3 DSM-oriented sub-scales from the CBCL and
969 ABCL, showing significantly increased pathology in 3q29Del participants in all 3 domains. ***,
970 $p<0.001$

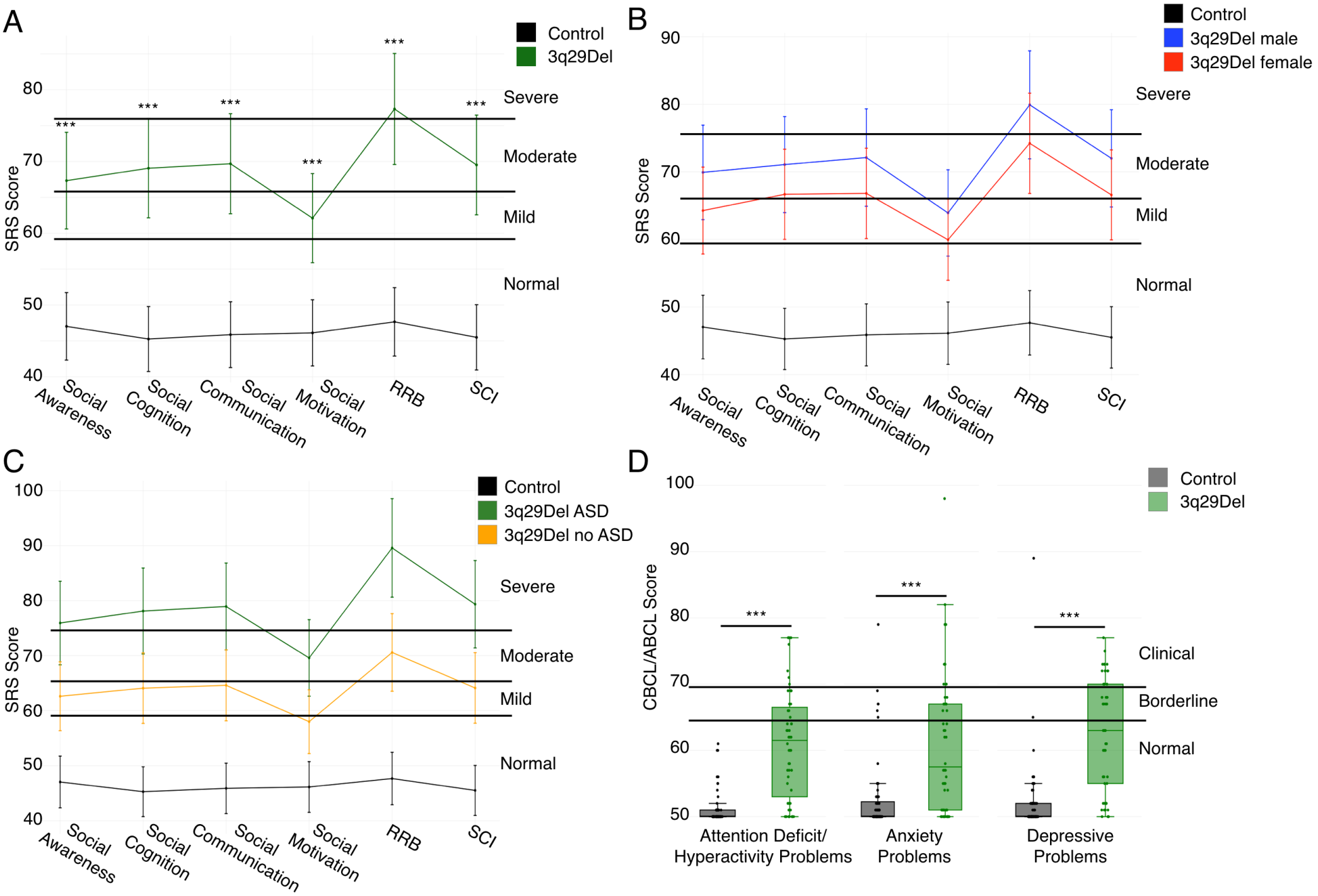

SRS
SCQ
ASSQ
CBCL/ABCL
Severe
Clinical
Clinical
Clinical
Normal
Normal
Normal
Normal
106
96
86
76
66
56
46
36
35
25
15
5
0
49
39
29
19
9
0
94
84
74
64
54
44
34
24
0
10
20
30
40
Age in years
ASD diagnosis, male
ASD diagnosis, female
No ASD diagnosis, male
No ASD diagnosis, female
Control


A
General population
3q29Del total
3q29Del male
3q29Del female
Percentage Reporting ASD Diagnosis
35
30
25
20
15
10
5
***
***
***
n=27
n=20
n=7


B
Control
No ASD reported
ASD reported
SRS Score
100
90
80
70
60
50
40
***
***
***
Severe
Moderate
Mild
Normal


Control
Female
Male
SRS Score
100
90
80
70
60
50
40
n.s.
***
***
Severe
Moderate
Mild
Normal


D
Control
Female, ASD reported
Female, no ASD reported
Male, ASD reported
Male, no ASD reported
SRS Score
100
90
80
70
60
50
40
***
***
Severe
Moderate
Mild
Normal


A
Control
3q29Del
SRS Score
Severe
Moderate
Mild
Normal
Social Awareness
Social Cognition
Social Communication
Social Motivation
RRB
SCI
B
Control
3q29Del male
3q29Del female
C
Control
3q29Del ASD
3q29Del no ASD
D
Control
3q29Del
CBCL/ABCL Score
Clinical
Borderline
Normal
Attention Deficit/ Hyperactivity Problems
Anxiety Problems
Depressive Problems