Full article title: Neuropsychiatric Phenotypes and Novel Features of ASD in 3q29 Deletion Syndrome: Results from the 3q29 Registry

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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) through the 3q29 registry (https://3q29deletion.patientcrossroads.org); typically developing controls (n=64, 51.6% male) were ascertained for comparison. 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 (30.1% vs. 1.47%, p<2.2E-16). Notably, 3q29 deletion confers a greater influence on risk for ASD in females (OR=49.4, p=1.09E-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 1.8:1 in our study sample. Although 70% 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 (26.9% 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 novel 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 presentation of ASD in this population is novel, with substantially less impaired social motivation compared to idiopathic ASD. Our study implies that ASD evaluation should be the standard of care for individuals with 3q29Del. From a research perspective, the novel 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

96 **BACKGROUND**

97 3q29 deletion syndrome (3q29Del) is a rare (~1 in 30,000) [1, 2] genomic disorder
98 characterized by a 1.6 Mb typically *de novo* deletion on chromosome 3 [3-5]. The interval
99 contains 21 distinct protein-coding genes, 3 antisense transcripts, 1 long noncoding RNA, and 1
100 microRNA. Our understanding of the syndrome phenotype continues to evolve. Initial reports
101 found developmental delay/intellectual disability universal among 3q29 deletion carriers, though
102 some case reports have since identified individuals without cognitive impairment [6]. The 3q29
103 deletion is associated with a 20-40-fold increased risk for schizophrenia (SZ), with multiple
104 replication studies supporting this association [7-11]. Case reports also indicate other
105 neuropsychiatric phenotypes may exist, including attention deficit/hyperactivity disorder
106 (ADHD) and bipolar disorder [3, 4, 12-16]. Previous work by our team examining self-report
107 data from 44 individuals with 3q29Del revealed a high prevalence (~20%) of generalized anxiety
108 disorder [5]. Further, case reports have long suggested an association with autism spectrum
109 disorder (ASD), and studies with large sample sizes indicate that the 3q29 deletion may confer a
110 19-fold increased risk for ASD ($p = 0.001$) [17, 18].

111 The range of neuropsychiatric manifestations in 3q29Del is consistent with other
112 genomic disorders. For example, the 22q11.2 deletion has a well-known association with
113 schizophrenia but is also associated with intellectual disability (ID), ASD, anxiety, mood
114 disorders, and ADHD [19, 20]. A similar constellation of phenotypes, including ASD, ADHD,
115 ID, SZ, and anxiety, has been identified in 16p11.2 deletion and duplication syndromes [21, 22],
116 7q11.23 duplication syndrome [23], and 1q21.1 deletion syndrome [24]. Thus, risk for multiple
117 neuropsychiatric phenotypes appears to be a feature common to many genomic disorders,
118 including 3q29 deletion syndrome.

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

128 **METHODS AND MATERIALS**

129 **Sample**

130 Individuals with 3q29Del were ascertained through the internet-based 3q29 deletion
131 registry (https://3q29deletion.patientcrossroads.org) as previously reported [5]. Briefly,
132 information about the registry was emailed to health care providers, medical geneticists, genetic
133 counselors, and support organizations; the registry is also advertised via Google AdWords,
134 where specific keywords were chosen to target the registry website in internet searches.
135 Participant recruitment, informed consent and assent, and data collection are all performed
136 through the registry website. Data were securely downloaded and de-identified for analysis.
137 After data cleaning of the electronic records (removing spam accounts, duplicate records, and
138 related individuals), 93 3q29Del registrants (58.1% male) were included in the present study,
139 ranging in age from 0.1-41.0 years (mean = 10.1±8.5 years). 64 typically developing controls
140 (51.6% male) were included, ranging in age from 1.0-41.0 years (mean = 9.9±7.2 years).
141 Controls were recruited via emails sent to intramural CDC and Emory listservs and invited to fill
142 out surveys in an identical fashion to cases. Controls reporting a clinical diagnosis of any

143 neurodevelopmental disorder were excluded (n = 1). Description of the study sample can be
144 found in Table 1. This study was approved by Emory University's Institutional Review Board
145 (IRB00064133).

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

	3q29 Deletion Syndrome	Control	P value
Age, years (mean ± SD)	10.1 ± 8.5	9.9 ± 7.2	0.902
Sex (n, %)			0.521
Male	54 (58.1%)	33 (51.6%)	
Female	39 (41.9%)	31 (48.4%)	
Race (n, %)			0.0001
White	81 (87.1%)	41 (64.1%)	
Black/African American	1 (1.1%)	12 (18.8%)	
Other	10 (10.8%)	9 (14.1%)	
Blank	1 (1.1%)	2 (3.1%)	
Heart Defect (n, %)			9.12E-06
Yes	24 (25.8%)	2 (3.1%)	
No	60 (64.5%)	61 (95.3%)	
Blank	9 (9.7%)	1 (1.6%)	
Age at Walking (n, %)			1.51E-09
Normal	42 (45.2%)	60 (93.8%)	
Delayed	22 (23.7%)	1 (1.6%)	
Extremely Delayed	14 (15.1%)	1 (1.6%)	
Unsure	10 (10.8%)	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 proportions 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]. Figures were generated using the plotly and
198 ggplot2 R packages [32, 33].

199 *Sensitivity Analysis*

200 The questionnaires for 90 participants with 3q29Del (96.8%) were completed by a parent
201 or guardian ("parent-registered"), while 3 participants with 3q29Del (3.2%) completed all
202 questionnaires themselves ("self-registered"). All control participants were parent-registered. To

assess whether responses from the self-registered 3q29Del participants were influencing the results, self-registrants were removed and the data were re-analyzed. Self-registrants were not found to have a significant effect on the analyses (Tables S2 and S3). All results include both parent- and self-registrants.

RESULTS

Self-report of neuropsychiatric diagnosis in 3q29Del

Self-report of neuropsychiatric diagnoses in our 3q29Del study subjects (Table 2) revealed a higher prevalence of neuropsychiatric disorder diagnoses compared to controls, including anxiety (26.9%), and compared to general population frequencies, including ASD (30.1%, Figure 2A) and GDD/MR (59.1%) (Table 2), confirming prior work by our group [5]. Reported rates of conduct disorder (3.2% vs. 3.5%) and oppositional defiant disorder (3.2% vs. 3.5%) were similar to those observed in the general population. While a small proportion of participants reported diagnoses of bipolar/manic depression (5.4%), depression (6.5%), and schizophrenia (4.3%), we focused on ASD due to the young age (mean = 10.1 years) of our study population, since many study participants have not reached the age of risk for schizophrenia and other adult-onset disorders. Despite this young age, the self-reported rate of SZ diagnoses in our adult study subjects (age > 18 years, n = 13) was 15-30 times higher than expected (15.4% compared to an expected 0.5-1% in the general population; n = 2) [34-38] and the frequency of bipolar disorder was ~1.8 times higher than expected [39]. A summary of neuropsychiatric diagnoses can be found in Table 2.

[Table 2 here]

SRS, SCQ, ASSQ, and CBCL/ABCL scores

In 3q29 deletion study subjects, the mean SRS score was in the moderate range (*T-score* = 71.8), the mean ASSQ score was in the clinical range (mean = 22.2), and the mean

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

233 **Standardized scores stratified by ASD diagnosis**

234 Next, we examined the relationship between SRS scores and reported ASD diagnosis, to
235 determine whether the score inflation we observed in our study population as a whole was
236 largely due to the increased prevalence of ASD. As expected, we observed that individuals with
237 3q29Del and an ASD diagnosis scored significantly higher than both controls and individuals
238 with 3q29Del without an ASD diagnosis (3q29Del with ASD *T-score* = 82.41, 3q29Del without
239 ASD *T-score* = 65.90, control *T-score* = 45.90, $p < 3.0E-13$, Figure 2B). We were interested to
240 observe that individuals with 3q29Del without an ASD diagnosis also scored significantly higher
241 than controls (3q29Del without ASD *T-score* = 65.90, control *T-score* = 45.90, $p = 2.16E-13$,
242 Figure 2B), indicating that increased SRS scores in individuals with 3q29Del are not driven by
243 ASD diagnostic status alone (Table S5). Similar features were observed in the contribution of
244 ASD diagnosis status to SCQ scores (Figure S1, Table S6).

245 **Standardized scores stratified by sex**

246 Both males and females with 3q29Del reported a significantly increased frequency of
247 ASD diagnoses, with a substantially greater burden for ASD on females with 3q29Del. Males
248 with 3q29Del are at 15-fold increased risk for ASD as compared to the general population
249 (37.0% vs. 2.34%, OR = 24.6, $p = 6.06E-09$) and females are at 39-fold risk compared to the
250 general population (20.5% vs. 0.52%, OR = 49.4, $p = 1.09E-05$) (figure 2A) [40], resulting in a

male:female ratio in our study population of 1.8:1, 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 *T-score* = 74.31, control male *T-score* = 45.80, p = 7.70E-11; 3q29Del female *T-score* = 68.73, control female *T-score* = 46.04, p = 7.42E-09), but they did not score significantly differently from each other (3q29Del male *T-score* = 74.31, 3q29Del female *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 participants without an ASD diagnosis had significantly higher scores than controls (3q29Del male without ASD *T-score* = 66.29, control male *T-score* = 45.80, p = 1.20E-06; 3q29Del female without ASD *T-score* = 65.69, control female *T-score* = 46.04, p = 5.04E-07; Figure 2D). Taken together, this suggests that increased SRS scores in individuals with 3q29Del are not driven by sex alone or by sex and ASD diagnosis status in combination (Table S5); rather, the presence of the deletion itself confers a greater risk for social disability. Furthermore, these data show an enrichment for female ASD in our study population, based on the reduction in male bias and the highly similar scores between males and females with 3q29Del, irrespective of ASD diagnosis status. Similar features were observed in the contribution of sex to SCQ scores (Figure S1, Table S6).

ASD presentation of 3q29Del

While total scores on the SRS, SCQ, ASSQ, and CBCL/ABCL can give an indication of the overall level of impairment of individuals, sub-scores can reveal nuanced deficits in specific behavioral domains. To this end, we analyzed all SRS sub-scales (Social Awareness, Social

Cognition, Social Communication, Social Motivation, Restricted Interests and Repetitive Behaviors, and Social Communication and Interaction) to better understand the extent of social disability in our study population; our goal was to determine whether our observed total score inflation was due to a specific severe deficit in a few domains, or if individuals with 3q29Del showed high scores across all sub-scales. The mean score for the Restricted Interests and Repetitive Behaviors sub-scale was in the severe range (*T-score* = 77.3). Mean scores for Social Awareness (*T-score* = 67.3), Social Cognition (*T-score* = 69.1), Social Communication (*T-score* = 69.7), and Social Communication and Interaction (*T-score* = 69.5) were all in the moderate 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 observed 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) [41]. 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/) 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 ($p > 0.05$, Figure 3B, Table 3). This shows that the social disability in 3q29Del is not qualitatively different between males and females, nor is the degree of social disability distinct between males and females.

ASD presentation stratified by ASD diagnosis

299 We then stratified our study subjects according to reported ASD diagnosis status and
300 examined subscale scores separately for 3q29Del individuals reporting a diagnosis of ASD and
301 those not reporting a diagnosis of ASD. We observed that the shape of the profile is shared
302 between 3q29Del individuals reporting a diagnosis of ASD and those not reporting a diagnosis of
303 ASD, with individuals reporting a diagnosis of ASD scoring on average 10-15 points higher on
304 every sub-scale (Figure 3C). As expected, 3q29Del participants with ASD scored significantly
305 higher on all sub-scales than 3q29Del participants without ASD ($p < 0.005$, Table 3); however,
306 3q29Del participants without ASD still scored significantly higher than controls on all sub-scales
307 ($p < 5.0E-05$, Table 3).
308 [Table 3 here]
309 **Additional neuropsychiatric phenotypes in 3q29Del**
310 To further assess behavioral features associated with the 3q29 deletion, we examined the
311 DSM-oriented Attention Deficit/Hyperactivity Problems, Anxiety Problems, and Depressive
312 Problems sub-scales from the CBCL and ABCL. These DSM-oriented sub-scales align with
313 neuropsychiatric diagnoses reported by individuals with 3q29Del [5]. Individuals with 3q29Del
314 scored significantly higher than typically developing controls on all three scales (3q29Del
315 Attention Deficit/Hyperactivity Problems *T-score* = 61.0, control Attention Deficit/Hyperactivity
316 Problems *T-score* = 51.3, 3q29Del Anxiety Problems *T-score* = 60.9, control Anxiety Problems
317 *T-score* = 52.9, 3q29Del Depressive Problems *T-score* = 62.7, control Depressive Problems *T-*
318 *score* = 52.3, all $p < 0.001$, Figure 3D, Table S7), supporting previous reports of increased risk
319 for neuropsychiatric phenotypes associated with the 3q29 deletion [5].
320 **Confounding due to heart defects and/or ID-related phenotypes**
321 A previous study of 3q29Del by our group showed that approximately 25% of individuals
322 reported a congenital heart defect [5]. Early hypoxic insult due to a heart defect has been

323 hypothesized to contribute to later neuropsychiatric and neurodevelopmental outcomes [42-47].
324 To determine if the high frequency of heart defects in our study population was driving adverse
325 neurodevelopmental outcomes within 3q29Del cases, we implemented generalized linear and
326 cumulative link models to assess the relationship between congenital heart defects and clinical
327 ASD diagnosis, GDD/MR diagnosis, and age at walking, which has been reported to be a
328 suitable proxy for ID in the absence of available IQ and adaptive behavior measures [48] .
329 Congenital heart defects were not associated with self-reported ASD or GDD/MR diagnoses or
330 age at walking ($p > 0.05$, Table S8). Individuals with 3q29Del are also commonly diagnosed
331 with mild to moderate ID [5]. To ask whether ASD phenotypes or ASD features were
332 disproportionately overrepresented in individuals with more pronounced ID-related phenotypes
333 and/or heart defects, we stratified the data according to these phenotypes. Within our 3q29Del
334 study population, congenital heart defects were associated with significantly increased scores on
335 the SCQ and CBCL/ABCL ($p < 0.05$); however, reported GDD/MR diagnosis and age at
336 walking were not significantly associated with scores on the SRS, SCQ, ASSQ, or CBCL/ABCL
337 ($p > 0.05$, Table S9). These data indicate that ID-related phenotypes were not driving the
338 increased scores in our study population.

339 **DISCUSSION**

340 Previous studies have found enrichment of the 3q29 deletion in large samples ascertained
341 based on clinical ASD diagnosis [17, 18]. We have approached the association of 3q29Del with
342 ASD from a different angle; by ascertaining subjects with 3q29Del and investigating the
343 prevalence of reported ASD diagnosis and ASD-related phenotypes, the current study
344 complements the existing literature, providing additional evidence for the 3q29 deletion as a
345 genetic risk factor for ASD. Notably, the male:female ratio of self-reported ASD diagnosis in our
346 study population is 1.8:1. This is a reduction from the 4:1 male bias observed in idiopathic ASD

347 in the general population. A substantial reduction in male bias in ASD prevalence has been
348 observed in studies of other CNVs and single-gene mutations; a recent study has shown that as
349 the severity of a mutation increases, the sex ratio in ASD prevalence approaches 1:1 [18]. Taken
350 together, this suggests that the 3q29 deletion is approaching the severe end of the spectrum of
351 ASD-associated mutations.

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

369 Based on the SRS sub-scales, participants with 3q29Del display a strikingly different
370 behavioral profile than children with idiopathic ASD [41]. Male and female 3q29Del individuals

371 show substantially less impaired social motivation in the context of an otherwise typical ASD
372 profile, with the most severe deficits in the Restricted Interests and Repetitive Behaviors domain.
373 This profile is also observed when dividing scores for 3q29Del participants based on reported
374 ASD diagnosis. This qualitative difference from idiopathic ASD may serve as an inroad to
375 therapeutic interventions in 3q29Del, as well as an investigative inroad to a novel subtype of
376 ASD. Because social motivation appears to be relatively well-preserved in 3q29Del, this
377 suggests that therapies such as cognitive-behavioral therapy to teach social skills and effective
378 strategies for social interaction may be particularly successful in this patient population.

379 Some facets of the difference in ASD features between 3q29Del and idiopathic ASD are
380 recapitulated by the scores on the Withdrawn sub-scale of the CBCL and ABCL. Previous
381 studies utilizing the CBCL in idiopathic ASD have found that mean scores for participants with
382 ASD are in the borderline range, with over 50% of subjects scoring in the borderline or clinical
383 range [49, 50]. While 3q29Del participants generally, as well as males and females separately,
384 score significantly higher than controls, their mean score is still in the normal range (Figure S2A
385 and B). However, 60% of 3q29Del participants reporting an ASD diagnosis score in the
386 borderline or clinical range (Figure S2C, Table S10), which is in line with what is expected
387 based on studies of idiopathic ASD [49, 50]. This is in conflict with the relatively well-preserved
388 social motivation in 3q29Del individuals with ASD identified in our analysis of the SRS sub-
389 scales and suggests that a more refined analysis is merited to identify the true degree of social
390 disability in this population.

391 We tested the hypothesis that the score inflation observed in our 3q29Del study subjects
392 may be due to the high prevalence of developmental delay or congenital heart defects [5]. Our
393 available data do not support this hypothesis, and instead reveal that social disability is equally
394 distributed in our study population. However, lack of direct measures of intellectual disability,

395 and errors or missing data in self-report measures, may obscure this relationship. Ongoing
396 studies with direct evaluation of study subjects [51] will address this question.
397 While this study is the most comprehensive study of behavioral phenotypes in 3q29Del to
398 date, it is not without limitations. All of the data used in the present study were collected from
399 questionnaires completed by the parents and guardians of individuals with 3q29Del, which
400 introduces several potential sources of bias. Some studies have questioned the validity and
401 reliability of parent-report data [52]; however, a recent study in Williams syndrome patients has
402 shown that parents are more accurate in predicting their child's social behaviors than the child
403 themselves [53]. The responses to the medical and demographic questionnaire are more likely to
404 include error due to the fact that the data is retrospective. By limiting our study to only a few key
405 points in the medical history (heart defects, age at walking, and ID/ASD diagnosis) we aimed to
406 reduce recall errors; however, we only had proxies for ID, rather than direct evaluation of
407 cognitive ability. Finally, there is likely ascertainment bias within our sample. First, our sample
408 of 93 individuals with 3q29Del is 87.1% white, indicating that we are not adequately reaching
409 minority populations. Second, parents that register their children and complete in-depth
410 questionnaires are likely to be highly motivated, possibly because their children experience
411 significant morbidity – a potential indication that we are sampling from the extreme of the
412 phenotypic distribution of 3q29Del. Thus, scores on the standardized questionnaires, as well as
413 rates of heart defects and clinical neuropsychiatric diagnoses, may be higher in our study sample
414 than in the general 3q29Del population. Additionally, the odds ratios calculated for the increased
415 risk for ASD associated with the 3q29 deletion may also be overestimated, due to the combined
416 effects of self-report data and ascertainment bias; however, if this increased risk is replicated
417 using gold-standard diagnostic measures, it could provide valuable insight into possible sex-
418 specific effects of the deletion. Finally, the lack of observed association between congenital heart

419 defects and neurodevelopmental outcomes may be obscured by the high rate of patent ductus
420 arteriosus in 3q29 deletion syndrome [5], which is a relatively mild heart defect; however, the
421 low number of participants with different types of heart defects rendered analyses to assess their
422 associations with neurodevelopment underpowered. Ongoing studies by the Emory 3q29 Project
423 (http://genome.emory.edu/3q29/), including direct in-person patient evaluations [51] aim to
424 address some of the weaknesses of the present work by performing comprehensive gold-standard
425 evaluations by expert clinicians.

426 While direct in-person evaluations are the ideal method to corroborate the findings of this
427 study, the low population frequency of the 3q29 deletion renders this approach infeasible for a
428 large number of study subjects. However, a small number of 3q29 deletion study subjects have
429 been directly assessed as part of the Emory 3q29 Project (http://genome.emory.edu/3q29/). We
430 confirm high concordance between registry-leveraged data and gold-standard direct evaluation,
431 as all participants qualifying for an ASD diagnosis based on gold-standard evaluation have
432 clinically significant scores on the SRS (Table S11). Five additional participants with a clinically
433 significant SRS score did not qualify for an ASD diagnosis, suggesting that the SRS is not
434 selectively identifying children with ASD in participants with 3q29Del, possibly due to the high
435 rates of reported anxiety in our study population. However, this comparison does suggest that our
436 analysis, though based on self-report data, reveals valid conclusions about behavioral phenotypes
437 in 3q29 deletion syndrome. For genetic syndromes with low population frequencies, data
438 collection through remote means such as online patient registries remains a valuable phenotyping
439 tool.

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

443 standardized questionnaires and best practices allowed rigorous assessment of phenotypes in our
444 cohort. Additionally, our online patient registry allows for remote data collection, which has
445 enabled us to expand our sample size. This study has shown that high-quality, comprehensive
446 medical history and symptomology data can be collected through an online patient registry,
447 effectively reducing the patient-ascertainment burden associated with studying rare disorders.
448 Taken together, these attributes make the present study an excellent complement to previously
449 published case reports on individuals with 3q29Del; by capturing a larger patient base with
450 systematic assessments, we are able to more accurately measure the presence of a variety of
451 neuropsychiatric and neurodevelopmental phenotypes associated with the 3q29 deletion. The
452 findings reported here imply that comprehensive neuropsychiatric and neurodevelopmental
453 assessments with gold standard tools are merited for individuals diagnosed with 3q29Del, and
454 that such assessments should be the standard of care for this patient population.

455 **CONCLUSIONS**

456 The present study confirms previous reports of phenotypes in 3q29Del, as well as
457 expanding the spectrum of behavioral phenotypes associated with the deletion. We found that
458 individuals with 3q29Del report a significantly higher prevalence of ASD diagnosis than the
459 general population, and significantly elevated scores on the SRS, SCQ, ASSQ, and CBCL/ABCL
460 irrespective of ASD diagnosis indicate significant social disability overall in our study
461 population. Further, 3q29Del participants showed a novel profile of ASD-related phenotypes on
462 the SRS sub-scales, marked by less impaired scores on the Social Motivation sub-scale and
463 extremely high scores on the Restricted Interests and Repetitive Behaviors sub-scale. This score
464 profile is consistent between 3q29Del males and females and between 3q29Del participants with
465 and without ASD, suggesting that it may be a hallmark behavioral feature of the syndrome and
466 providing a potential therapeutic inroad for the treatment of individuals with 3q29Del. Finally,

467 we identify a high degree of social disability in female 3q29Del participants; the 3q29 deletion
468 elevates the risk ASD in females (OR=49.4, p=1.09E-05) more substantially than in males
469 (OR=24.6, p=6.06E-09), and standardized scores for female participants are not significantly
470 different from male scores. These results demonstrate that there is a benefit to studying rare
471 CNVs such as 3q29Del; studying a single genomic variant with large effect allows us to control
472 for genetic etiology and unmask the mechanisms underlying the development of
473 neuropsychiatric and neurodevelopmental disorders.
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Abbreviations

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

515 **DECLARATIONS**

516 **Ethics approval and consent to participate**

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

519 **Consent for publication**

520 Not applicable.

521 **Availability of data and material**

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

524 **Competing interests**

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

527 **Funding**

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

530 **Authors' contributions**

531 RMP performed the statistical analysis, produced all figures and tables, and wrote the
532 manuscript. MMM collected the data. MPE helped with statistical analyses and interpretation.
533 MMM, CK, and CAS helped with data interpretation. MEZ and JGM edited the manuscript and
534 provided guidance on analyzing and interpreting data. JGM was the principle investigator
535 responsible for study direction. All authors participated in commenting on the drafts and have
536 read and approved the final manuscript.

537 **Acknowledgements**

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

752 File name: Supplemental Information

753 File format: Microsoft Word document (.docx)

754 Title of data: Supplementary figures and tables

755 Description of data: Supplementary figures are (S1) SCQ scores split by ASD status, sex, and
756 ASD status/sex and (S2) CBCL/ABCL Withdrawn sub-scale scores split by genotype, sex, and
757 ASD status. Supplementary tables are (S1) questionnaire demographics for the medical
758 questionnaire, SRS, SCQ, ASSQ, and CBCL/ABCL; (S2 and S3) sensitivity analysis description
759 and results for effect of 3q29Del self-registrants; (S4) comparison of scores on all four scales for
760 3q29Del versus control; (S5) SRS score comparison stratified by ASD status and sex; (S6) SCQ
761 score comparison stratified by ASD status and sex; (S7) CBCL/ABCL DSM-oriented sub-scale
762 score comparison; (S8) contribution of congenital heart defects to phenotypes of interest; (S9)
763 test for confounding factors contributing to symptomology questionnaire scores; (S10)
764 CBCL/ABCL Withdrawn sub-scale score comparison; and (S11) comparison of 3q29 registry-
765 leveraged and gold-standard phenotyping measures.

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775 **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	28 (30.1%)	20 (37.0%)	8 (20.5%)	1.47%*	2.34%*	0.52%*	
	No	65 (69.9%)	34 (63.0%)	31 (79.5%)	64 (100%)	33 (100%)	31 (100%)	
Anxiety (n, %)								0.001
	Yes	25 (26.9%)	14 (25.9%)	11 (28.2%)	4 (6.2%)	2 (6.1%)	2 (6.5%)	
	No	68 (73.1%)	40 (74.1%)	28 (71.8%)	60 (93.8%)	31 (93.9%)	29 (93.5%)	
Bipolar/Manic Depression (n. %)								0.080
	Yes	5 (5.4%)	2 (3.7%)	3 (7.7%)	0 (0%)	0 (0%)	0 (0%)	
	No	88 (94.6%)	52 (96.3%)	36 (92.3%)	64 (100%)	33 (100%)	31 (100%)	
Conduct Disorder (n, %)								0.271
	Yes	3 (3.2%)	1 (1.9%)	2 (5.1%)	0 (0%)	0 (0%)	0 (0%)	
	No	90 (96.8%)	53 (98.1%)	37 (94.9%)	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, %)								0.027
	Yes	13 (14.0%)	9 (16.7%)	4 (10.3%)	2 (3.2%)	0 (0%)	2 (6.5%)	
	No	80 (86.0%)	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%)

776 Characteristics of self-reported neuropsychiatric diagnoses in study participants with 3q29Del and controls. Asterisks indicate where
777 3q29Del was compared to general population prevalence values [40, 54]. P values were calculated with Fisher's exact test.
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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

793 Comparison of mean scores on the SRS sub-scales between study participants with 3q29Del and controls. 3q29Del participants were
794 stratified by ASD status and sex for further analysis. P values were calculated using simple linear regression, adjusting for age, race,
795 and sex.
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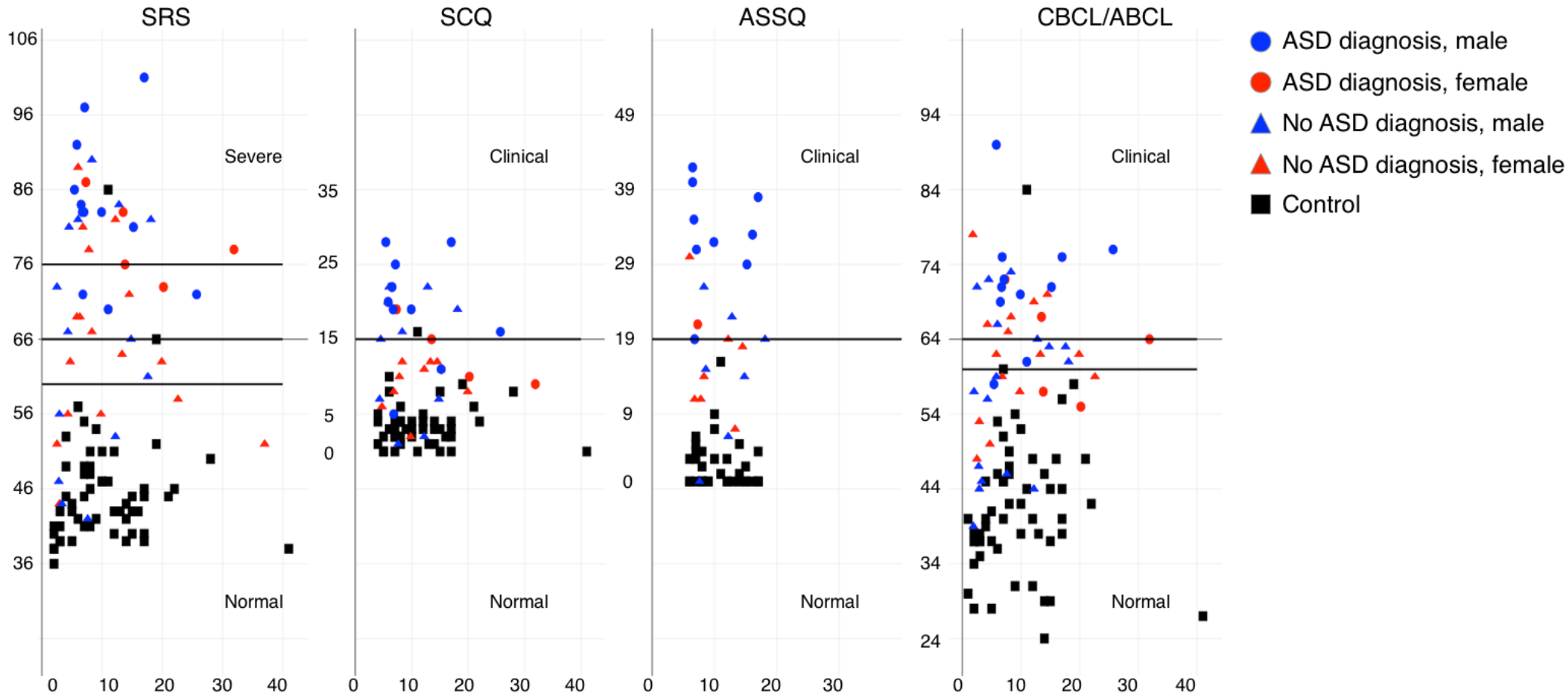
811 **Figure Titles and Legends**

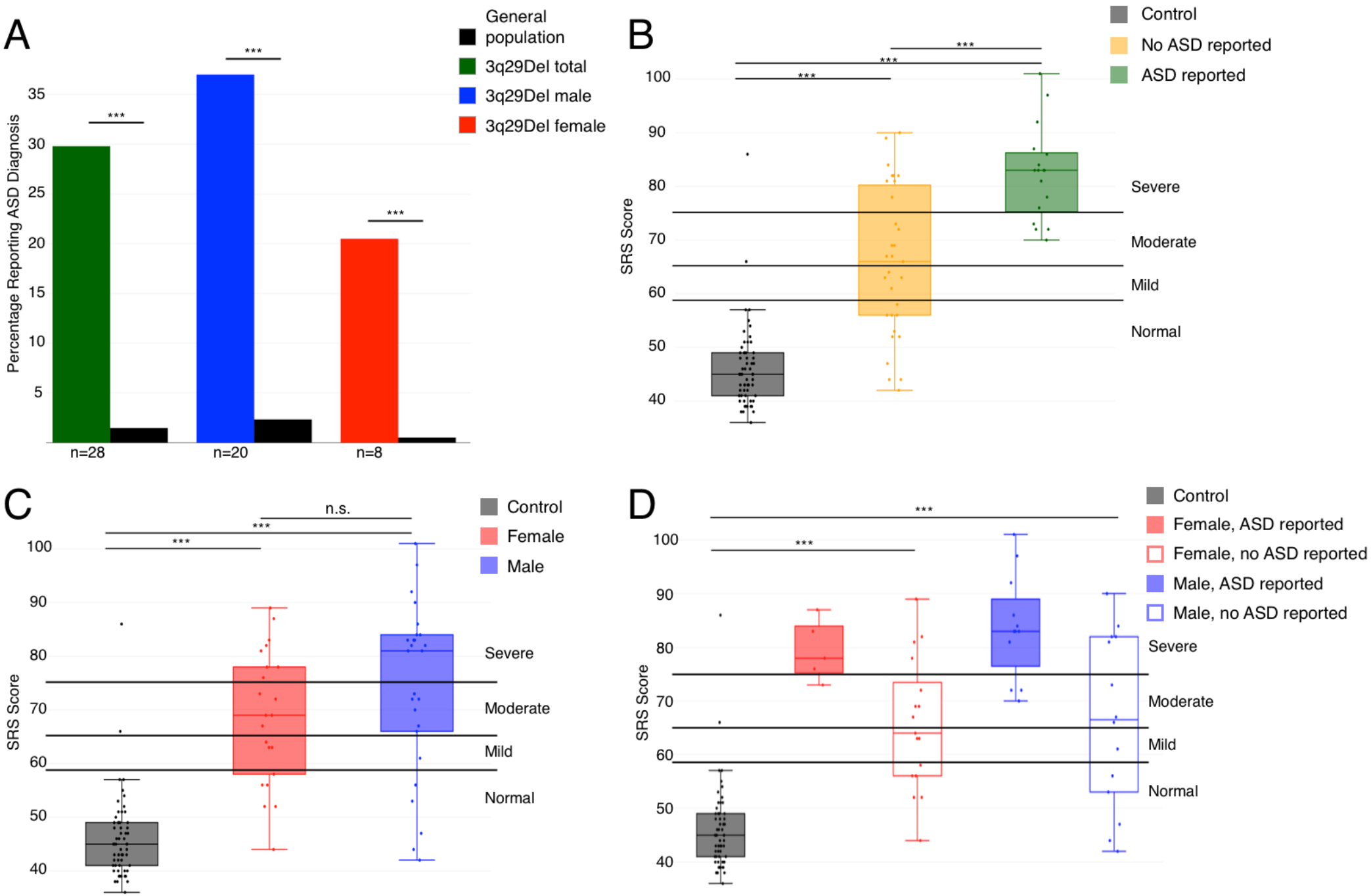
812 **Figure 1. Score distribution for 3q29Del and controls on the SRS, SCQ, ASSQ, and**
813 **CBCL/ABCL.** Total scores on the SRS (n=48 3q29Del, 56 control), SCQ (n=33 3q29Del, 46
814 control), ASSQ (n=24 3q29Del, 35 control), and CBCL/ABCL (n=48 3q29Del, 57 control) for
815 registry participants.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

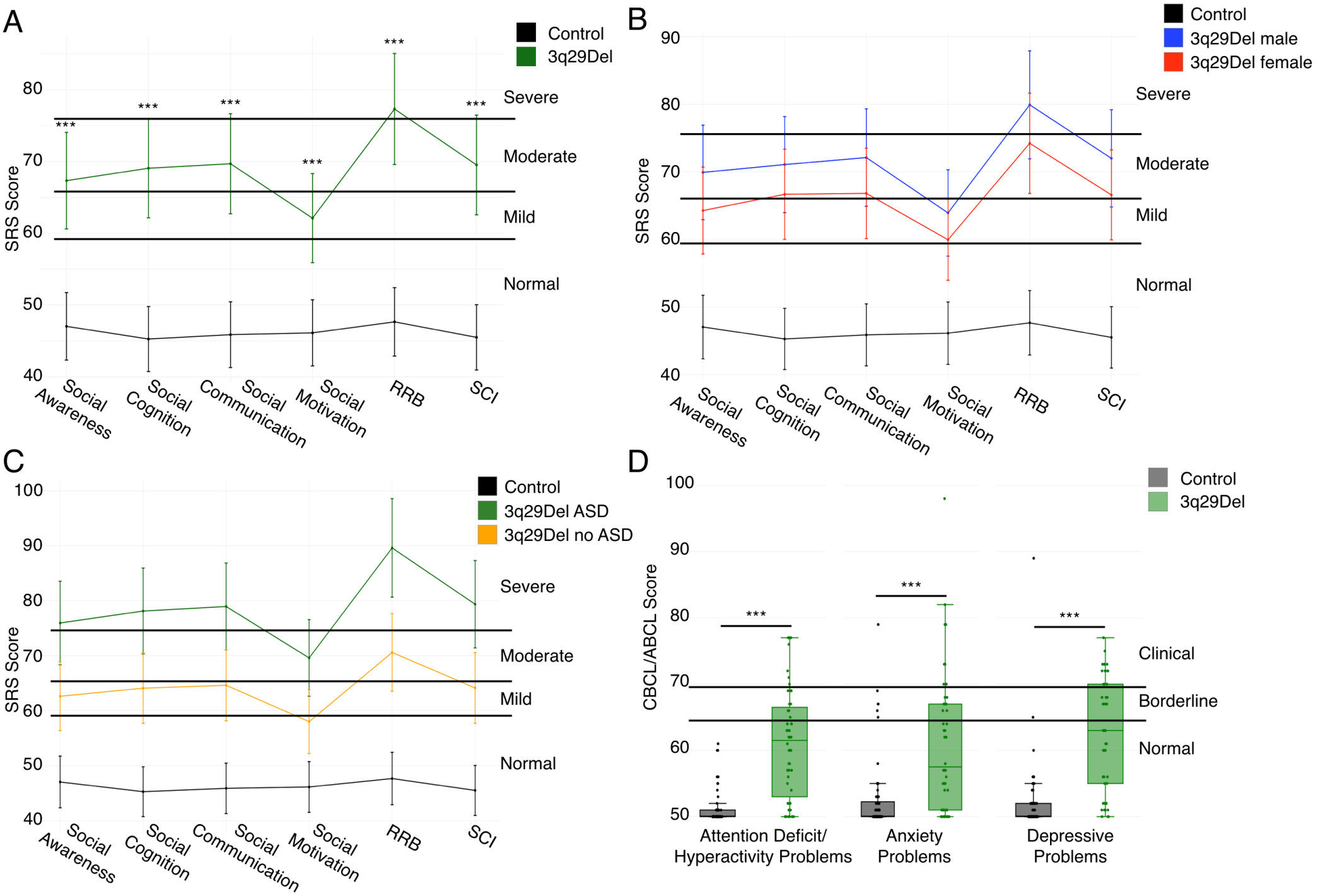
818 **Figure 2. Comparison of ASD prevalence and SRS scores between 3q29Del and controls. A)**
819 Proportion of participants with 3q29Del self-reporting a diagnosis of ASD (not all respondents
820 completed symptom questionnaires); 28 cases report an ASD diagnosis (green), comprised of 20
821 males (blue) and 8 females (red). Compared to general population frequencies (black), cases
822 report significantly higher incidence of ASD. **B)** SRS scores split by control (n=59), 3q29Del not
823 reporting an ASD diagnosis (n=31), and 3q29Del reporting an ASD diagnosis (n=17), showing a
824 significant association between self-reported diagnostic status and SRS score. **C)** SRS scores
825 split by sex, with control (n=59), 3q29Del female (n=22), and 3q29Del male (n=26), showing a
826 lack of sex bias in scores for 3q29Del participants. **D)** SRS scores split by sex and self-reported
827 diagnostic status, with control (n=59), 3q29Del female reporting ASD (n=5), 3q29Del female not
828 reporting ASD (n=17), 3q29Del male reporting ASD (n=12), and 3q29Del male not reporting
829 ASD (n=14), showing inflated scores for 3q29Del participants irrespective of sex or diagnostic
830 status. ***, $p<0.001$

831 **Figure 3. Comparison of SRS sub-scales and CBCL/ABCL DSM-oriented sub-scales**
832 **between 3q29Del and controls. A)** Profile of individuals with 3q29Del (n=48) and controls
833 (n=59) across SRS sub-scales, showing moderate to severe impairment of 3q29Del participants
834 in all domains except Social Motivation (RRB, Restricted Interests and Repetitive Behaviors;

835 SCI, Social Communication and Interaction). **B)** Profile of 3q29Del males (n=26) and females
836 (n=22) and controls (n=59) across SRS sub-scales, showing that 3q29Del males and females
837 both score significantly higher than controls and that there are no significant differences in score
838 between males and females. **C)** Profile of 3q29Del participants reporting an ASD diagnosis
839 (n=17) and participants not reporting an ASD diagnosis (n=31) and controls (n=59) across SRS
840 sub-scales, showing that 3q29Del participants score significantly higher than controls
841 irrespective of ASD status, with 3q29Del participants reporting an ASD diagnosis scoring
842 significantly higher than those not reporting an ASD diagnosis. **D)** Profile of 3q29Del
843 participants (n=48) and controls (n=57) across 3 DSM-oriented sub-scales from the CBCL and
844 ABCL, showing significantly increased pathology in 3q29Del participants in all 3 domains. ***,
845 $p<0.001$


SRS
SCQ
ASSQ
CBCL/ABCL
Severe
Normal
Clinical
Normal
Clinical
Normal
Clinical
Normal
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=28
n=20
n=8
B
Control
No ASD reported
ASD reported
SRS Score
100
90
80
70
60
50
40
***
***
***
Severe
Moderate
Mild
Normal
C
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