Clinical autism subscales have common genetic liability that is heritable, pleiotropic, and generalizable to the general population

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Abstract

The complexity of autism’s phenotypic spectra is well-known, yet most genetic research uses case-control status as the target trait. It is unclear whether clinical autism instruments such as the Social Communication Questionnaire (SCQ), Repetitive Behaviors Scale-Revised (RBS-R), and Developmental Coordination Disorder Questionnaire (DCDQ) are more genetically informative than case-control. We employed the SPARK autism cohort (N = 6,449) to illuminate the genetic etiology of these twelve subscales. In comparison to the heritability of autism case-control at 0.12, the RBS-R subscales were increased, ranging from 0.18 to 0.30 (all p < 0.05). Heritability of the DCDQ subscales ranged from 0.07 to 0.09 and the SCQ subscales from 0 to 0.09 (all p > 0.05). We also found evidence for genetic correlations among the RBS-R, SCQ, and DCDQ. GWAS followed by projection of polygenic scores (PGS) into ABCD revealed significant associations with CBCL social and thought problems, while the autism case-control PGS did not significantly associate. In phenotypic correlation analyses, the autism case-control PGS did not predict the subscales in SPARK, and sex-stratified correlations showed no effect in males and a surprising negative effect in females. Notably, other PGS did predict the subscales, with the strongest being educational attainment negatively correlated, while ADHD and major depression were positively correlated. Overall, our analyses suggest that clinical subscales are more genetically powerful than case-control, and that of the three instruments investigated, the RBS-R shows the greatest evidence of common genetic signal in both autistic and general population samples.

Introduction

Autism is a common and complex umbrella diagnosis affecting 1 in 59 children in the US [1], with comparable prevalence worldwide [2]. It has a strong genetic basis, with heritability estimated at over 80% [3]. The DSM-5 defines autism by two core domains of symptomatology: the social domain, which includes social communication difficulties, and the non-social domain which encompasses restricted and repetitive behaviors/interests [4]. However, other symptom domains are also characteristic and clinically meaningful in autism, including fine motor development [5], executive functioning [6], sleep [7], eating [8], and sensory issues [9]. Impaired fine motor skills are of particular interest due to being clinically detectable at twelve months, preceding deficits in the two core domains of autism, which emerge around two years of age [10]. Together, these multiple symptom domains imply that an accurate description of autism would be inherently multidimensional, rather than a simple binary diagnostic indicator. Because autism is strongly genetic, if these multiple symptom domains surpass the binary diagnosis in terms of their genetic signal, there may be broader implications for issues ranging from nosology to early identification [11] and individualized intervention [12].

Between autistic individuals there is extreme variance in these core and comorbid symptom domains, manifesting as heterogeneity in clinical presentation [13]. Clinical psychiatry has responded to this heterogeneity by developing numerous instruments to measure the social communication difficulties, restricted and repetitive behaviors/interests, and motor skills that are clinically relevant to autism. Those instruments with the heaviest clinical use, such as the ADOS [14] and the ADI-R [15], represent the gold standard in diagnosis. However, these rigorous assessments require lengthy and intensive interactions between patients and clinicians, and do not scale well to the power requirements of genetic studies. SPARK [16], a US-based study of autism with over 250,000 participants, collects a variety of background history and phenotypic information using online surveys. In this online setting, several clinically validated screening instruments have been used by SPARK to characterize participants, including the Social Communication Questionnaire (SCQ) [17], the Repetitive Behavior Scale-Revised (RBS-R) [18], and the Developmental Coordination Disorder Questionnaire (DCDQ) [19]. All three of these instruments and their subscales measure clinically relevant, quantitative traits that have important utility in the diagnosis of autism. Further investigation into the genetic characteristics of these clinical instruments could give insight into their best use cases as well as their potential limitations, potentially illuminating future autism diagnostic strategies and inherent biology.

Most genetic research on autism has favored diagnostic status as the target trait [20]. This is to be expected due to the relative ease of scaling a binary diagnostic phenotype to a large sample collected over many sites worldwide. Still, the traditional case-control approach in autism genetics is at odds with observations established by psychiatry and epidemiology in two major ways — first by
treating autism as a binary and homogeneous condition, and secondly by treating those without an autism diagnosis as true controls, despite the fact that autism is under-diagnosed (especially in females [21] and racial/ethnic minorities [22]) and many individuals without an autism diagnosis have autistic traits [23] [24]. While the case-control method has been successful in identifying a number of genes and biological processes associated with autism, variation in these genes has been found to represent broad neuropsychiatric risk with little specificity to autism [25]. As a result, studies relying on case-control status are inherently reducing statistical power, adding noise to the biological signal of core autism traits, and limiting the specificity of the results.

Somewhat ironically, genetic investigation of quantitative autism traits has gained the most traction in studies of the general population, yielding important insights. Most prominent are the recent genome-wide association studies (GWAS) in general population samples of systemizing behavior, an indicator of restricted and repetitive behaviors/interests, (N = 51,564) [26] and empathy, an indicator of social communication abilities, (N = 46,861) [27]. They did not observe significant genetic correlations between systemizing and empathy, but did find greater systemizing and lower empathy were both significantly genetically correlated with autism (PGC) [26]. Another study in a general population (N = 1,981) derived five autism trait factors, three of which were significantly associated with autism polygenic scores (PGS) [28]. These results suggest common genetic variation does play a significant role in dimensional autism traits that case-control autism studies are inherently overlooking. However, the interchangeability from general population samples to autistic cohorts is unknown. Results from genetic studies of autism traits in autistic cohorts are limited by sample size and the availability of clinically validated instruments as phenotypes. One study in N = 2,509 autistic individuals found significant heritabilities and autism PGS associations with their autism traits, but the phenotypes were derived factors from the Autism Diagnostic Interview-Revised, not clinically established subscales measuring distinct symptomatology [28]. The genetic etiology of autism traits in autistic samples is relatively indeterminate, especially with traits that have established clinical, psychological, and epidemiological usage in autism like the SCQ, RBS-R, and DCDQ subscales.

This analysis sought to investigate fundamental questions regarding the genetic etiology of dimensional autism traits captured by twelve subscales from three of the primary autism clinical assessments. First, these subscales are designed to measure autism traits that are meaningful at the clinical level, but are they measuring signal that is also meaningful at the genetic level in autistic individuals? Second, how heritable and genetically distinct are these quantitative traits, especially in an all-autistic cohort? Third, is the genetic signal discovered in this autistic cohort generalizable to related traits in a general population sample? Fourth, what are the pleiotropic relationships of these subscale traits with neuropsychiatric conditions, cognition, and dimensions of personality? Lastly, considering the observed sex differences that are pervasive in autism and other neuropsychiatric conditions, how do sex and PGS interact? We leveraged the SPARK autism cohort [16] to address these questions in N = 6,449 autistic children who all had the three DCDQ subscales, six RBS-R subscales, three SCQ subscales, and genetic data. The overview of our analyses steps are shown in Figure 1.

![Figure 1. Study overview.](https://example.com/figure1.png)

Twelve quantitative, clinical autism subscale traits were measured in N = 6,449 autistic children in the SPARK cohort: three DCDQ subscales, six RBS-R subscales, and three SCQ subscales. These traits were investigated both at the phenotype and genomic level, including SNP (h²) heritabilities, intra-cohort genetic correlations, genome-wide association studies (GWAS), and polygenic scores (PGS) associations with thirteen psychiatric, cognitive, and personality traits. Additionally, the GWAS were used to calculate PGS in a general population childhood cohort, ABCD (N = 6,559). These PGS were then compared to the autism case-control PGS in predicting two CBCL syndrome behavior traits shown to be elevated in autistic children.
Materials and methods

0.1 SPARK cohort description

The SPARK cohort is a nationwide autism study. The parent or legal guardian of the child with autism provided informed consent and completed the psychometric instruments on behalf of their child. This study was approved by the Western IRB (IRB# 20151664). We restricted the participants in our analysis with the following criteria: has an autism diagnosis, was between the ages of 3 and 17 at the time the instrument was completed, passed the SPARK validity check for each instrument, and had full subscale scores for each of the twelve traits, and only one participant per family ID. The cohort was further filtered to participants in which genetic data was available that passed our quality control and that clustered in the majority European cluster based on SNPs (genetic clustering described in further detail in the later section). After this filtering, 6,449 SPARK participants remained.

0.2 Dimensional autism trait phenotype measures

The Social Communication Questionnaire (SCQ) [17] is a well-established questionnaire of 40 yes/no items used as an autism screening tool in clinics [20]. The SCQ measures challenges with social interaction and communication and has three subscales: communication, reciprocal social interaction, and stereotyped behavior. The Restricted and Repetitive Behavior Scale-Revised (RBS-R) [18] is a 4 point questionnaire of 44 items that has been shown to be associated with several clinical features of autism [31]. The RBS-R has six subscales: compulsive behavior, self-injurious behavior, restricted behavior, ritualistic behavior, sameness behavior, and stereotyped behavior. The Developmental Coordination Disorder Questionnaire (DCDQ) [19] is a 5 point questionnaire of 15 items that is used clinically to screen for developmental coordination disorder [32]. The DCDQ has three subscales: control during movement, fine motor/handwriting, and general coordination. Subscale items within these scales were calculated as recommended by the scale publisher, including omitting the individual on a subscale due to missingness of item-level data within the subscale. In addition, the DCDQ was not completed if the proband was unable to move their hands. For the sake of consistent interpretation across the scales, the DCDQ scores were reversed so that higher scores indicated more coordination problems. In the cohort of N = 6,449 participants, these subscores were residualized for age in months using linear regression, then centered to have a mean of 0 and a standard deviation of 1. These normalized scores were used as the phenotypes in all analyses.

0.3 Phenotypic correlations and heterogeneous outgroups

The phenotypic correlations between subscale traits were calculated with the non-parametric Spearman method. P-values were corrected using the FDR method. The heterogeneous outgroups for each pair of traits were defined by the participant trait value being > 1 in one trait (1 standard deviation above the mean) and < 1 in the other trait (1 standard deviation below the mean).

0.4 Genotype quality control and imputation

The SNPs used in this study were based on the combined SPARK 2019 Version 3 release and the SPARK 2020 Version 4 release. These SNPs were merged using PLINK [33], then lifted from hg38 over to hg19. The SNP QC process was based on the recommendations by [34], using PLINK [33] and R [35]. First, 25,840 SNPs and 86 individuals were removed due to global missing rate greater than 20%. Second, the more stringent threshold of 5% global missing rate was used again which removed an additional 30,845 SNPs and 711 individuals. Third, 102,530 SNPs were removed because the minor allele frequency was less than 1%. Fourth, 47,825 SNPs were removed due to the HWE p-value less than 1 × 10^-10. Fifth, 1,180 individuals were removed because of missing rate greater than 5% on any autosome. Sixth, 1,115 individuals were removed due to their heterozygosity rate not within 3 standard deviations of the cohort mean heterozygosity. After this QC, the remaining SPARK cohort was merged and clustered with the 1,000 Genomes Phase 3. Clustering was based on the first 10 components from multi-dimensional scaling of the combined kinship matrix of the cohort and 1,000 Genomes. This combined cohort was clustered into 5 groups, representing the 5 distinct super-populations. 3,963 individuals were removed due to being more than 3 standard deviations away from any of the 5 mean multi-dimensional scaling components. In total, 36,154 individuals and 409,281 quality controlled (QC’d) SNPs remained. These remaining individuals and SNPs were imputed to the 1,000 Genomes Phase 3 ALL reference panel using the Genipe pipeline [36]. 29,443 individuals clustered in the majority European cluster. Genipe performed LD calculation and pruning with PLINK [33], genotype phasing with SHAPEIT [37], and genotype imputation by IMPUTE2 [38] using default parameters.

0.5 Additive SNP-based heritabilities

Additive SNP-based heritabilities (h^2) were calculated separately for the 12 traits using BOLT-REML on the QC’d SNPs. BOLT accounts for population stratification and relatedness, so genetic principal components were not used as covariates. However, sex at birth was used as a covariate. The BOLT-REML output provides the mean variance estimate (h^2) and the standard error. The 95% confidence intervals and p-values were then calculated based on the estimate and standard error.
0.6 Intra-cohort genetic correlations

Genetic correlations (rg) were calculated for between the 12 traits using BOLT-REML on the QC’d SNPs. Sex at birth was used as a covariate. The BOLT-REML output provides the mean variance estimate (rg) and the standard error. The 95% confidence intervals and p-values were then calculated based on the estimate and standard error.

0.7 Genome-wide association studies

Genome-wide association studies (GWAS) were performed separately for the 12 traits using the BOLT-LMM option --lmmForceNonInf on the QC’d and imputed SNPs. Sex at birth was used as a covariate. The summary statistics for each GWAS were then filtered to only include SNPs in which the imputation quality score (INFO) was $\geq 0.8$, effect allele frequency $\geq 1\%$ and $\leq 99\%$, unambiguous, and has an rsid. The SNPs were also processed so that the effect allele was the reference allele on the correct strand using Genomic Ranges [39]. After this quality control, each GWAS had 6,632,249 SNPs. Lead SNPs were pruned using the PLINK [33] command --clump with default parameters and 1000 Genomes European as the LD reference. The lead SNPs were mapped to genes using MAGMA [40] distance-based annotation.

0.7.1 ABCD cohort description

The ABCD cohort [41] is a typically-developing cohort was not recruited on the presence or absence of neuropsychiatric conditions. Release 2 data was used. The cohort was further filtered to participants in which genetic data was available that passed our quality control and that clustered in the majority European cluster based on SNPs (genetic clustering described in further detail in the later section). After this filtering, 6,559 ABCD participants remained.

0.7.2 ABCD behavior trait phenotype measures

The phenotypes were two Child Behavior Checklist Syndrome subscales, which were calculated by ABCD. The baseline intake year 1 scores were used. In the cohort of N = 6,559 participants, these scales were residualized for age in months using linear regression, then centered to have a mean of 0 and a standard deviation of 1. These normalized scores were used as the phenotypes in all analyses.

0.7.3 ABCD genotype quality control and imputation

The ABCD cohort was genotyped on the Affymetrix NIDA SmokeScreen Array and was processed through standard QC steps before release, including removing SNPs with low call rate and individuals with potential contamination problems or high missing data. The SNP QC process was based on the recommendations by [34] using PLINK [33] and R [35] with the same parameters used for the SPARK genotyping QC. In total, 399,016 SNPs and 9,324 individuals passed QC. After this QC, the remaining cohort was merged and clustered with the 1,000 Genomes Phase 3. Clustering was based on the first 10 components from multi-dimensional scaling of the combined kinship matrix of the cohort and 1,000 Genomes. This combined cohort was clustered into 5 groups, representing the 5 distinct super-populations. For genotype imputation, ambiguous SNPs were also removed, leaving 372,694 SNPs. These remaining individuals and SNPs were imputed to the 1,000 Genomes Phase 3 ALL reference panel using the Genipe pipeline [36]. 6,659 individuals clustered in the European cluster. Genipe performed LD calculation and pruning with PLINK [33], genotype phasing with SHAPEIT [37], and genotype imputation by IMPUTE2 [38] using default parameters.

0.7.4 ABCD polygenic scores generation and statistical analyses

Polygenic scores (PGS) were calculated using PRSice [43] using default parameters from the processed summary statistics for the 11 dimensional autism trait GWAS and the autism (PGC) [20] GWAS. PGS were calculated at the 0.05 SNP GWAS threshold for all individuals in the majority European cluster. The PGS were then filtered to only include individuals in the analysis and then centered to have a mean of 0 and standard deviation of 1. PGS prediction was assessed by a main effect linear model: \( \text{lm(trait} \sim \text{PGS + sex}) \). The \text{sex} is male coded as 1 and female coded as 0.

0.8 SPARK polygenic scores generation and statistical analyses

Polygenic scores (PGS) were calculated using PRSice [43] using default parameters from the following base GWAS: cognitive performance (SSGAC 2018) [44], educational attainment (SSGAC 2018) [44], ADHD (PGC 2018) [45], anorexia (PGC 2019) [46], autism (PGC 2019) [46], bipolar disorder (PGC 2018) [47], major depression (PGC 2018) [48], schizophrenia (PGC 2018) [47], agreeableness (GPC 2010) [49], conscientiousness (GPC 2010) [49], extraversion (GPC 2010) [49], neuroticism (SSGAC 2018) [50], and openness (GPC 2010) [49]. All base GWAS summary statistics SNPs were also processed so that the effect allele was the reference allele on the correct strand using Genomic Ranges [39]. PGS were calculated at the 0.05 SNP GWAS threshold for all individuals in the
majority European cluster. The PGS were then filtered to only include individuals in the analysis and then centered to have a mean of 0 and standard deviation of 1.

PGS were analyzed by two complementary methods: linear modeling and non-parametric Spearman correlations. Prediction was assessed by two linear models: the main effect model \( \text{lm(trait } \sim \text{ PGS + sex)} \) and the sex interaction model: \( \text{lm(trait } \sim \text{ PGS + sex + PGS : sex)} \). The \text{sex} is male coded as 1 and female coded as 0. The \text{PGS : sex} is the PGS by sex interaction term. Correlations were assessed by performing Spearman correlations, both within the cohort (\( N = 6,449 \)) and sex-stratified (\( N = 5,170 \) males and \( N = 1,279 \) females). For the grouped difference in means (proband vs. parent) of the PGS, the cohort was filtered to male probands in which their father also had genetic data available (\( N = 1,061 \) male probands and \( N = 686 \) fathers) and female probands in which their mother also had genetic data available (\( N = 475 \) female probands and \( N = 283 \) mothers). The PGS were re-normalized to have a mean of 0 and a standard deviation of 1 to include the parents in this analysis.

## Results

### 0.1 Investigation of phenotypic heterogeneity

This study leveraged the SPARK autism cohort [16], a nationwide cohort of autistic individuals and their parents and siblings. For our analyses of the autism subscale traits, we only included autistic children. The demographic summary of the SPARK cohort used for this analysis is shown in Table 1.

| variable      | % or mean (SD) |
|---------------|----------------|
| % male        | 80%            |
| cognitive imp.| 10%            |
| dx age        | 4.7 (2.7)      |
| DCDQ age      | 9.5 (3.3)      |
| RBS-R age     | 9.4 (3.4)      |
| SCQ age       | 8.9 (3.4)      |
| DCDQ coordination | 14 (4.3)    |
| DCDQ handwriting | 10.4 (4.4)  |
| DCDQ movement | 15.6 (5.9)     |
| RBS-R compulsive | 5.4 (4.3) |
| RBS-R injurious | 3.7 (4)      |
| RBS-R restricted | 4.2 (2.9)  |
| RBS-R ritualistic | 6 (4.1)    |
| RBS-R sameness | 9.5 (6.5)     |
| RBS-R stereotyped | 5.7 (3.5) |
| SCQ communication | 6.7 (2.3)    |
| SCQ interaction | 7.8 (3.8)    |
| SCQ stereotyped | 6.4 (2)      |

**Table 1. Autism subscale traits and demographic summary of SPARK participants.**

Ages in years. 17 participants did not have cognitive impairment data.

The autism traits used as the phenotypes were three subscales from the Developmental Coordination Disorder Questionnaire (DCDQ): coordination (general coordination), handwriting (fine motor handwriting), and movement (control during movement), six subscales from the Repetitive Behavior Scale-Revised (RBS-R): compulsive, injurious (self injurious), restricted, ritualistic, sameness, and stereotyped, and three subscales from the Social Communication Questionnaire (SCQ): communication, interaction (reciprocal interaction), and stereotyped. The raw subscales values are shown in Table 1. The phenotypes used for all analyses were these raw subscale values that were then residualized for age and then centered to have a mean of 0 and standard deviation of 1.

The Spearman correlations of the 12 autism subscales traits are shown in the red triangle in Figure 2A. All 12 of the traits were significantly correlated with each other after FDR correction, although most correlations were modest. As expected, correlations were strongest between traits within the same core domain. The highest Spearman correlation coefficient (\( \rho \)) was between RBS-R ritualistic and RBS-R sameness \( \rho = 0.72 \). The weakest correlation was between DCDQ handwriting and RBS-R ritualistic \( \rho = 0.11 \).
percentage of individuals within each pair of traits that are in the heterogeneous outgroup (defined as above 1 standard deviation from the mean in one trait and less than 1 standard deviation below the mean in the other trait) are shown in the purple triangle in Figure 2A. The DCDQ handwriting and RBS-R ritualistic pair of traits also the highest percentage of participants in the heterogeneous outgroup, with N = 343 participants being in the outgroup (5.3%). This example in particular highlight how individuals can have differences in their autistic traits, with some autistic individuals having strong difficulties in one core domain while having little difficulties in another. In comparison to the pair of traits most strongly correlated with each other, RBS-R sameness and RBS-R ritualistic had N = 4 individuals in the heterogeneous outgroup (0.062%).

Figure 2. Phenotypic heterogeneity of autism subscale traits. 
A The lower triangle in red is showing the Spearman ρ across traits. All traits were significantly correlated with each other after FDR correction. The upper triangle in purple is showing the % of individuals that are in the heterogeneous outgroup (defined as being > 1 SD in one trait and < 1 SD in the other trait).

B The two traits with the weakest correlation and the highest percentage in the heterogeneous outgroup (purple).

0.2 SNP-based heritability
Additive SNP-based heritability (h²) was calculated separately for the 12 autism subscale traits and are shown in Figure 3A. The mean h² estimate point is plotted with the whiskers being the 95% confidence intervals (CI). SNP heritability was calculated using BOLT-REML.

The overall heritability trend is the RBS-R traits having the highest heritabilities ranging from mean h² estimates of 0.18 to 0.30, the DCDQ traits having moderate heritabilities from 0.07 to 0.09, and the SCQ traits having the lowest heritabilities from 0 to 0.09. Despite large confidence intervals due to the sample size, all six RBS-R traits were significantly heritable: RBS-R injurious h² = 0.30 (CI: 0.16 – 0.44), RBS-R restricted h² = 0.23 (CI: 0.10 – 0.36), RBS-R sameness h² = 0.23 (CI: 0.10 – 0.37), RBS-R compulsive h² = 0.21 (CI: 0.07 – 0.34), RBS-R stereotyped h² = 0.21 (CI: 0.08 – 0.34), and RBS-R ritualistic h² = 0.18 (CI: 0.05 – 0.31). Notably, all of these significantly heritable traits has also have a mean estimated heritability higher than the autism (PGC) heritability h² = 0.12 (CI: 0.10 – 0.14), although the 95% confidence intervals around our trait estimates are substantially greater due to much smaller sample size. However, the lower 95% confidence bound for RBS-R injurious at 0.16 is above the upper bound for autism (PGC) at 0.10.
0.3 Genetic correlations

The genetic correlations (rg) are shown in Figure 3B, with the mean rg point plotted and the whiskers being the 95% confidence intervals. Genetic correlations were calculated using BOLT-REML, so the standard error (and hence significance of the estimate) are dependent on the heritability of each trait. Therefore, only nominally significant (unadjusted p-value < 0.05) are reported in the results and figure. The overall trend was the strongest genetic correlations being within the same core domain. However, a few traits in different domains were significantly genetically correlated. DCDQ coordination was genetically correlated with RBS-R compulsive rg = 1 (CI: 0.37 – 1), RBS-R restricted rg = 0.63 (CI: 0.00 – 1), RBS-R sameness rg = 0.92 (CI: 0.30 – 1), and RBS-R stereotyped rg = 0.98 (CI: 0.41 – 1). DCDQ handwriting was genetically correlated with RBS-R sameness rg = 1 (CI: 0.27 – 1) and RBS-R stereotyped rg = 0.78 (CI: 0.10 – 1). DCDQ movement was genetically correlated with RBS-R stereotyped rg = 0.83 (CI: 0.04 – 1). SCQ stereotyped was genetically correlated with RBS-R compulsive rg = 0.76 (CI: 0.17 – 1), RBS-R restricted rg = 1 (CI: 0.37 – 1), RBS-R ritualistic rg = 0.78 (CI: 0.10 – 1), RBS-R sameness rg = 1 (CI: 0.35 – 1), and RBS-R stereotyped rg = 1 (CI: 0.48 – 1). SCQ interaction was genetically correlated with RBS-R compulsive rg = 0.83 (CI: 0.08 – 1), RBS-R sameness rg = 0.69 (CI: 0.02 – 1), and RBS-R stereotyped rg = 0.77 (CI: 0.04 – 1).

0.4 Genome-wide association studies

Genome-wide association studies (GWAS) were ran separately for the 12 autism subscale traits and the Manhattan plots are shown in Figure 4A. For SCQ communication, the GWAS did not converge due to low heritability. The table of top SNPs with a p < 5 × 10^{-7} are shown in Table 2.

The SNP rs111395362 reached genome-wide significance in the RBS-R ritualistic GWAS (p = 7.7 × 10^{-9}), but it did not map within 100 kb of any gene. However, it is approximately 500 kb downstream of SPATA8, a gene that has been previously associated with
conduct disorders in an ADHD cohort [51]. The SNP rs75957257 also reached genome-wide significance in the RBS-R injurious GWAS ($p = 2.3 \times 10^{-8}$). It did not map within 100 kb of any gene, but it was approximately 200 kb upstream of SPATA8. The SNP rs76950800 was associated with RBS-R restricted ($p = 2.4 \times 10^{-7}$) is within E2F8, a transcription factor that was found to regulate SNRPN in a colorectal cancer study [52]. SNRPN is associated with several neurodevelopmental conditions [53], including autism [54]. The SNP rs10516589 was associated with RBS-R compulsive ($p = 4.3 \times 10^{-7}$) is within the ANK2 gene, which is a high-confidence autism risk gene [55]. The SNP rs62321572 was associated with RBS-R injurious ($p = 1.4 \times 10^{-7}$), which is within 50 kb of the genes USO1 and PPEF2. A CNV analysis associated this region containing these two genes with specific language impairment CNV analysis of specific language impairment associated a CNV region containing USO1 and PPEF2 [56]. The SNP rs6697359 was associated with RBS-R compulsive ($p = 4.8 \times 10^{-7}$) and is within 50 kb of SEC16B, which has been implicated with autism as a somatic mosaic mutation [57].

0.4.1 Polygenic scores derived from autism subscale trait GWAS and their associations in ABCD

Polygenic scores (PGS) from the 11 autism subscale trait GWAS, plus autism case-control (PGC) as a comparison, were calculated in the ABCD cohort (N = 6,559) to be leveraged as validation and generalizability of the genetic associations. The prediction of the PGS were assessed in two behavior traits from the Child Behavior Checklist [42] Syndrome Scales that have previously been shown to be 3 standard deviations higher in autistic children compared to undiagnosed children: social problems and thought problems [58]. The demographic summary of the ABCD cohort used for this analysis and the raw subscale values are shown in Table 3. The two subscale values were residualized for age using linear regression and then normalized to have a mean of 0 and a standard deviation of 1.

PGS performance was assessed using the main effects model $\text{lm}(\text{trait} \sim \text{PGS} + \text{sex})$, with the $\beta$ estimate from this model shown in Figure 4B, along with the 95% confidence interval around the $\beta$. The autism (PGC) PGS $\beta$ estimate is shown with the dashed line, and the autism subscale traits in which the lower 95% confidence bound is above this mean are highlighted in purple.

The autism (PGC) PGS $\beta$ estimate was not significant in predicting social problems $\beta = 0.02$ (CI: 0.00 – 0.04, $p = 0.09$) nor thought problems $\beta = 0.01$ (CI: -0.01 – 0.04, $p = 0.32$). However, six RBS-R traits and SCQ interaction were significant in predicting...
In order to assess pleiotropy of the autism subscale traits with neuropsychiatric conditions, cognitive traits, and behavior, we calculated polygenic scores main effects in SPARK. PGS main effects in SPARK from 13 publicly available GWAS. PGS at SNP threshold 0.05 were used for all analyses in associations with the phenotypes. PGS main effect analyses were performed with two approaches: linear models of main effects in polygenic scores (PGS) in the SPARK cohort from 13 publicly available GWAS. PGS at SNP threshold 0.05 were used for all analyses.

The overall trend of significant PGS effect estimates were highest with RBS-R traits, moderate with DCCQ traits, and lowest with SCQ traits. PGS main effects in SPARK. Table 4. For the reporting of p-values from tests in this results section, no asterisk indicates not significant, one asterisk indicates interaction $\beta = -0.09^{\ast}$ and $\rho$ including DCDQ coordination: $\beta = -0.03^{\ast}$ and $\rho$. Table 3. Top lead SNPs from GWAS. SNPs with $p < 5 \times 10^{-7}$. Genes mapped to the SNPs within the gene, 50 kb, or 100 kb are shown.

### Table 2. Top lead SNPs from GWAS.

| rsid          | CHR | $P$       | trait                  | genes (0kb) | genes (50 kb) | genes (100 kb) |
|---------------|-----|-----------|------------------------|-------------|---------------|----------------|
| rs111395362   | 15  | $7.7 \times 10^{-9}$ | RBS-R ritualistic     |             |               | ZNF138         |
| rs75957257    | 15  | $2.3 \times 10^{-8}$ | RBS-R injurious       |             |               | ZNF107, LOC44139, ZNF273 |
| rs6859290     | 5   | $2.8 \times 10^{-8}$ | DCCQ coordination     |             |               |                |
| rs61953951    | 13  | $8.1 \times 10^{-8}$ | RBS-R stereotyped     |             |               |                |
| rs142684193   | 7   | $1.2 \times 10^{-7}$ | RBS-R restricted      | ZNF138      |               |                |
| rs62321572    | 4   | $1.4 \times 10^{-7}$ | RBS-R injurious       | USO1, PPEF2 | NAAA          |                |
| rs10960087    | 9   | $2.1 \times 10^{-7}$ | SCQ stereotyped       |             |               |                |
| rs76950800    | 11  | $2.4 \times 10^{-7}$ | RBS-R restricted      | E2F8        | CSRP3         | ZDHHC13        |
| rs55686118    | 2   | $2.4 \times 10^{-7}$ | SCQ interaction       | TLK1, METTL8|               |                |
| rs112334346   | 3   | $2.8 \times 10^{-7}$ | RBS-R compulsive      | GXYLT2      | SHQ1, PPP4R2  |                |
| rs17720444    | 10  | $3.4 \times 10^{-7}$ | RBS-R ritualistic     | PLEKHS1, DCLRE1A, NHLRC2 | CASP7 | |
| rs10516589    | 4   | $4.3 \times 10^{-7}$ | RBS-R compulsive      | ANK2        |               |                |
| rs6697359     | 1   | $4.8 \times 10^{-7}$ | RBS-R compulsive      | SEC16B      |               |                |

### Table 3. Autism related behavior traits and demographic summary of ABCD participants.

Social problems: RBS-R injurious $\beta = 0.04$ (CI: 0.02 – 0.07), RBS-R ritualistic $\beta = 0.03$ (CI: 0.01 – 0.05), RBS-R sameness $\beta = 0.04$ (CI: 0.02 – 0.06), SCQ interaction $\beta = 0.04$ (CI: 0.02 – 0.06), with three of the seven traits having their lower 95% confidence bound not overlapping with the autism (PGC) $\beta$: RBS-R compulsive $\beta = 0.05$ (CI: 0.02 – 0.07), RBS-R restricted $\beta = 0.06$ (CI: 0.03 – 0.08), and RBS-R stereotyped $\beta = 0.05$ (CI: 0.03 – 0.07). The same seven traits were also significant in predicting thought problems: RBS-R compulsive $\beta = 0.03$ (CI: 0.01 – 0.06), RBS-R injurious $\beta = 0.04$ (CI: 0.01 – 0.06), SCQ interaction $\beta = 0.03$ (CI: 0.01 – 0.06), with four of the seven traits having their lower 95% confidence bound not overlapping with the autism (PGC) $\beta$: RBS-R compulsive $\beta = 0.05$ (CI: 0.02 – 0.07), RBS-R ritualistic $\beta = 0.05$ (CI: 0.02 – 0.07), RBS-R sameness $\beta = 0.05$ (CI: 0.02 – 0.07), and RBS-R stereotyped $\beta = 0.04$ (CI: 0.01 – 0.06).

### 0.5 Polygenic scores main effects in SPARK

In order to assess pleiotropy of the autism subscale traits with neuropsychiatric conditions, cognitive traits, and behavior, we calculated polygenic scores (PGS) in the SPARK cohort from 13 publicly available GWAS. PGS at SNP threshold 0.05 were used for all analyses in associations with the phenotypes. PGS main effect analyses were performed with two approaches: linear models of main effects $\text{lm(trait value ~ PGS value + sex)}$ and Spearman correlations. The PGS main effects from the linear models are shown in Figure 3A, with the $\beta$ estimate and 95% confidence interval around the $\beta$. Figure 3B is showing Spearman correlation $\rho$ for the trait with the highest number of nominally significant PGS effects, RBS-R sameness. The $\rho$ for both full cohort and sex-stratified correlations are in Table 3. For the reporting of $p$-values from tests in this results section, no asterisk indicates not significant, one asterisk indicates nominal significance and two asterisks indicates significance after FDR correction across the twelve traits and thirteen PGS.

The overall trend of significant PGS effect estimates were highest with RBS-R traits, moderate with DCCQ traits, and lowest with SCQ traits. The strongest effects were seen in educational attainment (SSGAC) PGS negatively associated with 9 autism traits, including DCDQ coordination: $\beta = -0.03^{\ast}$ and $\rho = -0.03^{\ast}$, DCDQ handwriting, $\beta = -0.07^{\ast\ast}$ and $\rho = -0.06^{\ast\ast}$, RBS-R compulsive $\beta = -0.11^{\ast\ast}$ and $\rho = -0.10^{\ast\ast}$, RBS-R injurious $\beta = -0.12^{\ast\ast}$ and $\rho = -0.11^{\ast\ast}$, RBS-R restricted $\beta = -0.12^{\ast\ast}$ and $\rho = -0.11^{\ast\ast}$, RBS-R ritualistic $\beta = -0.09^{\ast\ast}$ and $\rho = -0.08^{\ast\ast}$, RBS-R sameness $\beta = -0.11^{\ast\ast}$ and $\rho = -0.10^{\ast\ast}$, RBS-R stereotyped $\beta = -0.09^{\ast\ast}$ and $\rho = -0.09^{\ast\ast}$, and SCQ interaction $\beta = -0.04^{\ast\ast}$ and $\rho = -0.04^{\ast\ast}$. This effect was also seen in most of the cognitive performance (SSGAC) PGS as well.

Surprisingly, autism (PGC) PGS did not have a significant effects in any of the 12 traits. However, ADHD (PGC) had a positive
Figure 5. PGS main effects in predicting autism subscale traits in SPARK.

A The PGS β estimate from the main effects modeling is plotted with the 95% confidence interval. Names are cog = cognitive performance, edu = educational attainment, AN = anorexia nervosa, MDD = major depression disorder, SCZ = schizophrenia, agree = agreeableness, consc = conscientiousness, extra = extraversion, neurot = neuroticism, open = openness.

B Spearman correlations of PGS with RBS-R sameness.

- FDR < 0.05
- nominal < 0.05
- not significant

Effect in 8 traits: DCDQ handwriting $\beta = 0.05^{**}$ and $\rho = 0.04^{**}$, RBS-R compulsive $\beta = 0.05^{**}$ and $\rho = 0.05^{**}$, RBS-R injurious $\beta = 0.08^{**}$ and $\rho = 0.08^{**}$, RBS-R restricted $\beta = 0.05^{**}$ and $\rho = 0.05^{**}$, RBS-R ritualistic $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$, RBS-R sameness $\beta = 0.05^{**}$ and $\rho = 0.05^{**}$, RBS-R stereotyped $\beta = 0.05^{**}$ and $\rho = 0.05^{**}$, and SCQ stereotyped $\beta = 0.02^{*}$ and $\rho = 0.02$. Likewise, major depression (PGC) PGS had positive effects in 7 traits: DCDQ coordination $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$, DCDQ movement $\beta = 0.04^{**}$ and $\rho = 0.04^{**}$, RBS-R injurious $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$, RBS-R restricted $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$, RBS-R ritualistic $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$, RBS-R sameness $\beta = 0.04^{**}$ and $\rho = 0.04^{**}$, and SCQ stereotyped $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$. Bipolar disorder (PGC) PGS had positive effects in 3 traits: DCDQ handwriting $\beta = 0.02$ and $\rho = 0.03^{*}$, DCDQ movement $\beta = 0.04^{**}$ and $\rho = 0.04^{**}$, and SCQ interaction $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$. Schizophrenia (PGC) PGS had negative effects in 3 traits: RBS-R injurious $\beta = -0.02$ and $\rho = -0.03^{*}$, RBS-R restricted $\beta = -0.01$ and $\rho = -0.02^{*}$, and RBS-R sameness $\beta = -0.02$ and $\rho = -0.03^{*}$. Anorexia (PGC) PGS had a negative effect in RBS-R injurious $\beta = -0.04^{**}$ and $\rho = -0.05^{**}$.

Several personality PGS effects associations were significant as well. Agreeableness (GPC) PGS had negative effects in RBS-R compulsive $\beta = -0.03^{*}$ and $\rho = -0.02$, RBS-R injurious $\beta = -0.03^{*}$ and $\rho = -0.02$, and RBS-R sameness $\beta = -0.03^{*}$ and $\rho = -0.02$. Conscientiousness (GPC) PGS had positive effects in RBS-R compulsive $\beta = 0.03^{*}$ and $\rho = 0.02$, RBS-R ritualistic $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$, and RBS-R sameness $\beta = 0.03^{*}$ and $\rho = 0.02^{*}$. Neuroticism (SSGAC) PGS had positive effects in DCDQ handwriting $\beta = 0.02$ and $\rho = 0.03^{*}$, DCDQ movement $\beta = 0.03^{*}$ and $\rho = 0.03^{*}$, and RBS-R restricted $\beta = 0.03^{*}$ and $\rho = 0.02$. Openness (GPC) PGS had a positive effect in DCDQ movement $\beta = 0.04^{**}$ and $\rho = 0.04^{**}$. 

Table 4. PGS correlations with autism subscale traits.

Spearman $\rho$ coefficients of polygenic scores (PGS) with autism subscale traits. The full cohort ($N = 6,449$) and sex-stratified ($N = 5,170$ males and $N =$ 1,279 females) associations are shown, with nominal p-value $< 0.05$ indicated with one asterisk and FDR p-value $< 0.05$ indicated with two asterisks. Names are cog = cognitive performance, edu = educational attainment, AN = anorexia nervosa, MDD = major depression disorder, SCZ = schizophrenia, agree = agreeableness, consc = conscientiousness, extra = extraversion, neurot = neuroticism, open = openness.

| trait                  | cog | edu | ADHD | AN  | autism | bipolar | MDD  | SCZ  | agree | consc | extra | neurot | open |
|------------------------|-----|-----|------|-----|--------|---------|------|------|-------|-------|-------|--------|------|
| DCDQ coordination M    | 0.6 | 0.6 | 0.02 | 0   | 0.02   | 0.03    | 0    | 0.01 | 0     | 0     | 0.02  | -0.03  | 0.01 |
| F                      | 0   | 0   | -0.04| -0.04| 0.05   | 0.06    | 0.02 | 0.01 | 0     | 0     | 0     | 0.04   | 0.01 |
| DCDQ handwriting M     | -0.04| -0.06| 0.04 | 0   | -0.01| 0.03    | 0.01 | 0    | -0.01| 0     | 0     | 0.04   | 0    |
| F                      | -0.03| -0.06| -0.02| 0   | -0.07 | 0.04    | 0    | 0    | 0.01 | 0.01  | 0.03  | 0.04   | 0.01 |
| DCDQ movement M        | 0   | 0   | 0    | 0   | 0.01  | 0.02    | 0    | 0    | 0     | -0.01| 0     | 0.04   | 0    |
| F                      | 0   | 0   | -0.01| 0   | 0     | 0.02    | 0.01 | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| RBS-R compulsive M     | -0.05| -0.11| 0.05 | 0   | 0.02  | 0.01    | 0    | 0    | -0.01| 0.01  | 0.02  | 0.03   | 0.02 |
| F                      | -0.06| -0.12| 0.05 | 0   | 0.04  | -0.07   | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| RBS-R injurious M      | -0.05| -0.11| 0.08 | 0   | 0.02  | 0.03    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| F                      | -0.04| -0.13| 0.05 | 0   | 0.04  | -0.06   | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| RBS-R restricted M     | -0.02| -0.08| 0.03 | 0   | 0.01  | 0.04    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| F                      | -0.02| -0.08| 0.02 | 0   | 0.02  | 0.02    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| RBS-R ritualistic M    | -0.03| -0.08| 0.04 | 0   | 0.02  | 0.04    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| F                      | -0.03| -0.08| 0.02 | 0   | 0.04  | 0.09    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| RBS-R cannes M         | -0.04| -0.11| 0.05 | 0   | 0.02  | 0.03    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| F                      | -0.04| -0.11| 0.03 | 0   | 0.03  | -0.07   | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| RBS-R stereotyped M   | -0.04| -0.09| 0.05 | 0   | -0.01| 0       | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| F                      | -0.03| -0.08| 0.06 | 0   | 0.02  | 0.03    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| SCQ communication M    | -0.07| -0.12| 0.03 | 0   | -0.08 | 0.02    | 0    | 0    | 0.01 | 0.01  | -0.02| 0.03   | 0    |
| F                      | -0.01| -0.04| 0.01 | 0   | 0.02  | 0.01    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| SCQ interaction M      | 0   | 0   | 0    | 0   | 0.01  | 0.02    | 0    | 0    | -0.01| 0     | 0     | 0      | 0    |
| F                      | 0   | 0   | 0    | 0   | 0.01  | 0.02    | 0    | 0    | 0.01 | 0.01  | 0.02  | 0.03   | 0.01 |
| SCQ stereotyped M      | 0   | 0   | 0    | 0   | 0    | 0       | 0    | 0    | -0.01| 0     | 0     | 0      | 0    |

0.6 Polygenic scores sex interaction effects in SPARK

In order to analyze sex interactions of the autism subscale traits with neuropsychiatric, cognitive, and personality PGS, we first formally tested for sex interaction effects by linear models with a PGS by sex interaction term $\text{lm(trait} \sim \text{PGS value + sex + PGS value : sex})$. While this interaction modeling allows identification of significant PGS by sex interaction effects, it does not show how the interaction is manifested within each sex. For example, a positive $\beta$ estimate for the PGS by sex interaction term (note that male was coded as 1 and female was coded as 0) can be indicative of one of three possible mechanisms:

1. The PGS has a positive effect in males and negative effect in females.
2. The PGS has a positive effect in males and no effect in females.
3. The PGS has no effect in males and a negative effect in females.
Likewise, the three possible interpretations of a negative $\beta$ estimate (female direction) for the interaction term are:

1. The PGS has positive effect in females and negative effect in males.
2. The PGS has a positive effect in females and no effect in males.
3. The PGS has no effect in females and a negative effect in males.

Therefore, to further interrogate the sex interactions, we performed sex stratified Spearman correlations across all PGS and autism subscale traits.
subscale traits. The $\beta$ estimate from the PGS by sex interaction term is shown with the fill color in Figure 6A, with one dot indicating the term is nominally significant. A positive $\beta$ (green) indicates higher PGS by sex interaction for males, whereas a negative $\beta$ (purple) indicates higher PGS by sex interaction for females. For the significant terms, sex stratified correlations and best fit lines are shown in Figure 6B. The correlation $\rho$ coefficients for all PGS and traits are in Table 4. For the reporting of p-values from tests in this results section, no asterisk indicates not significant, one asterisk indicates nominal significance and two asterisks indicates significance after FDR correction across the 12 traits and 13 PGS. The p-values from the sex stratified analyses are important to understand in the context that there are four times as many males than females in the cohort.

Figure 6. PGS sex interaction effects in predicting autism subscale traits in SPARK.
A) The PGS $\beta$ estimate from the sex interaction modeling is shown with the fill color, with one dot indicating the PGS by sex interaction term was nominally significant. A positive $\beta$ (green) indicates higher PGS by sex interaction for males, whereas a negative $\beta$ (purple) indicates higher PGS by sex interaction for females.
B) Sex-stratified Spearman correlations in which the PGS by sex interaction term was nominally significant. The p-values on the plots are the nominal p-values.

The significant autism (PGC) PGS by sex interaction terms were in the male direction, as indicated by the positive $\beta$. Surprisingly, sex stratified correlations revealed a negative effect in females and a roughly neutral effect in males, meaning increasing polygenic risk for autism (PGC) had approximately no association with RBS-R traits in males and is actually associated with less RBS-R traits in females. This was evident for RBS-R compulsive: male $\rho = 0.01$ and female $\rho = -0.07^*$. RBS-R injurious: male $\rho = 0.01$ and female $\rho = -0.07^*$.
0.09**, RBS-R restricted: male $\rho = 0.00$ and female $\rho = -0.06*$, RBS-R sameness: male $\rho = -0.01$ and female $\rho = -0.07*$, and RBS-R stereotyped: male $\rho = 0.02$ and female $\rho = -0.08*$. To further interrogate the complex relationship between autism (PGC) PGS and sex, we analyzed the grouped means of the male probands in which their father also had genetic data available (N = 1,061 male probands and N = 686 fathers) and female probands in which their mother also had genetic data available (N = 475 female probands and N = 283 mothers). We expected autism case-control (PGC) to perform well in predicting case-control status in SPARK given that previous work performed an autism case-control GWAS in SPARK and found the genetic correlation with autism case-control (PGC) to be high [59]. We found that female probands had significantly higher autism (PGC) PGS than their mothers, and likewise male probands had significantly higher PGS than their fathers (see Table 5), with the mean difference between between female probands vs. mothers and male probands vs. fathers being very similar at 0.19. However, females overall have higher PGS, with the female proband mean at 0.14 and the male proband mean at 0.04. Likewise, the mother mean is -0.05 and the father mean is -0.15.

For ADHD PGS and DCDQ traits, the interaction term was in the male direction—DCDQ coordination: male $\rho = 0.03*$ and female $\rho = -0.04$, DCDQ handwriting: male $\rho = 0.05**$ and female $\rho = -0.02$, and DCDQ movement: male $\rho = 0.01$ and female $\rho = -0.07*$. The anorexia PGS and two DCDQ traits also had two significant interaction terms in the male direction—DCDQ handwriting: male $\rho = 0.01$ and female $\rho = -0.07$ and DCDQ movement: male $\rho = 0.02$ and female $\rho = -0.04$.

Several personality PGS by sex interaction terms were also significant. Conscientiousness (GPC) PGS correlations with RBS-R ritualistic were much stronger in females $\rho = 0.08*$ than in males $\rho = 0.02$. Extraversion (GPC) PGS were positive for females and negative for males in two DCDQ traits—DCDQ coordination: male $\rho = -0.04*$ and female $\rho = 0.05$ and DCDQ movement: male $\rho = -0.04$ and female $\rho = 0.03$. Neuroticism (SSGAC) PGS were positive for females and neutral for males in the same two DCDQ traits—DCDQ coordination: male $\rho = 0.00$ and female $\rho = 0.09**$ and DCDQ movement: male $\rho = 0.01$ and female $\rho = 0.09**$. Openness (GPC) PGS were positive for females and neutral in males—RBS-R compulsive: male $\rho = 0.02$ and female $\rho = -0.05$, RBS-R sameness: male $\rho = -0.07$ and female $\rho = -0.07$, and RBS-R stereotyped: male $\rho = -0.01$ and female $\rho = -0.07$.

| PGS   | sex | role | mean  | mean dif |
|-------|-----|------|-------|----------|
| ADHD  | M   | proband | 0.04  | 0.19**   |
|       | M   | parent   | -0.15 |          |
| autism| F   | proband  | 0.14  | 0.19**   |
|       | F   | parent   | -0.05 |          |
| edu   | M   | proband  | 0.07  | -0.01    |
|       | M   | parent   | -0.03 | 0.10*    |
| MDD   | F   | proband  | 0.05  |          |
|       | F   | parent   | -0.05 | 0.10     |
|       | M   | proband  | 0.06  | 0.16**   |
|       | M   | parent   | -0.10 |          |

Table 5. Difference in grouped mean PGS between the probands and their sex-matched parent.
The cohort was filtered to male probands in which their father also had genetic data available (N = 1,061 male probands and N = 686 fathers) and female probands in which their mother also had genetic data available (N = 475 female probands and N = 283 mothers). One asterisk indicates the grouped mean t-test between proband vs. parent was nominally significance, and two asterisks indicates FDR significance.

Discussion

Because autism is phenotypically complex and strongly genetic, our core motivation in this work was to characterize the genetic etiology of three widely-used and clinically valid instruments (SCQ, RBS-R, and DCDQ) and their subscales. We employed well-established approaches such as GWAS, SNP-heritability estimation, and genetic and phenotypic correlation to characterize these measures of core autism symptom domains. The findings from our analysis of N = 6,449 autistic children and N = 6,559 children sampled from the
general population, which are detailed below, show pronounced differences in genetic signal underlying the three assessments we investigated. These findings also underscore the limitations in signal strength (heritability) and in generalizability of genetic associations that are based on a binary diagnosis. Taken together, our results show that the future of autism genetics demands a focus on multidimensional, quantitative phenotypes.

Our phenotypic correlation analysis demonstrates the quantitative and heterogeneous nature of core autism traits in an autism-only cohort. Some traits were only moderately correlated with each other, especially subscales probing different core domains (Figure 2A). In addition, for some traits, up to 5% of individuals fell into a heterogeneous outgroup, meaning they were greater than 1 standard deviation above the mean in one trait and less than 1 standard deviation below the mean in the other trait (Figure 2B). Such discordances further demonstrate the complex and heterogeneous spectrum of autism (60) and that more detailed phenotypic characterization and new methods of integrating the phenotypic data (61) are important to further illuminate the biology of autism.

The autism subscales we investigated are positively genetically correlated to some extent, as revealed by our intra-cohort genetic correlation analysis (Figure 2B). Specifically, we observed that several RBS-R subscales are significantly genetically correlated among themselves, as well as with at least one SCQ trait and DCDQ trait. This is not unanticipated given that a diagnosis of autism requires determinations of both social communication problems and restricted and repetitive behaviors/interests. However, this finding contrasts with those from general population studies of latent autistic traits. Specifically, a recent GWAS in general population samples of systemizing behavior, an indicator of restricted and repetitive behaviors/interests, (N = 51,564) (22) and empathy, an indicator of social communication abilities, (N = 46,861) (27) did not observe significant genetic correlations between systemizing behavior and empathy, but did find that greater systemizing and lower empathy were both significantly genetically correlated with autism (PGC) (25).

Clinicians administering screening tools like the SCQ, the RBS-R, and the DCDQ might well wonder to what extent these instruments are detecting behaviors and traits that have bases in biology. Our analysis of SNP-heritability of each of these scales sheds light on such questions: A screen that is sensitive to relevant genetic factors should have higher heritability than a screen that is more environmentally influenced or more sensitive to state vs. trait. We found that SNP heritability varied widely across the 12 subscales, with most of the RBS-R subscales being more heritable than binary autism case-control (PGC) (Figure 2A). In contrast, the DCDQ and SCQ subscales had lower heritabilities than the RBS-R subscales and autism diagnosis (PGC). These results suggest that the RBS-R is more sensitive to relevant genetic factors than either the SCQ or DCDQ. Collapsing the phenotype to diagnostic status potentially diminishes the signal for several reasons, as our results demonstrate, due to variabilities both phenotypically and genetically among autistic individuals across these clinical subscales.

Given the recent progress in parsing the genetics of latent autism traits in large undiagnosed samples (26, 27), we were motivated to see if we could uncover evidence running in the other direction: could PGS based on our analysis of the twelve subscales in an autism-only cohort be used to predict autism-like phenotypes in a general population sample of children? We calculated PGS in ABCD (a general population sample) using our GWAS summary statistics for subscales in SPARK and correlated these estimates with those from general population studies of latent autistic traits. Specifically, a recent GWAS in general population samples of schizophrenia among cases. It was only predictive of the quantitative schizophrenia trait in controls (64). Interestingly, we found that the autism PGS had no association with autism severity among cases only: the diagnosis compresses the dynamic range of severity into autistic individuals across these clinical subscales.

Just as the autism case-control PGS (PGC) (20) did not translate to a general population sample (ABCD) to predict autism-related traits, it also failed to predict symptom severity across all 12 symptom subscales we investigated in SPARK (Figure 3A), again highlighting the limitations of diagnosis as a target phenotype in genetic research. Because the autism case-control (PGC) GWAS collapsed the heterogeneity in autism, it has the effect of pooling autism risk alleles broadly. It should therefore not be surprising that the autism PGS had no association with autism severity among cases: the diagnosis compresses the dynamic range of severity into autistic individuals across these clinical subscales. However, whereas the PGS has a correlation of roughly zero in males, it is anti-correlated with symptom severity in females. This unexpected finding warrants some discussion of potential explanations and, in particular, sex-specific explanations. It has been previously established that affected children (mostly sons) inherit more genetic autism risk from their mothers (65). Because the autism PGC cohort is heavily male-biased, the subset of risk alleles identified using this cohort can be seen as representing disproportionate “mother-to-son” alleles (alleles inherited from mothers to sons) or male-specific. Alternatively, these may be alleles that are female-specific or female-benign/protective alleles can provide one explanation for why the autism PGS predicts lower symptom severity in females. On the other hand, autistic females carry more rare genetic variants than autistic males (66). It has been established that PGS and rare variants interact additively in autism risk (67). It is possible that the
most severely affected autistic females in our cohort carry more rare variants that impact their severity, as recent analyses by [68] suggest. These two explanations are, of course, not mutually exclusive. Together, they present a strong case that it is crucial to consider sex in PGS applications, especially in sex-imbalanced cohorts where a sex-biased trait is studied [69].

Finally, we found that in SPARK, other neuropsychiatric, cognitive, and personality PGS were the significant predictors for autism symptomatology/severity in autistic individuals (Figure 5 and Table 4), as opposed to the autism (PGC) PGS. The strongest PGS associations we found were educational attainment (SSGAC) and cognitive performance (SSGAC) negatively predicting several autism traits, meaning higher polygenic propensity for cognitive abilities in autistic individuals is associated with reduced symptom burden. Interestingly, this contrasts with numerous reports of a positive genetic correlation between educational attainment and autism case-control (PGC) [20] [70] [71]. The ADHD (PGC) and major depression (PGC) PGS both positively predicted increased autism traits in the SPARK cohort, which is in line with previous work showing ADHD (PGC) and major depression (PGC) to be significantly genetically correlated with autism [20]. It is possible these two PGS are measuring the spectrum and/or subgroups in autism, similar to the schizophrenia (PGC) PGS predicting differences in neuroticism and psychological distress in a major depression cohort [22]. Beyond neuropsychiatric and cognitive PGS, we also wanted to assess the if Big Five personality PGS were predictive of autism traits in autistic individuals. There is extensive research on the Big 5 personality measures and their relationship to autism [73], including one study suggesting the Big Five personality measures can subtype autistic individuals based on severity [74], but it is unknown if the genetic signals of personality can also subtype autistic individuals. Other work has shown there is an overlap of genetic risk factors for neuroticism, autism, and ADHD quantitative traits [75], and the autism case-control (PGC) GWAS is positively genetically correlated with neuroticism [20]. Indeed, several Big Five personality PGS were predictive of autism subscales (Figure 5 and Table 4). Higher conscientiousness (GPC) PGS predicted increased RBS-R compulsive, RBS-R ritualistic, and RBS-R sameness traits. Lower agreeableness (GPC) PGS significantly was significantly associated with higher RBS-R compulsive, RBS-R injurious, and RBS-R sameness traits, and neuroticism (SSGAC) PGS was positively associated with DCDQ movement and RBS-R restricted traits. Again sex was an important variable, with significant PGS by sex interactions observed for eight of the Big Five personality PGS. These analyses suggest that optimizing predictors, e.g. for eventual use in personalizing care, will require the utilization of several neuropsychiatric, cognitive, and personality PGS, as well as consideration of interactions with sex.

Overall, our results show that these clinical autism subscales, particularly the RBS-R, are measuring meaningful common genetic signal that is more powerful, heritable, and generalizable than case-control status. Although historically the path to increased power GWAS discovery has been a quest for ever-larger case-control sample sizes, our results indicate that investing in better phenotypic characterization, even at the expense of sample size, might be a better value proposition for psychiatric genetics and autism research in particular.

0.7 Language choices

Many autistic self-advocates prefer identity-first language (i.e. autistic individuals), and some autistic individuals and their families prefer person-first language (i.e. individuals with autism). We chose to use identity-first language for this paper. Gender is distinct from sex and has important variance, especially in the autistic community [70] [77]. For language around sex and gender, we exclusively considered sex (meaning sex assigned at birth) and did not consider gender in our analyses.

Acknowledgments

We are grateful to all of the individuals and families in SPARK and ABCD, as well as the SPARK staff.
References

1. J. Baio, L. Wiggins, D. L. Christensen, M. J. Maenner, J. Daniels, Z. Warren, M. Kurzuis-Spencer, W. Zahorodny, C. Robinson, Rosenberg, T. White, M. S. Durkin, P. Imm, L. Nikolau, M. Yeargin-Allsopp, L.-C. Lee, R. Harrington, M. Lopez, R. T. Fitzgerald, A. Hewitt, S. Pettygrove, J. N. Constantino, A. Vehorn, J. Shenouda, J. Hall-Lande, K. Van, Naarden, Braun, and N. F. Dowling, “Prevalence of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network, 11 sites, united states, 2014.” MMWR. Surveillance Summaries, vol. 67, pp. 1–23, Apr. 2018.

2. F. Chiarotti and A. Venerosi, “Epidemiology of autism spectrum disorders: a review of worldwide prevalence estimates since 2014,” Brain sciences, vol. 10, no. 5, p. 274, 2020.

3. D. Bai, B. H. K. Yip, G. C. Windham, A. Sourander, R. Francis, R. Yoffe, E. Glasson, B. Mahjani, A. Suominen, H. Leonard, M. Gissler, J. D. Buxbaum, K. Wong, D. Schendel, A. Kodesh, M. Breshnahan, S. Z. Levine, E. T. Parner, S. N. Hansen, C. Hultman, A. Reichenberg, and S. Sandin, “Association of genetic and environmental factors with autism in a 5-country cohort,” JAMA Psychiatry, vol. 76, p. 1035, Oct. 2019.

4. American Psychiatric Association, Diagnostic and statistical manual of mental disorders: DSM-5. Washington, DC: Autor, 5th ed. ed., 2013.

5. M. Lloyd, M. MacDonald, and C. Lord, “Motor skills of toddlers with autism spectrum disorders,” Autism, vol. 17, pp. 133–146, May 2011.

6. L. Margari, F. Craig, F. Margari, A. Legrottaglie, R. Palumbi, and C. D. Giambattista, “A review of executive function deficits in autism spectrum disorder and attention-deficit/hyperactivity disorder,” Neuropsychiatric Disease and Treatment, p. 1191, May 2016.

7. M. C. Souders, T. B. A. Mason, O. Valladares, M. Bucan, S. E. Levy, D. S. Mandell, T. E. Weaver, and J. Pinto-Martin, “Sleep behaviors and sleep quality in children with autism spectrum disorders,” Sleep, vol. 32, pp. 1566–1578, Dec. 2009.

8. T. Koomar, T. R. Thomas, N. R. Pottscheidt, M. Lutter, and J. J. Michaelson, “Estimating the prevalence and genetic risk mechanisms of ARFID in a large autism cohort,” Frontiers in Psychiatry, vol. 12, June 2021.

9. E. J. MARCO, L. B. HINKLEY, S. S. HILL, and S. S. NAGARAJAN, “Sensory processing in autism: A review of neurophysiologic findings,” Pediatric Research, vol. 69, pp. 48R–54R, May 2011.

10. K. J. Varcin and S. S. Jeste, “The emergence of autism spectrum disorder,” Current Opinion in Psychiatry, vol. 30, pp. 85–91, Mar. 2017.

11. L. D. Wiggins, D. L. Robins, L. B. Adamson, R. Bakeman, and C. C. Henrich, “Support for a dimensional view of autism spectrum disorders in toddlers,” Journal of Autism and Developmental Disorders, vol. 42, pp. 191–200, Mar. 2011.

12. O. Ousley and T. Cermak, “Autism spectrum disorder: Defining dimensions and subgroups,” Current Developmental Disorders Reports, vol. 1, pp. 20–28, Dec. 2013.

13. S. H. Kim, S. Macari, J. Koller, and K. Chawarska, “Examining the phenotypic heterogeneity of early autism spectrum disorder: subtypes and short-term outcomes,” Journal of Child Psychology and Psychiatry, vol. 57, pp. 93–102, Aug. 2015.

14. C. Lord, S. Risi, L. Lambrecht, E. H. Cook, B. L. Leventhal, P. C. DiLavore, A. Pickles, and M. Rutter, “The autism diagnostic observation schedule—generic: A standard measure of social and communication deficits associated with the spectrum of autism,” Journal of autism and developmental disorders, vol. 30, no. 3, pp. 205–223, 2000.

15. M. Rutter, A. Le Couteur, C. Lord, et al., “Autism diagnostic interview-revised,” Los Angeles, CA: Western Psychological Services, vol. 29, no. 2003, p. 30, 2003.

16. P. Feliciano, A. M. Daniels, L. G. Snyder, A. Beaumont, et al., “SPARK: A US cohort of 50, 000 families to accelerate autism research,” Neuron, vol. 97, pp. 488–493, Feb. 2018.

17. M. Rutter, A. Bailey, and C. Lord, “The social communication questionnaire,” tech. rep., Western Psychological Services, 2003.

18. J. Bodfish, F. Symons, and M. Lewis, “The repetitive behavior scale,” tech. rep., Western Carolina Center Research Reports, 1999.

19. B. N. Wilson, S. G. Crawford, D. Green, G. Roberts, A. Aylott, and B. J. Kaplan, “Psychometric properties of the revised developmental coordination disorder questionnaire,” Physical & Occupational Therapy In Pediatrics, vol. 29, pp. 182–202, Jan 2009.

20. J. Grove, S. Ripke, T. D. Als, et al., “Identification of common genetic risk variants for autism spectrum disorder,” Nature Genetics, vol. 51, pp. 431–444, Feb. 2019.
21. A. B. Ratto, L. Kenworthy, B. E. Yerys, J. Bascom, A. T. Wieckowski, S. W. White, G. L. Wallace, C. Pugliese, R. T. Schultz, T. H. Ollendick, A. Scarpa, S. Seese, K. Register-Brown, A. Martin, and L. G. Anthony, “What about the girls? sex-based differences in autistic traits and adaptive skills,” *Journal of Autism and Developmental Disorders*, vol. 48, pp. 1698–1711, Dec. 2017.

22. L. Bishop-Fitzpatrick and A. J. Hill, “A scoping review of health disparities in autism spectrum disorder,” *Journal of Autism and Developmental Disorders*, vol. 47, pp. 3380–3391, July 2017.

23. E. Suksmithi, I. Roth, and R. A. Hoekstra, “Autistic traits below the clinical threshold: Re-examining the broader autism phenotype in the 21st century,” *Neuropsychiatric Review*, vol. 21, pp. 360–389, Oct. 2011.

24. E. B. Robinson, K. C. Koenen, M. C. McCormick, K. Munir, V. Hallett, F. Happé, R. Plomin, and A. Ronald, “Evidence that autistic traits show the same etiology in the general population and at the quantitative extremes (5%, 2.5%, and 1%),” *Archives of general psychiatry*, vol. 68, no. 11, pp. 1113–1121, 2011.

25. S. M. Myers, T. D. Challman, R. Bernier, T. Bourgeron, W. K. Chung, J. N. Constantino, E. E. Eichler, S. Jaconemont, D. T. Miller, K. J. Mitchell, H. Y. Zoghbi, C. L. Martin, and D. H. Ledbetter, “Insufficient evidence for “autism-specific” genes,” *The American Journal of Human Genetics*, vol. 106, pp. 587–595, May 2020.

26. V. Warrier, R. Toro, H. Won, C. S. Leblond, F. Cliquet, R. Delorme, W. De Witte, J. Bralten, B. Chakrabarti, A. D. Børglum, *et al.*, “Social and non-social autism symptoms and trait domains are genetically dissociable,” *Communications biology*, vol. 2, no. 1, pp. 1–13, 2019.

27. V. Warrier, R. Toro, B. Chakrabarti, A. D. Børglum, J. Grove, D. A. Hinds, T. Bourgeron, and S. Baron-Cohen, “Genome-wide analyses of self-reported empathy: correlations with autism, schizophrenia, and anorexia nervosa,” *Translational psychiatry*, vol. 8, no. 1, pp. 1–10, 2018.

28. J. Bralten, K. J. van Hulzen, M. B. Martens, T. E. Galesloot, A. A. Vasquez, L. A. Kiemeney, J. K. Buitelaar, J. W. Muntjewerff, B. Franke, and G. Poelmans, “Autism spectrum disorders and autistic traits share genetics and biology,” *Molecular Psychiatry*, vol. 23, pp. 1205–1212, May 2017.

29. A. Yousaf, R. Waltes, D. Haslinger, S. M. Klauck, E. Duketis, M. Sachse, A. Voran, M. Biscaldi, M. Schulte-Rüther, S. Cichon, *et al.*, “Quantitative genome-wide association study of six phenotypic subdomains identifies novel genome-wide significant variants in autism spectrum disorder,” *Translational psychiatry*, vol. 10, no. 1, pp. 1–11, 2020.

30. S. Chandler, T. Charman, G. Baird, T. Loucas, D. Meldrum, M. Scott, and A. Pickles, “Validation of the social communication questionnaire in a population cohort of children with autism spectrum disorders,” *Journal of the American Academy of Child & Adolescent Psychiatry*, vol. 46, no. 10, pp. 1324–1332, 2007.

31. R. L. Gabriels, M. L. Cuccaro, D. E. Hill, B. J. Ivers, and E. Goldson, “Repetitive behaviors in autism: relationships with associated clinical features,” *Research in Developmental Disabilities*, vol. 26, pp. 169–181, Mar. 2005.

32. B. Wilson, B. Kaplan, S. Crawford, and G. Roberts, “The developmental coordination disorder questionnaire 2007 (dcdq’07),” *Administrative manual for the DCDQ107 with psychometric properties*, pp. 267–272, 2007.

33. S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. de Bakker, M. J. Daly, and P. C. Sham, “PLINK: A tool set for whole-genome association and population-based linkage analyses,” *The American Journal of Human Genetics*, vol. 81, pp. 559–575, Sep 2007.

34. A. T. Marees, H. de Kluiver, S. Stringer, F. Vorspan, E. Curis, C. Marie-Claire, and E. M. Derks, “A tutorial on conducting genome-wide association studies: Quality control and statistical analysis,” *International Journal of Methods in Psychiatric Research*, vol. 27, p. e1608, Feb 2018.

35. R Core Team, *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2013.

36. L.-P. L. Perreault, M.-A. Legault, G. Asselin, and M.-P. Dubé, “genipe: an automated genome-wide imputation pipeline with automatic reporting and statistical tools,” *Bioinformatics*, p. btw487, Aug 2016.

37. O. Delaneau, J. Marchini, and J.-F. Zagury, “A linear complexity phasing method for thousands of genomes,” *Nature Methods*, vol. 9, pp. 179–181, Dec 2011.

38. B. N. Howie, P. Donnelly, and J. Marchini, “A flexible and accurate genotype imputation method for the next generation of genome-wide association studies,” *PLoS Genetics*, vol. 5, p. e1000529, Jun 2009.

39. M. Lawrence, W. Huber, H. Pagès, P. Aboyoun, M. Carlson, R. Gentleman, M. T. Morgan, and V. J. Carey, “Software for computing and annotating genomic ranges,” *PLoS Computational Biology*, vol. 9, p. e1003118, Aug 2013.

40. C. A. de Leeuw, J. M. Mooij, T. Heskes, and D. Posthuma, “MAGMA: Generalized gene-set analysis of GWAS data,” *PLoS Computational Biology*, vol. 11, p. e1004219, Apr. 2015.
[44] J. J. Lee, R. Wedow, A. Okbay, et al., “Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder,” Nature Genetics, vol. 51, pp. 63–75, Nov. 2018.

[45] H. J. Watson, Z. Yilmaz, L. M. Thornton, et al., “Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa,” Nature Genetics, vol. 51, pp. 1207–1214, July 2019.

[46] D. M. Ruderfer, S. Ripke, and A. McQuillin, “Genomic dissection of bipolar disorder and schizophrenia, including 28 subphenotypes,” Cell, vol. 173, pp. 337–349, e16, June 2018.

[47] D. M. Ruderfer, S. Ripke, and A. McQuillin, “Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression,” Nature Genetics, vol. 50, pp. 668–681, Apr. 2018.

[48] M. H. M. de Moor, P. T. Costa, A. Terracciano, R. F. Krueger, E. J. C. de Geus, T. Toshiko, B. W. J. H. Penninx, T. Esko, N. R. Wray, S. Ripke, M. Mattheisen, et al., “Multi-trait analysis of genome-wide association summary statistics using MTAG,” Nature Genetics, vol. 2, pp. 943–955, Dec. 2001.

[49] K. M. Lisdahl, K. J. Sher, K. P. Conway, R. Gonzalez, S. W. F. Ewing, S. J. Nixon, S. Tapert, H. Bartsch, R. Z. Goldstein, and M. Heitzeg, “Adolescent brain cognitive development (abcd) study: Overview of substance use assessment methods,” Developmental cognitive neuroscience, vol. 32, pp. 80–96, 2018.

[50] T. M. Achenbach, “The child behavior checklist and related instruments,” 1990.

[51] R. J. Anney, J. Lasky-Su, C. Ó'Dushláine, E. Kenny, B. M. Neale, A. Mulligan, B. Franke, K. Zhou, W. Chen, H. Christiansen, A. Arias-Vásquez, T. Banaschewski, J. Buitelaar, R. Elstein, A. Miranda, F. Mulas, R. D. Oades, H. Roeyers, A. Rothenberger, J. Sergeant, E. Sonuga-Barke, H. Steinhausen, P. Asherson, S. V. Faraone, and M. Gill, “Conduct disorder and ADHD: Evaluation of conduct problems as a categorical and quantitative trait in the international multicentre ADHD genetics study,” American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, vol. 147B, pp. 1369–1378, Dec. 2008.

[52] M. Ji, L. Ren, Y. Lv, X. Lao, Q. Feng, W. Tang, A. Zhuang, T. Liu, P. Zheng, and J. Xu, “Small nuclear ribonucleoprotein polypeptide n accelerates malignant progression and poor prognosis in colorectal cancer transcriptionally regulated by e2f8,” Scientific Reports, vol. 10, Nov. 2020.

[53] H. Li, P. Zhao, Q. Xu, S. Shan, C. Hu, Z. Qiu, and X. Xu, “The autism-related gene SNRPN regulates cortial and spine development via controlling nuclear receptor nr4a1,” The American Journal of Human Genetics, vol. 51, pp. 1207–1214, July 2019.

[54] S. E. Folstein and B. Rosen-Sheidley, “Genetics of autism: complex aetiology for a heterogeneous disorder,” Nature Reviews Genetics, vol. 2, pp. 943–955, Dec. 2001.

[55] I. Iossifov, B. J. O’Roak, S. J. Sanders, M. Ronemus, N. Krumm, D. Levy, H. A. Stessman, K. T. Witherspoon, L. Vives, K. E. Patterson, J. D. Smith, B. Paeper, D. A. Nickerson, J. Dea, S. Dong, L. E. Gonzalez, J. D. Mandell, S. M. Mane, M. T. Murtha, C. A. Sullivan, M. F. Walker, Z. Waqar, L. Wei, A. J. Willsey, B. Yamrom, Y. ha Lee, E. Grabowska, E. Dalkic, Z. Wang, S. Marks, P. Andrews, A. Leotta, J. Kendall, I. Hakker, J. Rosenbaum, B. Ma, L. Rodgers, J. Trobe, G. Narzisi, S. Yoon, M. C. Schatz, K. Ye, W. R. McCombie, J. Shendure, E. E. Eichler, M. W. State, and M. Wigler, “The contribution of de novo coding mutations to autism spectrum disorder,” Nature, vol. 515, pp. 216–221, Oct. 2014.

[56] N. H. Simpson, F. Ceroni, R. H. Reader, L. E. Covill, J. C. Knight, E. R. Hennessy, P. F. Bolton, G. Conti-Ramsden, A. O’Hare, G. Baird, S. E. Fisher, and D. F. Newbury, “Genome-wide association identifies a role for common copy number variants in specific language impairment,” European Journal of Human Genetics, vol. 23, pp. 1370–1377, Jan. 2015.

[57] D. R. Krupp, R. A. Barnard, Y. Duffourd, S. A. Evans, R. M. Mulqueen, R. Bernier, J.-B. Rivière, E. Fombonne, and B. J. O’Roak, “Exonic mosaic mutations contribute risk for autism spectrum disorder,” The American Journal of Human Genetics, vol. 101, no. 3, pp. 369–390, 2017.
58. A. A. Arias, M. M. Rea, E. J. Adler, A. D. Haendel, and A. V. V. Hecke, “Utilizing the child behavior checklist (CBCL) as an autism spectrum disorder preliminary screener and outcome measure for the PEERS® intervention for autistic adolescents,” *Journal of Autism and Developmental Disorders*, May 2021.

59. N. Matoba, D. Liang, H. Sun, N. Aygün, J. C. McAfee, J. E. Davis, L. M. Rafffield, H. Qian, J. Piven, Y. Li, S. Kosuri, H. Won, and J. L. Stein, “Common genetic risk variants identified in the SPARK cohort support DDHD2 as a candidate risk gene for autism,” *Translational Psychiatry*, vol. 10, Aug. 2020.

60. D. H. Geschwind and P. Levitt, “Autism spectrum disorders: developmental disconnection syndromes,” *Current opinion in neurobiology*, vol. 17, no. 1, pp. 103–111, 2007.

61. M. V. Lombardo, M.-C. Lai, and S. Baron-Cohen, “Big data approaches to decomposing heterogeneity across the autism spectrum,” *Molecular Psychiatry*, vol. 24, pp. 1435–1450, Jan. 2019.

62. E. B. Robinson, B. S. Pourcain, V. Anttila, J. A. Kosnicki, B. Bulik-Sullivan, J. Grove, J. Maller, K. E. Samocha, S. J. Sanders, S. Ripke, J. Martin, M. V. Hollegaard, T. Verge, D. M. Hougaard, B. M. Neale, D. M. Evans, D. Skuse, P. B. Mortensen, A. D. Berglum, A. Ronald, G. D. Smith, and M. J. Daly, “Genetic risk for autism spectrum disorders and neuropsychiatric variation in the general population,” *Nature Genetics*, vol. 48, pp. 552–555, Mar. 2016.

63. K. Nayar, J. M. Sealock, N. Maltman, L. Bush, E. H. Cook, L. K. Davis, and M. Losh, “Elevated polygenomic burden for autism spectrum disorder is associated with the broad autism phenotype in mothers of individuals with autism spectrum disorder,” *Biological Psychiatry*, vol. 89, pp. 476–485, Mar. 2021.

64. R. Shafee, P. Nanda, J. L. Padmanabhan, N. Tandon, N. Alliey-Rodriguez, S. Kalapurakkel, D. J. Weiner, R. E. Gur, R. S. E. Keefe, S. K. Hill, J. R. Bishop, B. A. Clementz, C. A. Tammenga, E. S. Gershon, G. D. Pearlson, M. S. Keshavan, J. A. Sweeney, S. A. McCarron, and E. B. Robinson, “Polygenic risk for schizophrenia and measured domains of cognition in individuals with psychosis and controls,” *Translational Psychiatry*, vol. 8, Apr. 2018.

65. J. N. Constantino, A. Todorov, C. Hilton, P. Law, Y. Zhang, E. Molloy, R. Fitzgerald, and D. Geschwind, “Autism recurrence in half siblings: strong support for genetic mechanisms of transmission in ASD,” *Molecular Psychiatry*, vol. 18, pp. 137–138, Feb. 2012.

66. Y. Zhang, N. Li, C. Li, Z. Zhang, H. Teng, Y. Wang, T. Zhao, L. Shi, K. Zhang, K. Xia, J. Li, and Z. Sun, “Genetic evidence of gender difference in autism spectrum disorder supports the female-protective effect,” *Translational Psychiatry*, vol. 10, Jan. 2020.

67. D. J. Weiner, E. M. Wigdor, S. Ripke, R. K. Walters, J. A. Kosnicki, J. Grove, K. E. Samocha, J. I. Goldstein, A. Okbay, J. Bybjerg-Grauholm, T. Verge, D. M. Hougaard, J. Taylor, D. Skuse, B. Devlin, R. Anney, S. J. Sanders, S. Bishop, P. B. Mortensen, A. D. Berglum, G. D. Smith, M. J. Daly, and E. B. R. and, “Polygenic transmission disequilibrium confirms that common and rare variation act additively to create risk for autism spectrum disorders,” *Nature Genetics*, vol. 49, pp. 978–985, May 2017.

68. D. Antaki, A. Maihofer, M. Klein, J. Guevara, J. Grove, C. Carey, O. Hong, M. Arranz, A. Hervas, C. Corsello, A. Muotri, L. Iakoucheva, E. Couchesne, K. Pierce, J. Gleeson, E. Robinson, C. Nievorgelt, and J. Sebat, “A phenotypic spectrum of autism is attributable to the combined effects of rare variants, polygenic risk, and sex,” Apr. 2021.

69. E. A. Khramtsova, L. K. Davis, and B. E. Stranger, “The role of sex in the genomics of human complex traits,” *Nature Reviews Genetics*, vol. 20, pp. 173–190, Dec. 2018.

70. T.-K. Clarke, M. K. Lupton, A. M. Fernandez-Pujals, J. Starr, G. Davies, S. Cox, A. Pattie, D. C. Liewald, L. S. Hall, D. J. MacIntyre, B. H. Smith, L. J. Hocking, S. Padmanabhan, P. A. Thomson, C. Hayward, N. K. Hansell, G. W. Montgomery, S. E. Medland, N. G. Martin, M. J. Wright, D. J. Porteous, I. J. Deary, and A. M. McIntosh, “Common polygenic risk for autism spectrum disorder (ASD) is associated with cognitive ability in the general population,” *Molecular Psychiatry*, vol. 21, pp. 419–425, Mar. 2015.

71. J. W. Trampus, M. L. Z. Yang, J. Yu, E. Knowles, G. Davies, D. C. Liewald, J. M. Starr, S. Djurovic, I. Melle, K. Sundet, A. Christoforou, I. Reinvang, P. DeRosse, A. J. Lundervold, V. M. Steen, T. Espeseth, K. Räikkönen, E. Widen, A. Palotie, J. G. Eriksson, I. Giegling, B. Korte, P. Roussos, S. Giakoumaki, K. E. Burdick, A. Payton, W. Ollier, M. Horan, O. Chiba-Falek, D. K. Attix, A. C. Need, E. T. Cirulli, A. N. Voinoskos, N. C. Stefanis, D. Avramopoulos, A. Hatzimanolis, D. E. Arking, N. Smyrnis, R. M. Bilder, N. A. Freimer, T. D. Cannon, E. London, R. A. Poldrack, F. W. Sabb, E. Congdon, E. D. Conley, M. A. Scult, D. Dickinson, R. E. Straub, G. Donohoe, D. Morris, A. Corvin, M. Gill, A. R. Hariri, D. R. Weinberger, N. Pendleton, P. Bitsios, D. Rujescu, J. Lahti, S. L. Hellard, M. C. Keller, O. A. Andreassen, I. J. Deary, D. C. Glahn, A. K. Malhotra, and T. Lencz, “GWAS meta-analysis reveals novel loci and genetic correlates for general cognitive function: a report from the COGENT consortium,” *Molecular Psychiatry*, vol. 22, pp. 336–345, Jan. 2017.

72. H. C. Whalley, M. J. Adams, L. S. Hall, T.-K. Clarke, A. M. Fernandez-Pujals, J. Gibson, E. Wigmore, J. Hafferty, S. P. Hagenaaars, G. Davies, A. Campbell, C. Hayward, S. M. Lawrie, D. J. Porteous, I. J. Deary, and A. M. McIntosh, “Dissection of major depressive disorder using polygenic risk scores for schizophrenia in two independent cohorts,” *Translational Psychiatry*, vol. 6, pp. e938–e938, Nov. 2016.
73. J. Lodi-Smith, J. D. Rodgers, S. A. Cunningham, C. Lopata, and M. L. Thomeer, “Meta-analysis of big five personality traits in autism spectrum disorder,” *Autism*, vol. 23, no. 3, pp. 556–565, 2019.

74. B. C. Schwartzman, J. J. Wood, and S. K. Kapp, “Can the five factor model of personality account for the variability of autism symptom expression? multivariate approaches to behavioral phenotyping in adult autism spectrum disorder,” *Journal of autism and developmental disorders*, vol. 46, no. 1, pp. 253–272, 2016.

75. S.-H. Park, A. J. Guastella, M. Lysnkey, A. Agrawal, J. N. Constantino, S. E. Medland, Y. J. C. Song, N. G. Martin, and L. Colodro-Conde, “Neuroticism and the overlap between autistic and ADHD traits: Findings from a population sample of young adult australian twins,” *Twin Research and Human Genetics*, vol. 20, pp. 319–329, July 2017.

76. J. F. Strang, A. I. van der Miesen, R. Caplan, C. Hughes, S. daVanport, and M.-C. Lai, “Both sex-and gender-related factors should be considered in autism research and clinical practice,” 2020.

77. V. Warrier, D. M. Greenberg, E. Weir, C. Buckingham, P. Smith, M.-C. Lai, C. Allison, and S. Baron-Cohen, “Elevated rates of autism, other neurodevelopmental and psychiatric diagnoses, and autistic traits in transgender and gender-diverse individuals,” *Nature communications*, vol. 11, no. 1, pp. 1–12, 2020.