

Polygenic transmission disequilibrium confirms that common and rare variation act additively to create risk for autism spectrum disorders

Daniel J. Weiner^{1,2,3}, Emilie M. Wigdor^{1,2,3}, Stephan Ripke^{1,2,3,4}, Raymond K. Walters^{1,2}, Jack A. Kosmicki^{1,2,3,5}, Jakob Grove^{6,7,8,9}, Kaitlin E. Samocha^{1,2,3}, Jacqueline Goldstein^{1,2,3}, Aysu Okbay¹⁰, Jonas Bybjerg-Grauholm^{7,11}, Thomas Werge^{7,12,13}, David M. Hougaard¹¹, Jacob Taylor^{1,2,3,14}, iPSYCH-Broad Autism Group, Psychiatric Genomics Consortium Autism Group, David Skuse¹⁵, Bernie Devlin¹⁶, Richard Anney¹⁷, Stephan Sanders¹⁸, Somer Bishop¹⁸, Preben Bo Mortensen^{6,7,19}, Anders D. Børghlum^{6,7,8}, George Davey Smith²⁰, Mark J. Daly^{1,2,3}, and Elise B. Robinson^{1,2,3,*}

¹Analytic and Translational Genetics Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

²Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

³Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

⁴Department of Psychiatry and Psychotherapy, Charité, Campus Mitte, Berlin, Germany.

⁵Program in Genetics and Genomics, Biological and Biomedical Sciences, Harvard Medical School, Boston, Massachusetts, USA.

⁶Department of Biomedicine (Human Genetics), Aarhus University, Aarhus, Denmark.

⁷Lundbeck Foundation Initiative for Integrative Psychiatric Research, iPSYCH, Copenhagen, Denmark.

⁸Centre for Integrative Sequencing, iSEQ, Aarhus University, Aarhus, Denmark.

⁹Bioinformatics Research Centre, Aarhus University, Aarhus, Denmark.

¹⁰Department of Complex Trait Genetics, Center for Neurogenetics and Cognitive Research, Vrije Universiteit Amsterdam, Amsterdam, 1081 HV, The Netherlands.

¹¹Danish Center for Neonatal Screening, Department for Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark.

¹²Institute of Biological Psychiatry, Mental Health Center Sct. Hans, Mental Health Services Copenhagen, Copenhagen, Denmark.

¹³Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark.

¹⁴Department of Psychiatry, Brigham and Women's Hospital, Boston, Massachusetts, USA.

¹⁵Behavioural Sciences Unit, Institute of Child Health, University College London, London, UK.

¹⁶Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA.

¹⁷Division of Psychological Medicine and Clinical Neurosciences, Cardiff University, Cardiff, Wales, UK.

¹⁸Department of Psychiatry, University of California, San Francisco, San Francisco, California, USA.

¹⁹National Centre for Register-based Research, University of Aarhus, Aarhus, Denmark.

²⁰Medical Research Council Integrative Epidemiology Unit, University of Bristol, Bristol, UK.

*Please address correspondence to: erob@broadinstitute.org

Autism spectrum disorder (ASD) risk is influenced by both common polygenic and *de novo* variation. The purpose of this analysis was to clarify the influence of common polygenic risk for ASDs and to identify subgroups of cases, including those with strong acting *de novo* variants, in which different types of polygenic risk are relevant. To do so, we extend the transmission disequilibrium approach to encompass polygenic risk scores, and introduce the polygenic transmission disequilibrium test. Using data from more than 6,400 children with ASDs and 15,000 of their family members, we show that polygenic risk for ASDs, schizophrenia, and greater educational attainment is over transmitted to children with ASDs in two independent samples, but not to their unaffected siblings. These findings hold independent of proband IQ. We find that common polygenic variation contributes additively to risk in ASD cases that carry a very strong acting *de novo* variant. Lastly, we find evidence that elements of polygenic risk are independent and differ in their relationship with proband phenotype. These results confirm that ASDs' genetic influences are highly additive and suggest that they create risk through at least partially distinct etiologic pathways.

Risk for autism spectrum disorders (ASDs) is strongly genetically influenced, and reflects several types of genetic variation¹⁻⁷. Common polygenic variation, distributed across the genome, accounts for at least 20% of ASD liability^{2,5,8,9}. *De novo* single nucleotide and copy number variants can strongly affect the individuals who carry them^{1,3,4}, but account for less liability at a population level ($< 10\%$)². Over the last several years, the common polygenic and *de novo* influences on ASD risk have been increasingly well characterized, particularly in terms of the distribution of their phenotypic effects. Most consistently, ASD-associated *de novo* variants have been strongly linked to intellectual disability, as well as other indicators of global neurodevelopmental impact (e.g., seizures; motor delay)^{1,10}. Indeed ASD-associated *de novo* mutations that yield protein truncations are far more commonly observed in global developmental delay than in autism itself¹¹.

Recent studies have suggested that the common polygenic component of ASDs has a different, perhaps surprising, relationship with cognition. Polygenic risk for ASDs has been positively associated with intelligence and educational attainment in several reports^{6,12-14}. In other words, in the general population, greater common variant risk for ASDs is associated with higher IQ. These findings are difficult to interpret – on average, IQ in ASD is at least a standard deviation below the population mean^{1,15}. Further, ASDs share approximately 25% of their common variant influences with schizophrenia, and schizophrenia itself shows a negative genetic correlation with IQ^{6,14}. While genetic correlation analyses are not expected to be transitive, this particularly complex network of common variant associations has led to concerns about confounding resulting from ascertainment and case heterogeneity.

Here we attempt to clarify the influence of common variant risk for ASDs, and to better understand the subgroups of ASD individuals for whom polygenic variation is risk contributing. We thus sought to employ a robust family-based design that would be immune to many of the potential confounders that can arise in attempting to construct a well-matched case-control comparison. To advance this analysis we extended the transmission disequilibrium approach to encompass polygenic risk scores; we call the resulting methodology the polygenic transmission disequilibrium test (pTDT). Using pTDT we then show that common polygenic predictors of (i) ASDs, (ii) schizophrenia, and (iii) years of educational attainment are unambiguously associated with ASD risk, independent of the presence of intellectual disability in cases. We find that common polygenic variation still contributes to ASD risk in cases that carry a very strong acting *de novo* event. Lastly, we find that the three aforementioned polygenic risk factors have independent and distinct effects on phenotypic heterogeneity in ASD, suggesting that components of common polygenic variation also behave additively and operate through at least partially distinct etiologic pathways.

Polygenic Transmission Disequilibrium. Children are expected to inherit half of their parents' risk alleles for a trait. This expectation forms the basis of several commonly used tests of genetic association. The classic transmission disequilibrium test, for example, examines the frequency with which single genetic variants are transmitted from parents to their children¹⁶. Variants transmitted significantly more than half of the time from unaffected parents to children affected with some trait or condition are nominated for

association with that trait – only the ascertainment on a trait in the offspring and the association of the allele with the trait introduces deviation from the 50-50 chance of inheriting either allele from a heterozygous parent. Transmission disequilibrium tests have several convenient properties. First, they are immune to confounding by ancestry. They are also less vulnerable to bias from other potential differences between cases and controls, such as socioeconomic background or other factors commonly related to case ascertainment since the ‘controls’ are, in effect, the perfectly matched untransmitted chromosomes.

In this study, we extend the transmission disequilibrium test to polygenic risk scores and introduce the polygenic transmission disequilibrium approach. A polygenic risk score (PRS) provides a quantitative measure of an individual’s genome-wide common variant predisposition (or “risk”) for a trait (**Online Methods: Polygenic Risk Scoring**). PRS are normally distributed in the population, which means that some degree of common variant risk for complex traits like ASDs is present in all of us. Since a child has a 50% chance of inheriting either allele from a heterozygous parent, it is algebraically defined that the expected value of a child’s PRS for any trait will be equal to the average of their parents’ PRS (mid-parent PRS). To use height as a conceptual illustration, one expects the average PRS for a group of a children to be the sum of their mothers’ PRS for height and their fathers’ PRS for height, divided by two (**Figure 1a**). This corresponds to the phenotypic observation that children’s height averages to the sex-standardized mean height of their parents¹⁷. Of course, children are often taller or shorter than expected; if one were to select a subgroup of children each several inches taller than expected, their average height PRS should exceed that of their parents (**Figure 1b**). A statistically significant deviation between (i) the mid-parent PRS distribution and (ii) the mean of the child PRS distribution would provide strong evidence that common polygenic influences contributed to high height in the offspring. We refer to this deviation as polygenic transmission disequilibrium.

Similar logic can be applied to neuropsychiatric disorders such as ASDs¹⁸. Individuals diagnosed with ASDs should, on average, have more common variant risk for ASDs than their unaffected family members⁹. By comparing ASD individuals’ PRS for various traits against those of their unaffected parents, one can unambiguously associate specific types of common variant risk (e.g., polygenic risk for schizophrenia, educational attainment) with ASDs, and query the subsets of cases in which those risk factors are most relevant.

We used two independent ASD family cohorts to examine transmission of polygenic risk (**Supplementary Table 1; Online Methods: Sample Description**). The Simons Simplex Collection (SSC) is a resource of more than 2,500 families with a single child diagnosed with ASD¹⁹. No other family members to the level of first cousins had an ASD diagnosis. Genotype data were available for the parents, the affected child, and an unaffected sibling for 2,091 SSC families (quad families). An additional 493 SSC families had available data for the parents and the affected child alone (trio families). Most genotyped individuals in SSC were exome sequenced in previous studies (89.0%). Our independent Psychiatric Genomics Consortium ASD (PGC ASD) sample consisted of 3,870 genotyped parent-child trios from the Psychiatric Genomics Consortium Autism Group

(**Supplementary Table 2**). The PGC ASD cohort described here does not include individuals from the SSC, and the data set does not include exome sequence information.

Using a standard approach²⁰, we calculated common polygenic risk for ASDs, educational attainment (EA) and schizophrenia (SCZ) for all genotyped family members in the SSC and PGC ASD datasets (**Online Methods: Polygenic Risk Scoring**). To do so, we used summary statistics from the largest genome-wide association studies of each phenotype (**Supplementary Table 3**). All discovery GWAS (including ASD) were independent, in that they did not include any SSC or PGC ASD individuals. Using the calculated polygenic risk scores, we first examined properties of their distribution in the parents. Analyzing the SSC and PGC ASD cohorts separately, we did not observe any consistent correlations between the parents' PRS, for any pair of PRS, and based on that no PRS-based evidence of assortative mating (**Supplementary Method 1, Supplementary Tables 4a-b**). We also found no evidence that the mothers and fathers of children with ASD differ with regard to the common variant risk that they carry (**Supplementary Methods 2a, Supplementary Table 5**). Lastly, there was no evidence that mid-parent polygenic risk differs by proband sex (**Supplementary Methods 2b, Supplementary Table 6**).

The pTDT is a t-test asking whether the mean of the offspring PRS distribution is consistent with its parentally-derived expected value (**Online Methods: pTDT**). In brief, for each trio, one averages the parent PRS for a given trait, ASD for example, and then subtracts that value from the proband's PRS for the same trait. To standardize and improve interpretability, we divide the resulting difference by the standard deviation of the (observed) mid-parent distribution. This yields the estimated pTDT deviation, and the pTDT then tests whether the average pTDT deviation across all offspring differs from zero. A mean pTDT deviation of 0.1 for the ASD PRS, for example, would indicate that the offspring's ASD PRS is on average 0.1 standard deviations higher than that of their parents.

For each of ASD, SCZ, and EA, we calculated the average pTDT deviation for each affected child in SSC and PGC ASD, as well as for the unaffected siblings in the SSC. The primary pTDT results are shown in **Figure 2a**. In both the SSC and PGC ASD samples, polygenic risk for each of ASD, EA, and SCZ was significantly over transmitted to affected children ($P < 1E-06$ for all comparisons), but not to SSC unaffected siblings ($P > 0.05$ for all comparisons; **Figure 2a**). This means that common polygenic risk defined in GWAS studies of ASD, SCZ, and greater EA are each unambiguously associated with autism spectrum disorders. The results did not change when the SSC and PGC ASD samples were restricted to families of European ancestry (**Supplementary Methods 3; Supplementary Table 7a**). We repeated the analysis in SSC and PGC ASD using a PRS for body mass index (BMI) – a polygenic risk category unassociated with ASD⁶ – as a negative control (**Online Methods: Polygenic Risk Scoring**). As expected, we found that neither ASD probands nor unaffected siblings over inherited BMI PRS ($P > 0.05$ for all comparisons) (**Supplementary Table 8**).

The degree of polygenic over transmission did not differ between SSC and PGC ASD probands for any comparison ($P > 0.05$ for all comparisons; **Supplementary Table 7b**), so we combined the samples to improve the power of subsequent subgroup analyses. Using the combined data, each PRS is over transmitted to both male and female probands ($P < 0.05$ for all comparisons; **Figure 2b**). In **Figure 2c**, we also see that each of the three PRS are significantly, and equally, over transmitted to ASD cases with measured IQs in the intellectual disability range ($IQ < 70$, (**Online Methods: *pTDT***; **Supplementary Tables 9a-b**) when compared with those without intellectual disability. Most probands in the intellectual disability group do not have an observed genetic event that might explain their low IQ, for example a *de novo* mutation from an ASD-associated class, and repeating the analysis in the low IQ SSC probands with *de novo* carriers removed did not change the observed associations (**Supplementary Table 10; Online Methods: De novo variant analyses**).

Common and rare variant additivity. In order to best interrogate additivity between common polygenic and rare, strong acting variation, we defined a group of *de novo* mutations of large effect (**Supplementary Table 11**). We previously identified a subclass of *de novo* protein truncating variants (contributing PTVs) responsible for almost all of the PTV association to ASDs²¹ (**Online Methods: De novo variant analyses**). *De novo* PTVs in this class are a) absent from the Exome Aggregation Consortium database, a reference sample of over 60,000 exomes, and b) found within a gene predicted to be intolerant of heterozygous loss of function variation (probability of loss of function intolerance (pLI) ≥ 0.9)²². In the SSC, *de novo* PTVs in this class were found in 7.1% of cases and 2.1% of unaffected siblings ($P = 4.12E-14$). As *de novo* copy number variants (CNVs) that delete a gene should have the same, if not greater, molecular impact as a PTV in that gene, we were motivated to investigate whether we could similarly refine ASDs' association with *de novo* deletions (**Online Methods: De novo variant analyses**). In the SSC, we found that ASDs were strongly associated with *de novo* deletions from two categories: a) deletions that include a constrained gene, as defined above, and b) uncommon, large (≥ 500 kilobase) deletions that do not contain a constrained gene (from here: contributing CNV deletions; **Supplementary Figure 1**). Contributing CNV deletions were seen in 2.4% of SSC cases and 0.5% of SSC unaffected siblings ($P = 2.73E-07$). All other types of *de novo* deletions were observed in 1.5% of cases and 1.2% of unaffected siblings, and were not associated with ASD risk ($P = 0.50$; **Supplementary Figure 2**).

Together, these refined classes of *de novo* PTVs and deletions form a category of strong acting *de novo* variants (from here: contributing *de novo* variants (CDNVs); odds ratio (OR) = 3.91, $P = 6.56E-20$; case rate = 9.4%; control rate = 2.6%). Consistent with a hallmark of ASD-associated *de novo* variation, **Figure 3a** shows that the rate of CDEVs in SSC varies substantially based on the probands' counts of co-occurring adverse neurodevelopmental events, in this case the count of: delayed walking, a history of seizures, or intellectual disability (**Supplementary Table 12a; Online Methods: De novo variant analyses**). ASD probands without any of those comorbid traits were more than 3 times (OR = 3.15; $P = 3.88E-10$) as likely to carry a CDEV than their unaffected siblings; ASD probands with all three of these comorbid traits were approximately 15

times as likely to carry a CDNV than their unaffected siblings (OR = 15.05; $P = 9.08E-10$). Because *de novo* events observed in cases and controls differ with regard to their average severity, their effect size cannot be directly estimated using the case-control carrier ratio. Using the male:female carrier approach described by de Rubeis et al.³, we estimate that, on average, the CDNVs defined here confer an approximate 20-fold increase in risk for an ASD diagnosis (**Online Methods: De novo variant analyses**). Their effect size, however, likely varies as the male:female ratio of the carriers declines with increasing neurodevelopmental comorbidities (**Supplementary Table 12b**). In other words, the CDNVs seen in the ASD individuals with multiple comorbid neurodevelopmental traits are likely, on average, more deleterious than those seen in probands with ASD alone.

Shown in **Figure 3b**, we used pTDT to determine whether polygenic risk for ASD, SCZ, and EA was also over transmitted to CDNV carriers (**Online Methods: De novo variant analyses**). ASD cases with a CDNV ($n = 221$ cases) indeed carried more polygenic risk for ASD and SCZ than expected based on parent background ($P < 0.05$ for both comparisons). There was no evidence of over transmission of EA PRS ($P = 0.80$). This provides clear evidence for additivity between common and rare variation in creating risk for ASDs. We did not see a difference in polygenic over transmission between probands with zero versus one or more co-occurring neurodevelopmental events ($P > 0.05$ for all comparisons; **Supplementary Table 12c**), further supporting the consistent influence of polygenic risk factors.

Additivity among common polygenic risk factors. In the context of three orthogonal risk distributions, each of which is associated with ASDs, one does not have to maintain an extreme position on any single distribution to carry a cumulatively uncommon amount of ASD risk (**Figure 4a**). For example, being in the top 20% of three uncorrelated ASD risk distributions would result in a cumulative amount of risk seen in less than 1% of people in the population ($0.2^3 = 0.008$).

Common polygenic risk for ASDs, SCZ, and EA is partially correlated⁶. As shown in **Figure 4b**, however, we observed little evidence of correlation between the ASD, SCZ, and EA PRS in terms of either a) correlation at the mid-parent level, above the diagonal or b) correlation in the degree to which the scores were transmitted to the probands (pTDT deviation), below the diagonal. The lack of strong associations is likely a function of both ascertainment effects and attenuation due to limited predictive ability of the PRS.

Given limited association between the three PRS, we were able to examine additivity among largely distinct common polygenic risk factors. First, we saw significant evidence that each of the three PRS were independently over transmitted ($P < 2E-04$ in all SSC + PGC ASD comparisons; **Online Methods: Genetic heterogeneity; Supplementary Table 13**). This means that ASD risk is influenced by elements unique to each of the scores, as well as by elements that are shared among them. Independent influences are further suggested by the scores' relationship with proband IQ (**Supplementary Table 14**). In **Figure 4c**, we show the association between each of the three PRS and full scale proband IQ in European ancestry SSC probands, controlling for sex, presence of CDNVs,

the other two PRS, and the first 10 principal components of ancestry (**Online Methods: Genetic heterogeneity**). The EA and SCZ PRS are associated with proband IQ in opposite directions, consistent with the patterns observed in the general population (**Supplementary Table 14**)¹⁴.

In addition to statistical evidence of unique contributions, the phenotypic associations suggest that the three PRS are influencing ASD risk through at least partially different processes. Polygenic risk for SCZ influences ASD liability in a manner that negatively influences cognition. Polygenic risk for EA influences ASD liability in a manner that positively influences cognition. These findings reinforce the idea that ASD heterogeneity is shaped not only by rare variants of strong effect, but also by common variant risk acting through multiple biological pathways.

Despite longstanding evidence for common polygenic influences on ASD risk, many have questioned those associations, particularly the recently published – and counterintuitive – findings from genetic correlation analysis. Using pTDT, we have shown an unambiguous association between ASD risk and the common polygenic influences on: ASDs themselves, schizophrenia, and greater educational attainment. These effects were evident in affected males and affected females, as well as ASD individuals with and without intellectual disability. Because of the strong association between polygenic predictors of educational attainment and intelligence¹⁴, this finding means that, on average, individuals with ASD and intellectual disability were genetically predicted to have higher IQ than their parents and unaffected siblings. That association, which replicated in independent ASD cohorts and held in probands without an ASD-associated *de novo* event, will require further study. With regard to proband phenotype, the finding furthers existing questions of whether an IQ measured below 70 in someone with an ASD might be different in some ways from an IQ measured below 70 in someone without. With regard to better understanding the mechanisms through which genetic risk is conferred, we need to examine how and when certain amounts of risk are beneficial (e.g., a strong interest in the arts or sciences that increases one's educational attainment), and how and when they are deleterious (e.g., an overwhelming interest in a topic that disrupts healthy and necessary activities). Genetic risk and phenotypic traits relevant to neuropsychiatric disease exist on continua¹⁸, for which effective research and treatment paradigms will likely need to account.

These findings also highlight important differences between the common and rare variant contributors to ASD risk. Strong acting, *de novo* variant risk for ASDs impacts a limited subset of cases. The phenotypic preferences of those types of variants are now well established; they are associated with intellectual disability, seizures, and global neurodevelopmental impact¹. Common variant risk factors, on the other hand, appear more pervasively influential. Common variant risk appears similarly relevant to ASD individuals with high and low IQ, and with and without a large acting *de novo* mutation. The common polygenic influences also appear comparatively neurologically gentle. They are, in many cases, in fact associated with better educational and cognitive outcomes in the population. These differences strongly suggest that *de novo* and common polygenic variation may confer risk for ASD in different ways. Particularly as common polygenic

risk is the more consistent contributor to ASD liability across cases, it will be critically important to take common variation into account in creating animal or stem cell models of ASD.

iPSYCH-Broad Autism Group

Marie Bækvad-Hansen^{1,2}, Ashley Dumont³, Christine Hansen^{1,2}, Thomas F. Hansen^{1,4}, Daniel Howrigan^{3,5,6}, Manuel Mattheisen^{1,7,8}, Jennifer Moran³, Ole Mors^{1,9}, Merete Nordentoft^{1,10}, Bent Norgaard-Pedersen^{1,2}, Timothy Poterba^{3,5,6}, Jesper Poulsen^{1,2}, and Christine Stevens³

¹iPSYCH, The Lundbeck Foundation Initiative for Integrative Psychiatric Research, Denmark.

²Center for Neonatal Screening, Department for Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark.

³Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

⁴Institute of Biological Psychiatry, MHC Sct. Hans, Mental Health Services Copenhagen, Roskilde, Denmark.

⁵Analytic and Translational Genetics Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

⁶Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

⁷iSEQ, Centre for Integrative Sequencing, Aarhus University, Aarhus, Denmark.

⁸Department of Biomedicine - Human Genetics, Aarhus University, Aarhus, Denmark.

⁹Psychosis Research Unit, Aarhus University Hospital, Risskov, Denmark.

¹⁰Mental Health Services in the Capital Region of Denmark, Mental Health Center Copenhagen, University of Copenhagen, Copenhagen, Denmark.

Psychiatric Genomics Consortium Autism Group

Veneri Anttila^{1,2}, Peter Holmans³, Hailiang Huang^{1,2}, Lambertus Klei⁴, Phil H. Lee^{1,2,5}, Sarah E. Medland⁶, Benjamin Neale^{1,2}, Lauren A. Weiss^{7,8}, Lonnie Zwaigenbaum⁹, Timothy W. Yu¹⁰, Kerstin Wittemeyer¹¹, A. Jeremy Willsey⁷, Ellen M. Wijsman^{12,13}, Thomas H. Wassink¹⁴, Regina Waltes¹⁵, Christopher A. Walsh^{10,16,17,18,19}, Simon Wallace²⁰, Jacob A. S. Vorstman²¹, Veronica J. Vieland²², Astrid M. Vicente^{23,24}, Herman van Engeland²¹, Kathryn Tsang^{7,8}, Ann P. Thompson²⁵, Peter Szatmari²⁶, Oscar Svantesson²⁷, Stacy Steinberg²⁸, Kari Stefansson²⁸, Hreinn Stefansson²⁸, Matthew W. State⁷, Latha Soorya^{29,30,31}, Teimuraz Silagadze³², Stephen W. Scherer^{33,34,35}, Gerard D. Schellenberg³⁶, Sven Sandin²⁷, Evald Saemundsen³⁷, Guy A. Rouleau³⁸, Bernadette Rogé³⁹, Kathryn Roeder^{40,41}, Wendy Roberts⁴², Jennifer Reichert^{29,30}, Abraham Reichenberg^{29,30}, Karola Rehnström⁴³, Regina Regan^{44,45}, Fritz Poustka¹⁵, Christopher S. Poultny^{29,30}, Joseph Piven⁴⁶, Dalila Pinto^{29,30,47,48,49,50}, Margaret A. Pericak-Vance⁵¹, Milica Pejovic-Milovancevic⁵², Marianne G. Pedersen^{53,54,55}, Carsten B. Pedersen^{53,54,55}, Andrew D. Paterson^{33,35,56}, Jeremy R. Parr^{57,58}, Alistair T. Pagnamenta⁵⁹, Guiomar Oliveira^{60,61}, John I. Nurnberger^{62,63,64}, Merete Nordentoft^{53,65}, Michael T. Murtha⁶⁶, Susana Mouga^{60,61}, Ole Mors^{53,67}, Eric M. Morrow⁶⁸, Daniel Moreno-De-Luca⁶⁸, Anthony P. Monaco^{59,69}, Nancy Minshew⁴, Alison Merikangas⁷⁰, William M. McMahon⁷¹, Susan G. McGrew⁷², Manuel Mattheisen^{53,73,74}, Igor Martenskovsky⁷⁵, Donna M. Martin⁷⁶, Shrikant M. Mane⁷⁷, Pall Magnusson⁷⁸, Tiago Magalhaes^{44,45}, Elena Maestrini⁷⁹, Jennifer K. Lowe^{80,81,82}, Catherine Lord⁸³, Pat Levitt⁸⁴, Christa Lese Martin⁸⁵, David H. Ledbetter⁸⁶, Marion Leboyer^{87,88,89,90}, Ann S. Le Couteur^{57,58}, Christine Ladd-Acosta⁹¹, Alexander Kolevzon^{29,30,48,50}, Sabine M. Klauck⁹², Suma Jacob^{93,94}, Bozenna Iliadou²⁷, Christina M. Hultman²⁷, Irva Hertz-Picciotto^{95,96}, Robert Hendren⁷, Christine S. Hansen^{53,97}, Jonathan L. Haines⁹⁸, Stephen J. Guter⁹³, Dorothy E. Grice³⁰, Jonathan M. Green^{99,100}, Andrew Green^{45,101}, Arthur P. Goldberg^{47,49}, Christopher Gillberg¹⁰², John Gilbert⁵¹, Louise Gallagher⁷⁰, Christine M. Freitag¹⁵, Eric Fombonne¹⁰³, Susan E. Folstein¹⁰⁴, Bridget Fernandez¹⁰⁵, M. Daniele Fallin¹⁰⁶, A. Gulhan Ercan-Sencicek⁶⁶, Sean Ennis^{45,101}, Frederico Duque^{60,61}, Eftichia Duketis¹⁵, Richard Delorme^{87,107,108,109}, Silvia De Rubeis^{29,30}, Maretha V. De Jonge²¹, Geraldine Dawson^{110,111}, Michael L. Cuccaro⁵¹, Catarina T. Correia^{23,24}, Judith Conroy^{45,112}, Ines C. Conceição^{23,24}, Andreas G. Chiocchetti¹⁵, Patrícia B. S. Celestino-Soper^{63,113}, Jillian Casey^{45,112}, Rita M. Cantor^{114,115}, Cátia Café⁶⁰, Sean Brennan⁷⁰, Thomas Bourgeron^{87,107,108,116}, Patrick F. Bolton^{117,118}, Sven Bölte^{15,119,120}, Nadia Bolshakova⁷⁰, Catalina Betancur^{121,122,123}, Raphael Bernier¹²⁴, Arthur L. Beaudet¹¹³, Agatino Battaglia¹²⁵, Vanessa H. Bal⁷, Gillian Baird¹²⁶, Anthony J. Bailey^{20,127}, Marie Bækvad-Hansen^{53,97}, Joel S. Bader¹²⁸, Elena Bacchelli⁷⁹, Evdokia Anagnostou¹²⁹, David Amaral^{96,130,131}, Joana Almeida⁶⁰, Joseph D. Buxbaum^{29,30,47,48,50,132}, Aravinda Chakravarti¹²⁸, Edwin H. Cook⁹³, Hilary Coon⁷¹, Daniel H. Geschwind^{80,81,82,115}, Michael Gill⁷⁰, Hakon Hakonarson^{133,134}, Joachim Hallmayer¹³⁵, Aarno Palotie⁴³, Susan Santangelo¹³⁶, James S. Sutcliffe^{98,137}, and Dan E. Arking¹²⁸

¹Analytic and Translational Genetics Unit, Dept. of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA.

²Stanley Center for Psychiatric Research and Program in Medical and Population Genetic, Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA.

³MRC Centre for Neuropsychiatric Genetics & Genomics, Cardiff University, Cardiff, CF24 4HQ, UK.

- ⁴Dept. of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA.
- ⁵Dept. of Psychiatry, Harvard Medical School, Boston, MA 02115, USA.
- ⁶Queensland Institute of Medical Research, Brisbane, QLD, 4006, Australia.
- ⁷Dept. of Psychiatry, University of California San Francisco, San Francisco, CA 94143, USA.
- ⁸Inst. Human Genetics, University of California San Francisco, San Francisco, CA 94143, USA.
- ⁹Dept. of Pediatrics, University of Alberta, Edmonton, AB, T6G 1C9, Canada.
- ¹⁰Division of Genetics, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA.
- ¹¹School of Education, University of Birmingham, Birmingham, B15 2TT, UK.
- ¹²Dept. of Medicine, University of Washington, Seattle, WA 98195, USA.
- ¹³Dept. of Biostatistics, University of Washington, Seattle, WA 98195, USA.
- ¹⁴Dept. of Psychiatry, Carver College of Medicine, Iowa City, IA 52242, USA.
- ¹⁵Dept. of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, JW Goethe University Frankfurt, Frankfurt am Main, 60528, Germany.
- ¹⁶Program in Genetics and Genomics, Harvard Medical School, Boston, MA 02115, USA.
- ¹⁷Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA.
- ¹⁸Dept. of Pediatrics, Harvard Medical School, Boston, MA 02115, USA.
- ¹⁹Dept. of Neurology, Harvard Medical School, Boston, MA 02115, USA.
- ²⁰Dept. of Psychiatry, University of Oxford and Warneford Hospital, Oxford, OX3 7JX, UK.
- ²¹Dept. of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, 3584 CG, The Netherlands.
- ²²Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205, USA.
- ²³Instituto Nacional de Saude Dr. Ricardo Jorge, Lisboa, 1600, Portugal.
- ²⁴Center for Biodiversity, Functional and Integrative Genomics, Campus da FCUL, Lisboa, 1649, Portugal.
- ²⁵Dept. of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, ON, L8S 4L8, Canada.
- ²⁶Dept. of Psychiatry, University of Toronto, ON, M5T 1R8, Canada.
- ²⁷Karolinska Institutet, Solna, SE-171 77, Sweden.
- ²⁸deCODE Genetics, Reykjavik, IS-101, Iceland.
- ²⁹Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
- ³⁰Dept. of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
- ³¹Dept. of Psychiatry, Rush University Medical Center, Chicago, IL 60612, USA.
- ³²Dept. of Psychiatry and Drug Addiction, Tbilisi State Medical University, Tbilisi, 0186, Georgia.
- ³³The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, M5G 1L4, Canada.
- ³⁴McLaughlin Centre, University of Toronto, Toronto, ON, M5G 0A4, Canada.
- ³⁵Dept. of Molecular Genetics, University of Toronto, Toronto, ON, M5S 1A8, Canada.
- ³⁶Dept. of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19102, USA.
- ³⁷State Diagnostic and Counseling Centre, Kopavogur, IS-201, Iceland.
- ³⁸Montreal Neurological Institute, Dept of Neurology and Neurosurgery, McGill University, Montreal, QC, H3A 2B4, Canada.
- ³⁹Centre d'Etudes et de Recherches en Psychopathologie, Toulouse University, Toulouse, 31058, France.
- ⁴⁰Dept. of Computational Biology, Carnegie Mellon University, Pittsburgh, PA 15213, USA.
- ⁴¹Dept. of Statistics, Carnegie Mellon University, Pittsburgh, PA 15213, USA.
- ⁴²Autism Research Unit, The Hospital for Sick Children, Toronto, ON, M5G 1L4, Canada.
- ⁴³Sanger Institute, Hinxton, CB10 1SA, UK.
- ⁴⁴National Childrens Research Centre, Our Lady's Hospital Crumlin, Dublin, D12, Ireland.
- ⁴⁵Academic Centre on Rare Diseases, University College Dublin, Dublin, D4, Ireland.
- ⁴⁶University of North Carolina, Chapel Hill, NC 27599, USA.
- ⁴⁷Dept. of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
- ⁴⁸The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
- ⁴⁹The Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
- ⁵⁰Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.

- ⁵¹The John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL 33101, USA.
- ⁵²Institute of Mental Health and Medical Faculty, University of Belgrade, Belgrade, 11 000, Serbia.
- ⁵³iPSYCH, Lundbeck Foundation Initiative for Integrative Psychiatric Research, Aarhus, Denmark.
- ⁵⁴National Centre for Register-based Research, Aarhus University, Aarhus, Denmark.
- ⁵⁵Centre for Integrated Register-based Research, Aarhus University, Aarhus, Denmark.
- ⁵⁶Dalla Lana School of Public Health, Toronto, ON, M5T 3M7, Canada.
- ⁵⁷Institute of Neuroscience, Newcastle University, Newcastle Upon Tyne, NE2 4HH, UK.
- ⁵⁸Institute of Health and Science, Newcastle University, Newcastle Upon Tyne, NE2 4AX, UK.
- ⁵⁹Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, OX3 7BN, UK.
- ⁶⁰Unidade de Neurodesenvolvimento e Autismo do Serviço do Centro de Desenvolvimento da Criança and Centro de Investigação e Formação Clínica, Pediatric Hospital, Centro Hospitalar e Universitário de Coimbra, Coimbra, 3041-80, Portugal.
- ⁶¹University Clinic of Pediatrics and Institute for Biomedical Imaging and Life Science, Faculty of Medicine, University of Coimbra, Coimbra, 3041-80, Portugal.
- ⁶²Institute of Psychiatric Research, Dept. of Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46202, USA.
- ⁶³Dept. of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202, USA.
- ⁶⁴Program in Medical Neuroscience, Indiana University School of Medicine, Indianapolis, IN 46202, USA.
- ⁶⁵Mental Health Services in the Capital Region of Denmark, Mental Health Center Copenhagen, University of Copenhagen, Copenhagen, Denmark.
- ⁶⁶Programs on Neurogenetics, Yale University School of Medicine, New Haven, CT 06520, USA.
- ⁶⁷Psychosis Research Unit, Aarhus University Hospital, Risskov, Denmark.
- ⁶⁸Dept. of Psychiatry and Human Behaviour, Brown University, Providence, RI 02912, USA.
- ⁶⁹Tufts University, Boston, MA 02155, USA.
- ⁷⁰Dept. of Psychiatry, Trinity College Dublin, Dublin, D8, Ireland.
- ⁷¹Dept. of Psychiatry, University of Utah, Salt Lake City, UT 84108, USA.
- ⁷²Dept. of Pediatrics, Vanderbilt University, Nashville, TN 37232, USA.
- ⁷³iSEQ, Centre for Integrative Sequencing, Aarhus University, Aarhus, DK-8000, Denmark.
- ⁷⁴Dept. of Biomedicine - Human Genetics, Aarhus University, Aarhus, DK-8000, Denmark.
- ⁷⁵Dept. of Child, Adolescent Psychiatry and Medical-Social Rehabilitation, Ukrainian Research Institute of Social Forensic Psychiatry and Drug Abuse, Kyiv, 04080, Ukraine.
- ⁷⁶Dept. of Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI 48109, USA.
- ⁷⁷Yale Center for Genomic Analysis, Yale University School of Medicine, New Haven, CT 06516, USA.
- ⁷⁸Dept. of Child and Adolescent Psychiatry, National University Hospital, Reykjavik, IS-101, Iceland.
- ⁷⁹Dept. of Pharmacy and Biotechnology, University of Bologna, Bologna, 40126, Italy.
- ⁸⁰Center for Autism Research and Treatment, Semel Institute, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA 90095, USA.
- ⁸¹Program in Neurogenetics, Dept. of Neurology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA.
- ⁸²Center for Neurobehavioral Genetics, Semel Institute, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA.
- ⁸³Dept. of Psychiatry, Weill Cornell Medical College, Cornell University, New York, NY 10065, USA.
- ⁸⁴Dept. of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA 90027, USA.
- ⁸⁵Autism & Developmental Medicine Institute, Geisinger Health System, Danville, PA 17837, USA.
- ⁸⁶Chief Scientific Officer, Geisinger Health System, Danville, PA 17837, USA.
- ⁸⁷FondaMental Foundation, Créteil, 94000, France.
- ⁸⁸INSERM U955, Paris, 94010, France.
- ⁸⁹Faculté de Médecine, Université Paris Est, Créteil, 94000, France.
- ⁹⁰Dept. of Psychiatry, Henri Mondor-Albert Chenevier Hospital, Assistance Publique – Hôpitaux de Paris, Créteil, 94000, France.
- ⁹¹Dept. of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA.
- ⁹²Division of Molecular Genome Analysis and Working Group Cancer Genome Research, Deutsches Krebsforschungszentrum, Heidelberg, D-69120, Germany.
- ⁹³Institute for Juvenile Research, Dept. of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612, USA.

USA.

⁹⁴Institute of Translational Neuroscience and Dept. of Psychiatry, University of Minnesota, Minneapolis, MN 55454, USA.

⁹⁵Dept. of Public Health Sciences, School of Medicine, University of California Davis, Davis, CA 95616, USA.

⁹⁶The MIND Institute, School of Medicine, University of California Davis, Davis, CA 95817, USA.

⁹⁷Center for Neonatal Screening, Dept. for Congenital Disorders, Statens Serum Institut, Copenhagen, DK-2300, Denmark.

⁹⁸Dept. of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN 37232, USA.

⁹⁹Manchester Academic Health Sciences Centre, Manchester, M13 9NT, UK.

¹⁰⁰Institute of Brain, Behaviour, and Mental Health, University of Manchester, Manchester, M13 9PT, UK.

¹⁰¹Centre for Medical Genetics, Our Lady's Hospital Crumlin, Dublin, D12, Ireland.

¹⁰²Gillberg Neuropsychiatry Centre, University of Gothenburg, Gothenburg, S-405 30, Sweden.

¹⁰³Dept. of Psychiatry and Institute for Development and Disability, Oregon Health & Science University, Portland, OR 97239, USA.

¹⁰⁴Division of Child and Adolescent Psychiatry, Dept. of Psychiatry, Miller School of Medicine, University of Miami, Miami, FL 33136, USA.

¹⁰⁵Memorial University of Newfoundland, St. John's, NL, A1B 3X9, Canada.

¹⁰⁶Dept. of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA.

¹⁰⁷Human Genetics and Cognitive Functions Unit, Institut Pasteur, Paris, 75015, France.

¹⁰⁸Centre National de la Recherche Scientifique URA 2182 Institut Pasteur, Paris, 75724, France.

¹⁰⁹Dept. of Child and Adolescent Psychiatry, Robert Debré Hospital, Assistance Publique – Hôpitaux de Paris, Paris, 75019, France.

¹¹⁰Duke Center for Autism and Brain Developments, Duke University School of Medicine, Durham, NC 27705, USA.

¹¹¹Duke Institute for Brain Sciences, Duke University School of Medicine, Durham, NC 27708, USA.

¹¹²Temple Street Children's University Hospital, Dublin, D1, Ireland.

¹¹³Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA.

¹¹⁴Dept. of Psychiatry, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA 90095, USA.

¹¹⁵Dept. of Human Genetics, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA 90095, USA.

¹¹⁶University Paris Diderot, Sorbonne Paris Cité, Paris, 75013, France.

¹¹⁷Institute of Psychiatry, Kings College London, London, SE5 8AF, UK.

¹¹⁸South London & Maudsley Biomedical Research Centre for Mental Health, London, SE5 8AF, UK.

¹¹⁹Dept. of Women's and Children's Health, Center of Neurodevelopmental Disorders, Karolinska Institutet, Stockholm, SE-113 30, Sweden.

¹²⁰Child and Adolescent Psychiatry, Center for Psychiatry Research, Stockholm County Council, Stockholm, SE-171 77, Sweden.

¹²¹INSERM U1130, Paris, 75005, France.

¹²²CNRS UMR 8246, Paris, 75005, France.

¹²³Sorbonne Universités, UPMC Univ Paris 6, Neuroscience Paris Seine, Paris, 75005, France.

¹²⁴Dept. of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195, USA.

¹²⁵Stella Maris Institute for Child and Adolescent Neuropsychiatry, Pisa, 56018, Italy.

¹²⁶Paediatric Neurodisability, King's Health Partners, Kings College London, London, SE1 7EH, UK.

¹²⁷Mental Health and Addictions Research Unit, University of British Columbia, Vancouver, BC, V5Z 4H4, Canada.

¹²⁸McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD 21218, USA.

¹²⁹Bloorview Research Institute, University of Toronto, Toronto, ON, M4G 1R8, Canada.

¹³⁰Dept. of Psychiatry, School of Medicine, University of California Davis, Davis, CA 95817, USA.

¹³¹Dept. of Behavioural Sciences, School of Medicine, University of California Davis, Davis, CA 95817, USA.

¹³²Dept. of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.

¹³³The Center for Applied Genomics and Division of Human Genetics, Children's Hospital of

Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

¹³⁴Dept of Pediatrics, University of Pennsylvania, Philadelphia, PA 19104, USA.

¹³⁵Dept. of Psychiatry, Stanford University, Stanford, CA 94305, USA.

¹³⁶Maine Medical Center Research Institute, Portland, ME 04074, USA.

¹³⁷Center for Human Genetics Research, Vanderbilt University, Nashville, TN 37232, USA.

[†]Please address correspondence to: pgc.autism@gmail.com

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Conflicts of Interest

The authors have no conflicts of interest to report.

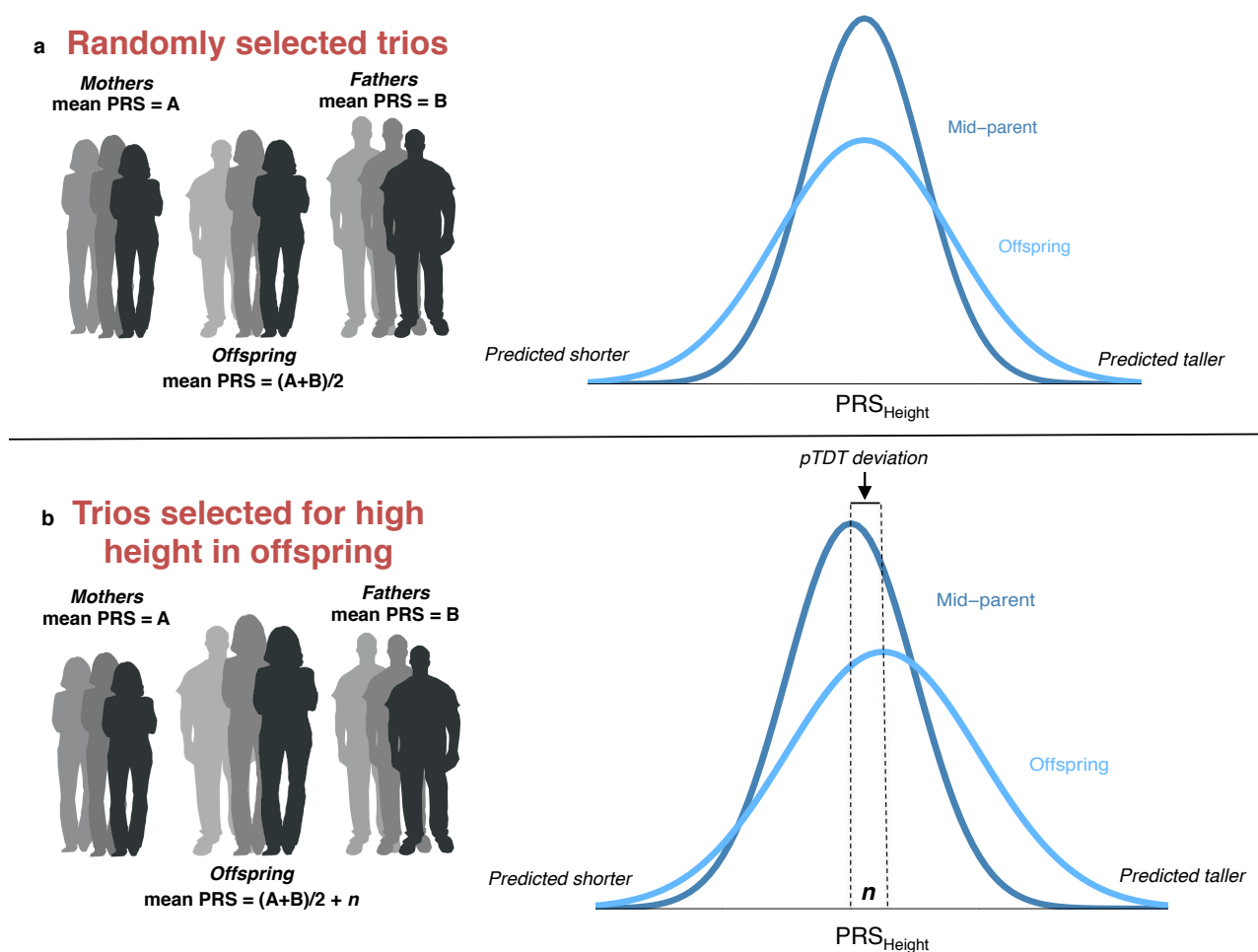


Figure 1 (a) In a randomly selected cohort of parent-child trios, a child's genetic predisposition for height (polygenic risk score, or PRS) is expected to be the average of their parents' PRS (mid-parent PRS). This corresponds to the phenotypic observation that children's height is expected to be the sex-standardized average height of their parents. (b) In a cohort of trios selected for very high height in the offspring, we correspondingly expect offspring PRS to exceed mid-parent PRS. The difference between the mean of the offspring PRS distribution and mid-parent PRS distribution, n , we refer to as polygenic transmission disequilibrium.

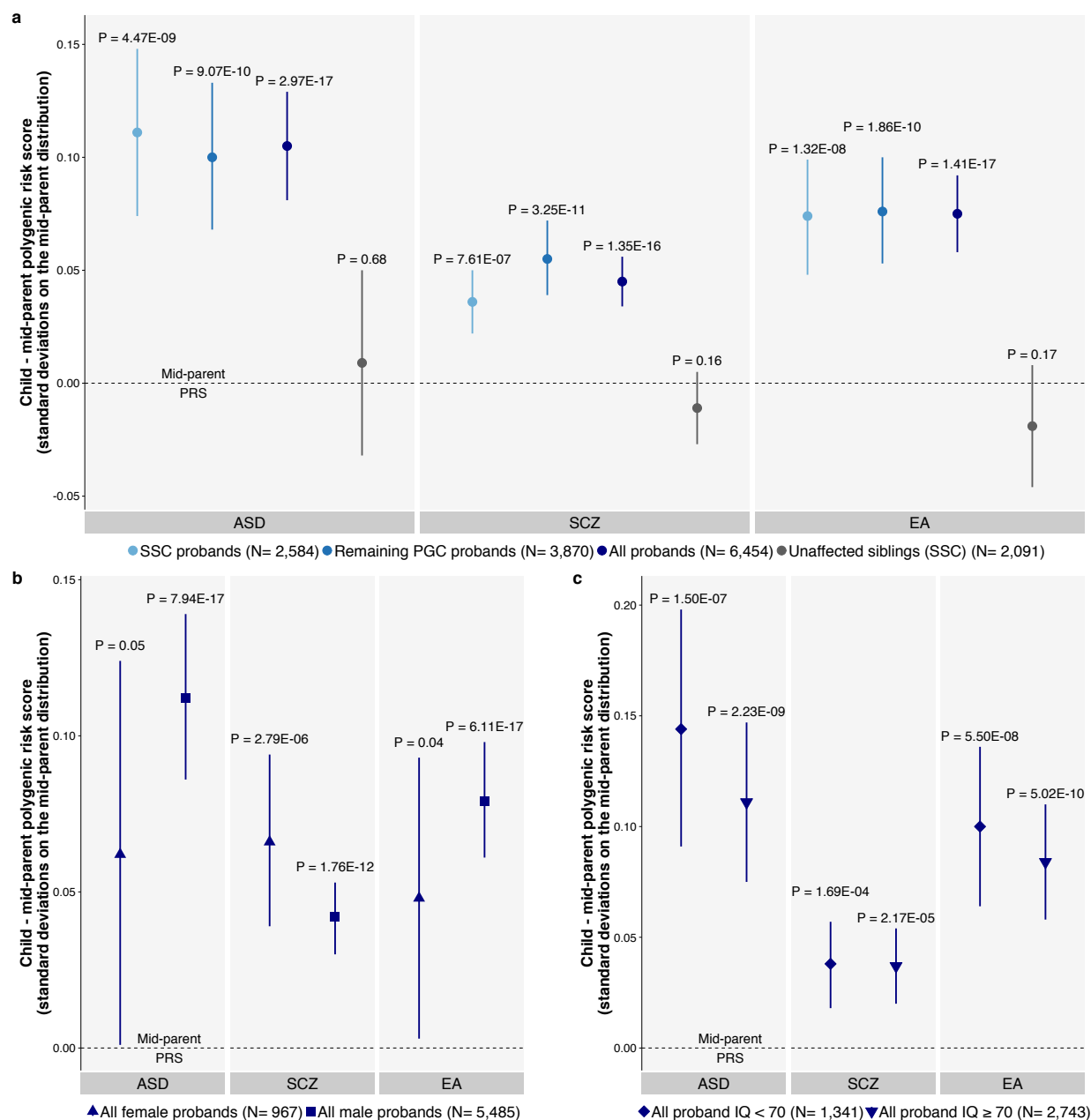


Figure 2 ASD probands over inherit polygenic risk for ASD, schizophrenia, and educational attainment. Transmission is shown in terms of standard deviations on the average parent distribution ± 1.96 SE (95% confidence intervals). P-values denote the probability that the mean of the pTDT deviation distribution is 0 (one-sample t-test). **(a)** ASD probands over inherit ASD associated polygenic risk in the Simon Simplex Collection (SSC), Psychiatric Genomics Consortium Autism Group (PGC ASD), and combined cohorts. Unaffected siblings in SSC do not over inherit ASD associated polygenic risk. **(b)** Both male and female probands over inherit ASD associated polygenic risk in the combined cohort. **(c)** ASD probands with and without intellectual disability (full-scale IQ < 70) over inherit ASD associated polygenic risk in the combined cohort.

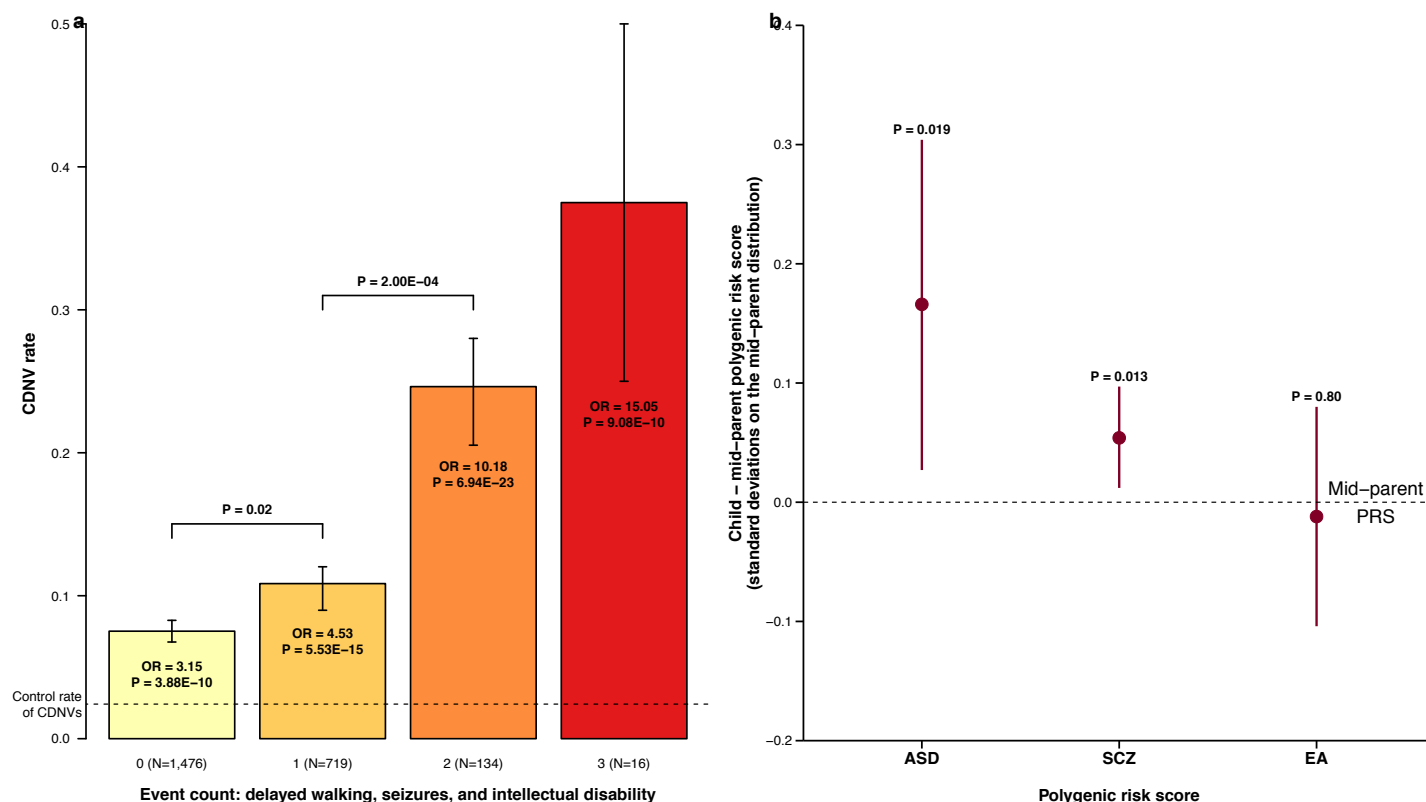


Figure 3 Contributing *de novo* mutations are associated with adverse neurological and developmental events and act additively with polygenic burden to influence ASD risk. **(a)** Simons Simplex Collection (SSC) probands are grouped by their count of the following: delayed walking (≥ 18 months); presence of seizures; intellectual disability (full-scale IQ < 70). Case and control contributing *de novo* variant (CDNV) rate is calculated by dividing the count of CDNVs by the count of individuals in the group. The odds ratio (OR) compares the proband CDNV rate in each event bin to the overall control CDNV rate. P-values in the shaded bars indicate the probability that the CDNV rate in cases is not different from the CDNV rate in controls. **(b)** pTDT analysis for SSC CDNV proband carriers ($n = 221$). Transmission shown in terms of standard deviations on mid-parent distribution ± 1.96 SE (95% confidence intervals). P-values denote the probability that the mean of the pTDT distribution is 0.

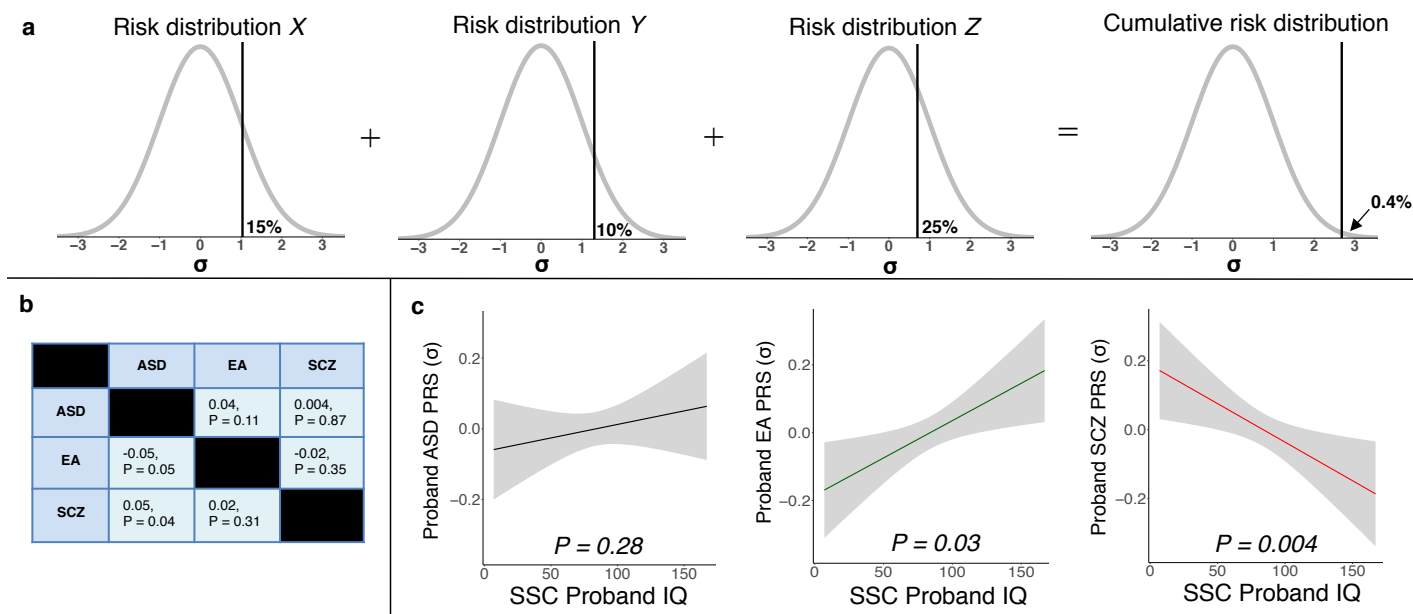


Figure 4 (a) Additivity among orthogonal risk factors can yield high cumulative risk. (b) Polygenic risk scores (PRS) for ASDs, schizophrenia (SCZ), and educational attainment (EA) are not strongly associated at either the mid-parent level (above diagonal) or the pTDT deviation level (below diagonal). The table contains Pearson correlation coefficients and p-values indicating the probability with which the true correlation is 0. PRS are from European ancestry SSC families. (c) Polygenic risk factors for ASD exhibit independent, distinct effects on IQ. P-values indicate the probability with which there is no association between each polygenic risk score (PRS) and SSC proband IQ.

Online Methods

Sample Description. The analytic cohorts are presented in **Supplementary Table 1**. The Simons Simplex Collection (SSC) is a resource of more than 2,500 families with a child diagnosed with an autism spectrum disorder (ASD)¹⁹. Each member of the family was genotyped on one of the following platforms: Illumina Omni2.5, Illumina 1Mv3 or Illumina 1Mv1¹. We analyzed 2,091 SSC quads, defined as families with both parents, the proband, and a designated unaffected sibling genotyped, and 493 SSC trios, defined as families with both parents and the proband genotyped. Most SSC families were also whole exome sequenced to detect rare coding variation (82.7% of quads, an additional 8.0% of quads without sibling sequencing; 91.1% of trios).

The Psychiatric Genomics Consortium Autism Group (PGC ASD) sample consisted of parent-proband trios from the Psychiatric Genomics Consortium Autism Group (**Supplementary Table 2**). Our PGC ASD analytic sample excluded the SSC families present in the PGC ASD genome-wide association study (GWAS)²³. The PGC ASD sample described here is accordingly independent from the SSC sample and included 3,870 parent-proband trios. In brief, the PGC ASD data included ASD trio cohorts from: Autism Center of Excellence UCLA ($n = 215$), Autism Genome Project ($n = 2,254$), Montreal/Boston Collection ($n = 138$), Johns Hopkins University ($n = 764$), and the Children's Hospital of Philadelphia ($n = 499$)²³. All genotype data (SSC and PGC ASD) were imputed using the 1000 Genomes reference panel and Ricopili pipeline, which are publicly available and have been reported on extensively^{20,24,25}.

Though trio approaches are broadly immune to confounding through ancestry, we isolated European-only subsets of both the SSC and PGC ASD samples to a) ensure that our primary results did not change in an ancestrally homogeneous subset of the data and b) conduct comparisons across probands or across parents, which might be sensitive to ancestry (as opposed to comparisons between probands and parents in a trio). In the SSC, we first selected probands with parent-reported white non-Hispanic ancestry ($n = 1,912$). We merged the genotype data from those individuals with the Hapmap III dataset²⁶ and generated principal components of ancestry using GCTA²⁷. Through visual inspection (**Supplementary Figure 8**), we defined an ancestrally European SSC subcohort, leaving 1,851 probands (and, by extension, 1,851 families). We calculated principal components of ancestry distinctly within the derived SSC European ancestry subcohort and used these as covariates in the non-trio analysis (e.g., genotype to phenotype analyses among probands).

Self-reported ancestry was not available for the PGC ASD samples. To conservatively isolate a European ancestry PGC ASD subcohort, we identified those families in which both parents were of European ancestry. To do so, we merged the PGC ASD parent data with Hapmap III and similarly generated principal components of ancestry using GCTA. By visual inspection, we identified European ancestry individuals, leaving 6,742 of the original 7,740 PGC ASD parents (**Supplementary Figure 9**). Both parents were of European ancestry in 3,209 families, and those families comprised the European ancestry PGC ASD subcohort. We again calculated principal components of ancestry within the derived PGC ASD European ancestry subcohort for use as analytic covariates in non-trio analyses.

Polygenic Risk Scoring. A polygenic risk score (PRS) provides a quantitative estimate of an individual's genetic predisposition ("risk") for a given phenotype based on common variant genotype data and independent GWAS results for the target phenotype (e.g., schizophrenia or educational attainment)²⁸. The score provides a relative, not absolute, measure of risk. For example, individual A ($\text{PRS}_{\text{schizophrenia}} = 8$) is at higher estimated genetic risk for schizophrenia than individual B ($\text{PRS}_{\text{schizophrenia}} = 6$), but a PRS of 8 or 6 is not independently interpretable.

We calculated polygenic risk for ASD, educational attainment (EA), schizophrenia (SCZ) and body mass index (BMI) for all individuals with available genotype data in the SSC and PGC ASD datasets. To do so, we used summary statistics from the largest available, independent GWAS of each phenotype (**Supplementary Table 3**). None of the subjects in the SSC or PGC ASD cohort were included in any of the four GWAS discovery samples. We selected SCZ and EA because of their robust, well-replicated associations with ASD risk^{6,14,23}. We selected BMI as a negative control due to its lack of association with ASD risk⁶. We used ASD summary statistics from a GWAS of a Danish population-based sample of 7,783 cases and 11,359 controls from the first 10 genotyping waves of the iPSYCH-Broad Autism project¹⁸. The SCZ summary statistics were from the 2014 GWAS of 36,989 cases and 113,075 controls from the Psychiatric Genomics Consortium²⁰. The EA summary statistics were from a population-based GWAS of years of schooling ($n = 328,917$, discovery and replication meta-analysis, excluding 23andMe)¹². The BMI summary statistics were from a population-based GWAS of body mass index ($n = 322,154$, European ancestry meta-analysis)²⁹.

To construct the polygenic risk scores, we first gathered the summary statistics from the GWAS described above. The summary statistics included effect sizes and p-values for each single nucleotide polymorphism (SNP) in the GWAS. We employed the widely used Ricipili pipeline to generate the PRS. In brief, we removed SNPs within 500 kilobases of and correlated ($r^2 \geq 0.1$) with a more significantly associated SNP in the GWAS²⁰. We used the 1000 Genomes Reference panel to estimate SNP correlations²⁴. For complex, polygenic traits, only a small fraction of SNPs pass the genome-wide significance threshold of $P = 5.00\text{E-}08$; the majority of the signal resides in SNPs that are less significantly associated^{6,12,20,27}. In order to maximally capture common, polygenic influence, we relaxed the p-value threshold for SNPs in the PRS until doing so added more noise than signal (threshold options: $P \leq 1, 5\text{E-}1, 2\text{E-}1, 1\text{E-}1, 5\text{E-}2, 1\text{E-}2, 1\text{E-}3, 1\text{E-}4, 1\text{E-}6$, and $5\text{E-}8$). We identified the optimal p-value threshold as that which explained the most phenotypic variation for each trait in an independent sample. For the ASD PRS, we found that the threshold of $P = 0.1$ explained the most case-pseudocontrol variance in SSC (as the SSC does not have independent control data, we generated pseudocontrol genotypes from the untransmitted parental alleles)^{18,23}. For the SCZ and EA PRS, we used the threshold identified by the discovery GWAS analyses as explaining the most variance. In the PGC's 2014 schizophrenia analyses, a p-value threshold of $P = 0.05$ most commonly explained the most SCZ case-control variance in 40 leave-one-out analyses²⁰. For educational attainment, constructing a PRS using a threshold of $P = 1$ explained the most variance in number years of education achieved in an independent sample¹². For BMI, constructing a PRS using a threshold of $P = 0.2$ explained the most variance in phenotypic body mass index in the Simons Simplex Collection (**Supplementary Methods 9**). These thresholds create the strongest polygenic risk scores for ASD, EA,

SCZ and BMI, leaving us maximally powered to investigate the relationship between these four traits and ASDs.

Next, we excluded SNPs that were poorly imputed in either SSC or PGC ASD (info score < 0.6 in either cohort). The exception to this filtering rule was in SSC-specific analyses, (e.g., analysis of *de novo* variation) where our info threshold of 0.6 was the minimum in the SSC imputation only. For each trait, the polygenic risk scores for individuals in SSC and PGC ASD were then calculated as the product of the GWAS effect size (log odds or beta) at that SNP by the individual's count of reference alleles at that SNP (0, 1 or 2), summed across all remaining markers. We implemented this scoring protocol using the score function in Plink³⁰. If an individual was missing genetic data at a SNP in the summary statistics file, Plink calculated the expected score based on the cohort-wide allele frequency.

pTDT. We define pTDT deviation as:

$$pTDT\ deviation = \frac{PRS_C - PRS_{MP}}{SD(PRS_{MP})}$$

where PRS_C is the polygenic risk score for the child (proband or unaffected sibling). PRS_{MP} is the mid-parent polygenic risk score:

$$PRS_{MP} = \frac{PRS_{mother} + PRS_{father}}{2}$$

$SD(PRS_{MP})$ is the standard deviation of the sample-specific mid-parent PRS. For example, in SSC analysis, the $SD(PRS_{MP})$ was the standard deviation of the mid-parent PRS distribution in SSC parents. The approach can be adapted to PRS from unaffected siblings, but using mid-parent PRS improves statistical power (**Supplementary Method 4, Supplementary Table 17**).

To evaluate whether the pTDT deviation is significantly different than 0, we defined the pTDT test statistic (t_{pTDT}) as:

$$t_{pTDT} = \frac{mean(pTDT\ deviation)}{SD(pTDT\ deviation)/\sqrt{n}}$$

where n is the number of families included in the pTDT. We evaluate t_{pTDT} as a one-sample t-test.

We performed pTDT using the ASD, EA, SCZ and BMI PRS described above in four groups: SSC probands ($n = 2,584$), SSC unaffected siblings ($n = 2,091$), PGC ASD probands ($n = 3,870$), and the combination of SSC and PGC ASD probands ($n = 6,454$). As described in the main text (**Figure 2a**), each of the ASD, EA, and SCZ PRS were significantly over transmitted from parents to probands, but not to unaffected siblings ($P < 1E-06$ for all parent to proband comparisons in either SSC or PGC ASD; $P < 1E-15$ for all parent to proband comparisons in combined SSC and PGC ASD; $P > 0.05$ for all unaffected sibling comparisons). In contrast, BMI PRS was not over transmitted to

probands ($P > 0.05$). In a complementary permutation test, we randomly assigned case/control labels to the affected and unaffected children in SSC. We then counted the number of times that the simulated difference in ASD PRS between affected and unaffected SSC children exceeded the observed difference (86 of 1,000,000 permutations = 0.0086%), consistent with the primary results (**Figure 2a**).

In our primary associations (**Figure 2a, Supplementary Table 7a**), there were no statistically significant pTDT differences between the SSC and PGC ASD cohorts (**Supplementary Table 7b**). We accordingly combined SSC and PGC ASD for the analyses stratified by sex and IQ to increase statistical power (**Figures 2b, 2c**). In SSC, full scale IQ (FSIQ; SSC variable: *sscfsiq*) was derived from a number of scales and available for almost all probands (99.8%) (Differential Ability Scales, Second Edition³¹; Mullen Scales of Early Learning³²; Wechsler battery³³). Our full scale SSC IQ estimates were taken from the SSC's 'full scale deviation IQ' variable when it was available, and 'full scale ratio IQ' when it was not³⁴. FSIQ was measured heterogeneously across the contributing PGC ASD cohorts (**Supplementary Table 2**). To accommodate measurement differences, FSIQ was converted to broad groups for PGC consortium-level analyses (for the most part, numeric IQ values from PGC ASD were not available for this analysis). PGC ASD probands were assigned FSIQ levels 1-4 as follows: (1) $FSIQ < 35$; (2) $35 \leq FSIQ < 70$; (3) $70 \leq FSIQ < 90$; (4) $90 \leq FSIQ$. In PGC ASD, 38.9% of probands had estimated IQ available. We divided each of SSC and PGC ASD by presence/absence of intellectual disability (ID) in the proband so that they could be analyzed together (SSC ID: $IQ < 70$; PGC ASD ID: $IQ = 1$ or $IQ = 2$). We repeated the IQ stratified analyses in SSC and PGC ASD separately, which further suggested no differences between the cohorts, despite the limited FSIQ data available in PGC ASD (**Supplementary Tables 9a and 9b**).

De novo variant analyses. We defined a group of *de novo* mutations strongly associated with ASD risk (**Supplementary Table 11**). We performed this analysis exclusively in SSC; we could not perform the analysis in PGC ASD because only common variant (GWAS) data was available. As detailed in the main text, our previous work has identified a subclass of *de novo* protein truncating variants (PTVs) that are a primary source of association to ASD²¹. PTVs not in this contributing class are not associated with proband IQ ($P = 0.67$) (**Supplementary Figure 6**) and are unassociated with ASD risk (observed in 7.8% of cases and 6.9% of unaffected siblings, $P = 0.33$).

As detailed in the main text, *de novo* copy number variants (CNVs) that delete a gene should have the same molecular impact as a PTV in that same gene. The associations between CNV categories and ASD risk are presented in the supplement and did not differ substantially when controlling for parental age at birth of proband (**Supplementary Table 16**). Contributing deletions outside of our defined class were not associated with proband IQ ($P = 0.34$, **Supplementary Figure 4**). In contrast, *de novo* duplications of constrained genes are not disproportionately associated with ASD risk ($P = 0.49$; **Supplementary Figure 3, Supplementary Methods 5**).

We identified a subset of *de novo* CNV deletions, primarily those containing loss of function intolerant genes, that account for most of the category's association to ASDs (contributing CNV deletions; **Supplementary Figure 1**). Together, contributing PTVs and contributing CNV deletions formed a strong acting *de novo* variant category in SSC,

known from here as contributing *de novo* variants (CDNVs). Multiple lines of evidence suggest CDNVs are robustly associated with ASD risk. CDNVs were seen in 9.4% of SSC cases ($n = 221$ of 2,346) that were both sequenced and genotyped and 2.6% of SSC unaffected siblings ($n = 45$ of 1,736) that were both sequenced and genotyped ($P = 6.56E-20$). CDNVs were very strongly associated with proband IQ ($P = 5.92E-06$; controlling for proband sex), while all other *de novo* PTVs and deletions (those not in the CDNV category) were not associated with proband IQ ($P = 0.44$, controlling for proband sex; **Supplementary Figure 5**).

As *de novo* variants are on average more severe when observed in ASD cases than in controls³⁵, we could not estimate the amount of ASD risk conferred by CDNVs directly from the case-unaffected sibling odds ratio. As noted in the main text, we used the male:female CDNV carrier ratio to re-estimate the effect size for the event class, as described in De Rubeis et al., 2014. In brief, the approximately 4:1 male:female ratio among ASD cases suggests a different ASD liability threshold for males and females in the population. Regardless of whether that difference reflects etiology or ascertainment, it results in a direct mathematical relationship between the expected effect size of an event class and the male:female carrier ratio of cases. In the case of CDNVs, a variant class observed twice as frequently in female probands than in male probands (17.4% v 8.2%; $P = 1.35E-06$; **Supplementary Figure 7**), we estimate an OR of approximately 20³. This estimate far exceeds that directly suggested by the CDNV case-control excess of 3.63.

We next examined the rate of CDNVs in SSC probands based on the number of adverse proband co-occurring neurological and developmental events (**Figure 3a**). Previous studies have demonstrated that *de novo* PTVs and *de novo* CNVs are both associated with intellectual disability ($IQ < 70$) in ASD probands and positively associated with history of seizures^{1,15}. Motor delay is also a neurodevelopmental outcome associated with autism spectrum disorder³⁶. Here, we defined motor delay for SSC probands as failing to walk unaided before 18 months after birth, the age by which the great majority of children have begun to walk³⁷. As hypothesized, motor delay, seizures, and low IQ were independently associated with CDNV rate in SSC probands after controlling for proband sex (**Supplementary Table 12a**). CDNV rate was positively associated with the number of these adverse events experienced by probands (**Figure 3a**, **Supplementary Table 12b**). The decreasing male:female ratio among CDNV carriers as count of co-occurring neurodevelopmental outcomes increases suggests that the co-occurring neurodevelopmental categories are capturing a severe phenotype (**Supplementary Method 6, Supplementary Table 12b**). Multiple lines of evidence, including associations with IQ, male:female carrier ratio, and adverse co-occurring neurodevelopmental events, suggest that CDNVs are strongly associated with ASD risk.

Using pTDT, we evaluated polygenic transmission in probands carrying at least one CDNV (**Figure 3b, Supplementary Table 10**). As this CDNV analysis is specific to SSC, the polygenic risk scores generated for the CDNV analysis used info score cutoffs from SSC imputation in order to increase the number of well-imputed SNPs retained for PRS. We restricted our analysis to those families with genotyped parents and probands with both genotype and exome sequence data ($n = 2,345$; $n = 221$ with CDNV). The cohort of probands with a CDNV is too small to determine whether the difference in transmission between probands that do and do not carry a CDNV is statistically significant. We also analyzed whether polygenic over transmission was seen in a more

broadly defined set of *de novo* events (**Supplementary Methods 7, Supplementary Table 10**).

Genetic heterogeneity and proband phenotype. We analyzed whether polygenic risk for ASD, EA and SCZ were each independently over transmitted to ASD cases. For each of SSC ($n = 2,584$ trios), PGC ASD ($n = 3,870$ trios) and SSC & PGC ASD combined ($n = 6,454$ trios), we performed a logistic regression predicting proband/average parent status from polygenic risk for ASD, EA and SCZ. We confirmed that the over transmission acts independently in each PRS ($P < 2E-04$ for all PRS in SSC + PGC ASD cohort; **Supplementary Table 13**).

To calculate the relationship between proband polygenic risk, CDNVs and ASD IQ, we performed a linear regression using proband sex, the three polygenic risk scores and CDNV count to predict full-scale IQ of SSC probands of European ancestry. The results were consistent with previously published association between the three polygenic risk scores and IQ in the general population (**Supplementary Table 14**)¹⁴. This relationship between polygenic risk and proband IQ holds when using mid-parent PRS and controlling for proband CDNV status (**Supplementary Methods 8, Supplementary Table 15**).

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