

Identification of common genetic risk variants for autism spectrum disorder

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Autism spectrum disorder (ASD) is a highly heritable and heterogeneous group of neurodevelopmental phenotypes diagnosed in more than 1% of children. Common genetic variants contribute substantially to ASD susceptibility, but to date no individual variants have been robustly associated with ASD. With a marked sample-size increase from a unique Danish population resource, we report a genome-wide association meta-analysis of 18,381 individuals with ASD and 27,969 controls that identified five genome-wide-significant loci. Leveraging GWAS results from three phenotypes with significantly overlapping genetic architectures (schizophrenia, major depression, and educational attainment), we identified seven additional loci shared with other traits at equally strict significance levels. Dissecting the polygenic architecture, we found both quantitative and qualitative polygenic heterogeneity across ASD subtypes. These results highlight biological insights, particularly relating to neuronal function and corticogenesis, and establish that GWAS performed at scale will be much more productive in the near term in ASD.

ASD is the term for a group of pervasive neurodevelopmental disorders characterized by impaired social and communication skills along with repetitive and restrictive behavior. The clinical presentation is highly heterogeneous, including individuals with severe impairment and intellectual disability (ID) as well as individuals with above-average intelligence quotient (IQ) and high levels of academic and occupational functioning. ASD affects 1–1.5% of individuals and is highly heritable, and both common and rare variants contribute to its etiology^{1–4}. Common variants have been estimated to account for a major part of ASD liability²,

as has been observed for other common neuropsychiatric disorders. In contrast, de novo mutations, mostly copy number variants (CNVs) and gene-disrupting point mutations, have larger individual effects but collectively explain <5% of the overall liability^{1–3} and far less of the heritability. Although a number of genes have been convincingly implicated via excess statistical aggregation of de novo mutations, the largest genome-wide association study (GWAS) to date ($n=7,387$ cases scanned)—although providing compelling evidence for the bulk contribution of common variants—did not conclusively identify single variants at genome-wide significance^{5–7}.

A full list of affiliations appears at the end of the paper.

These results underscore that common variants, as in other complex diseases such as schizophrenia, individually have low impact and that a substantial scale-up in sample numbers would be needed.

Here we report what are, to our knowledge, the first common risk variants robustly associated with ASD, by more than doubling the discovery sample size relative to that in previous GWAS^{5–8}. We describe strong genetic correlations between ASD and other complex disorders and traits, confirming shared etiology, and we show results indicating differences in the polygenic architecture across clinical subtypes of ASD. Leveraging these relationships and recently introduced computational techniques⁹, we identify additional previously undescribed ASD-associated variants that are shared with other phenotypes. Furthermore, by integrating with complementary data from Hi-C chromatin-interaction analysis of fetal brains and brain transcriptome data, we explore the functional implications of our top-ranking GWAS results.

Results

GWAS. As part of the iPSYCH project¹⁰, we collected and genotyped a Danish nationwide population-based case-cohort sample including nearly all individuals born in Denmark between 1981 and 2005 and diagnosed with ASD (according to the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10)) before 2014. We randomly selected controls from the same birth cohorts (Supplementary Table 1). We previously validated registry-based ASD diagnoses^{11,12} and demonstrated the accuracy of genotyping DNA extracted and amplified from blood spots collected shortly after birth^{13,14}. Genotypes were processed with Ricopili¹⁵, performing stringent quality control of data, removal of related individuals, exclusion of ancestry outliers based on principal component analysis (PCA), and imputation by using the 1000 Genomes Project phase 3 reference panel. After this processing, genotypes from 13,076 cases and 22,664 controls from the iPSYCH sample were included in the analysis. As is now standard in human complex-trait genomics, our primary analysis was a meta-analysis of the iPSYCH ASD results with five family-based trio samples of European ancestry from the Psychiatric Genomics Consortium (PGC; 5,305 cases and 5,305 pseudocontrols)¹⁶. All PGC samples had been processed with the same Ricopili pipeline for quality control, imputation, and analysis as used here.

Supporting the consistency between the study designs, the iPSYCH population-based and PGC family-based analyses showed a high degree of genetic correlation with $r_G = 0.779$ (s.e.m. = 0.106; $P = 1.75 \times 10^{-13}$), findings similar to the genetic correlations observed between datasets in other mental disorders¹⁷. Likewise, polygenicity, as assessed by polygenic risk scores (PRSs), showed consistency across the samples, thus supporting homogeneity of the effects across samples and study designs (results below regarding PRSs on a five-way split of the sample). The SNP heritability (h_G^2) was estimated to be 0.118 (s.e.m. = 0.010), for a population prevalence of 0.012 (ref. ¹⁸).

The main GWAS meta-analysis included a total of 18,381 ASD cases and 27,969 controls, and applied an inverse-variance-weighted fixed-effects model. To ensure that the analysis was well powered and robust, we examined markers with minor-allele frequency (MAF) ≥ 0.01 and imputation INFO score ≥ 0.7 , which were supported by an effective sample size in $>70\%$ of the total. This final meta-analysis included results for 9,112,387 autosomal markers and yielded 93 genome-wide-significant markers in three separate loci (Fig. 1, Table 1a and Supplementary Figs. 1–44). Each locus was strongly supported by both the Danish case-control and the PGC family-based data. Although modest inflation was observed ($\lambda = 1.12$, $\lambda_{1000} = 1.006$), linkage disequilibrium (LD)-score regression analysis¹⁹ indicated that this finding arose from polygenicity ($>96\%$; Methods) rather than confounding.

The strongest signal among 294,911 markers analyzed on the X chromosome was $P = 7.8 \times 10^{-5}$.

We next obtained replication data for the top 88 loci with P values $< 1 \times 10^{-5}$ in five cohorts of European ancestry, including a total of 2,119 additional cases and 142,379 controls (Supplementary Table 2 and 3). An overall replication of the direction of effects was observed (53 of 88 (60%) of $P < 1 \times 10^{-5}$; 16 of 23 (70%) at $P < 1 \times 10^{-6}$; sign tests, $P = 0.035$ and $P = 0.047$, respectively), and two additional loci achieved genome-wide significance in the combined analysis (Table 1a). More details on the identified loci can be found in Supplementary Table 4, and selected candidates are described in Box 1.

Correlation with other traits and multitrait GWAS. To investigate the extent of genetic overlap between ASD and other phenotypes, we estimated the genetic correlations with a broad set of psychiatric and other medical diseases, disorders, and traits available at LD Hub²⁰, by using bivariate LD-score regression (Fig. 2 and Supplementary Table 5). Significant correlations were found for several traits including schizophrenia¹⁵ ($r_G = 0.211$, $P = 1.03 \times 10^{-5}$) and measures of cognitive ability, especially educational attainment²¹ ($r_G = 0.199$, $P = 2.56 \times 10^{-9}$), thus indicating a substantial genetic overlap with these phenotypes and corroborating previous reports^{5,22–24}. In contrast to findings in previous reports¹⁶, we find a strong and highly significant correlation with major depression²⁵ ($r_G = 0.412$, $P = 1.40 \times 10^{-25}$), and we report a novel and prominent overlap with ADHD²⁶ ($r_G = 0.360$, $P = 1.24 \times 10^{-12}$). Moreover, we confirm the genetic correlation with social communication difficulties at age 8 in a non-ASD population sample previously reported and based on a subset of the ASD sample²⁷ ($r_G = 0.375$, $P = 0.0028$).

To leverage these observations for the discovery of loci that may be shared between ASD and these other traits, we selected three particularly well-powered and genetically correlated phenotypes. These were schizophrenia ($n = 79,641$)¹⁵, major depression ($n = 424,015$)²⁵, and educational attainment ($n = 328,917$)²¹. We used the recently introduced MTAG method⁹ which, in brief, generalizes the standard inverse-variance-weighted meta-analysis for multiple phenotypes. In this case, MTAG takes advantage of how, given an overall genetic correlation between ASD and a second trait, the effect-size estimate and evidence for association to ASD can be improved by appropriate use of the association information from the second trait. The results of these three ASD-anchored MTAG scans are correlated to the primary ASD scan (and to each other), but given the exploration of three scans, we used a more conservative threshold of 1.67×10^{-8} for declaring significance across these secondary scans giving an estimated maximum false discovery rate (maxFDR) of 0.021. In addition to stronger evidence for several of the ASD hits defined above, variants in seven additional regions achieved genome-wide significance, including three loci shared with educational attainment and four shared with major depression (Table 1b, Box 1, Supplementary Table 6 and Supplementary Figs. 49–55). We note that in these seven instances, the effect-size estimate is stronger in ASD than the secondary trait, and the result is not characteristic of the strongest signals in these other scans (Supplementary Table 7–9) (and in fact, three of these seven were not significant in the secondary trait and constitute potentially novel findings). Moreover, we benchmarked against MTAG running two very large and heritable traits (height²⁸, $n = 252,288$ and body mass index (BMI)²⁹, $n = 322,154$) with no expected links to ASD, and no significant loci were added to the list of ASD-only significant associations.

Gene and gene-set analysis. Next, we performed gene-based association analysis on our primary ASD meta-analysis by using MAGMA³⁰, testing for the joint association of all markers within a locus (across all protein-coding genes in the genome). This analysis identified 15 genes surpassing the significance threshold

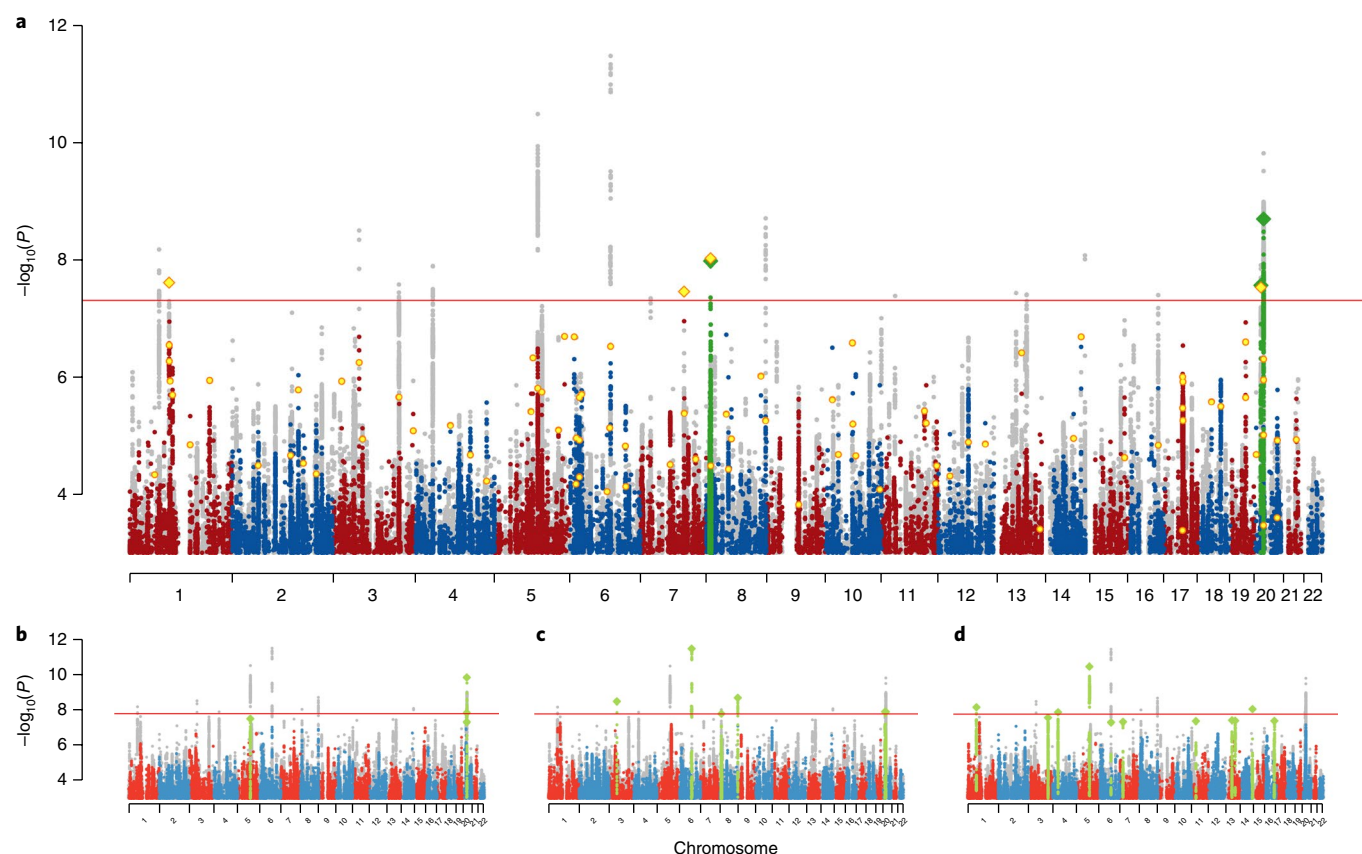


Fig. 1 | Manhattan plots. The x axis shows genomic position (chromosomes 1–22), and the y axis shows statistical significance as $-\log_{10}(P)$ of z statistics. **a**, The main ASD scan (18,381 cases and 27,969 controls), with the results of the combined analysis with the follow-up sample (2,119 cases and 142,379 controls) in yellow in the foreground. Genome-wide-significant clumps are green, and index SNPs are shown as diamonds. **b–d**: Manhattan plots for three MTAG scans of ASD together with schizophrenia¹⁵ (34,129 cases and 45,512 controls; **b**), educational attainment²¹ ($n = 328,917$; **c**) and major depression²⁵ (111,902 cases and 312,113 controls; **d**). Full-size plots are shown in Supplementary Figs. 45–48. In all panels, the results of the composite of the five analyses (consisting of the minimal P value of the five for each marker) is shown in gray in the background.

(Supplementary Table 10). As expected, most of these genes were located within the genome-wide-significant loci identified in the GWAS, but seven genes were located in four additional loci: *KCNN2*, *MMP12*, *NTM*, and a cluster of genes on chromosome 17 (*KANSL1*, *WNT3*, *MAPT*, and *CRHR1*) (Supplementary Figs. 57–71). In particular, *KCNN2* was strongly associated ($P = 1.02 \times 10^{-9}$), far beyond even single-variant statistical thresholds, and is included in the descriptions in Box 1.

Enrichment analyses using gene coexpression modules from human neocortex transcriptomic data (M13, M16, and M17 from Parikshak et al.³¹) and loss-of-function intolerant genes (probability of loss-of-function intolerance, $pLI > 0.9$)^{32,33}, for which there is evidence of enrichment in neurodevelopmental disorders^{26,31,34}, yielded only nominal significance for the latter ($P = 0.014$) and M16 ($P = 0.050$) (Supplementary Table 11). Genes implicated in ASD by studies or rare variants in Sanders et al.³⁵ were just shy of showing nominally significant enrichment ($P = 0.063$), whereas enrichment in the curated gene list from the SPARK consortium³⁶ was significant ($P = 0.0034$). Likewise, analysis of Gene Ontology sets^{37,38} for molecular function from the Molecular Signatures Database (MsigDB)³⁹ showed no significant sets after Bonferroni correction for multiple testing (Supplementary Table 12).

Dissection of the polygenic architecture. Because ASD is a highly heterogeneous disorder, we explored how h_G^2 partitioned across phenotypic subcategories in the iPSYCH sample, and we estimated the genetic correlations among these groups by using GCTA⁴⁰.

We examined cases with ($n = 1,873$) and those without ID and the ICD-10 diagnostic subcategories of childhood autism (F84.0, $n = 3,310$), atypical autism (F84.1, $n = 1,607$), Asperger's syndrome (F84.5, $n = 4,622$), and other/unspecified pervasive developmental disorders (PDDs, F84.8–9, $n = 5,795$), reducing to nonoverlapping groups when performing pairwise comparisons (Supplementary Table 13). Whereas the pairwise genetic correlations were consistently high among all subgroups (95% confidence intervals (CIs) including 1 in all comparisons), the h_G^2 of Asperger's syndrome ($h_G^2 = 0.097$, s.e.m. = 0.001) was found to be twice the h_G^2 of both childhood autism ($h_G^2 = 0.049$, s.e.m. = 0.009, $P = 0.001$) and the group of other/unspecified PDDs ($h_G^2 = 0.045$, s.e.m. = 0.008, $P = 0.001$) (Supplementary Tables 14 and 15 and Supplementary Figs. 82 and 83). Similarly, the h_G^2 of ASD without ID ($h_G^2 = 0.086$, s.e.m. = 0.005) was three times higher than that for cases with ID ($h_G^2 = 0.029$, s.e.m. = 0.013, $P = 0.015$).

To further examine the apparent polygenic heterogeneity across subtypes, we investigated how PRSs trained on different phenotypes were distributed across distinct ASD subgroups. We focused on phenotypes showing strong genetic correlation with ASD (for example, educational attainment) but also included traits with little or no correlation to ASD (for example, BMI) as negative controls. In this analysis, we regressed the normalized scores on ASD subgroups while including covariates for batches and principal components (PCs) in a multivariate regression. Of the eight phenotypes evaluated, only the cognitive phenotypes showed strong heterogeneity (educational attainment²¹, $P = 1.8 \times 10^{-8}$; IQ⁴¹, $P = 3.7 \times 10^{-9}$)

Table 1 | Genome-wide-significant loci from ASD scans and MTAG analyses

	Index variant	Chr	BP	Analysis	P	β	s.e.	A1/A2	FRQ	Support from other scans			Nearest genes
										Scan	P	β	
a	rs910805	20	21248116	ASD	2.04×10^{-9}	-0.096	0.016	A/G	0.760	ASD-SCZ	1.5×10^{-10}	-0.069	KIZ, XRN2,
										ASD-Edu*	2.0×10^{-8}	-0.061	NKX2-2, NKX2-4
	rs10099100	8	10576775	ASD	1.07×10^{-8}	0.084	0.015	C/G	0.331	Comb ASD	9.6×10^{-9}	0.078	C8orf74, SOX7,
										ASD-Edu	1.6×10^{-8}	0.056	PINX1
	rs201910565	1	96561801	Comb ASD	2.48×10^{-8}	-0.077	0.014	A/AT	0.689	ASD	3.4×10^{-7}	-0.033	LOC102723661, PTBP2
b	rs71190156	20	14836243	ASD	2.75×10^{-8}	-0.078	0.014	GTTTT TTT/G	0.481	Comb ASD	3.0×10^{-8}	-0.072	MACROD2
										ASD-Edu	1.2×10^{-8}	0.053	
	rs111931861	7	104744219	Comb ASD	3.53×10^{-8}	-0.216	0.039	A/G	0.966	ASD	1.1×10^{-7}	-0.094	KMT2E, SRPK2
	rs2388334	6	98591622	ASD-Edu	3.34×10^{-12}	-0.065	0.009	A/G	0.517	ASD	1.0×10^{-6}	-0.068	MMS22L, POU3F2
	rs325506	5	104012303	ASD-MD	3.26×10^{-11}	0.057	0.009	C/G	0.423	ASD	3.5×10^{-7}	0.071	NUD12
	rs11787216	8	142615222	ASD-Edu	1.99×10^{-9}	-0.058	0.010	T/C	0.364	ASD	2.6×10^{-6}	-0.030	MROH5
	rs1452075	3	62481063	ASD-Edu	3.17×10^{-9}	0.061	0.010	T/C	0.721	ASD	2.1×10^{-7}	0.035	CADPS
	rs1620977	1	72729142	ASD-MD	6.66×10^{-9}	0.056	0.010	A/G	0.260	ASD	1.2×10^{-4}	0.062	NEGR1
	rs10149470	14	104017953	ASD-MD	8.52×10^{-9}	-0.049	0.008	A/G	0.487	ASD	8.5×10^{-5}	-0.056	MARK3, CKB, TRMT61A, BAG5, APOPT1, KLC1, XRCC3
	rs16854048	4	42123728	ASD-MD	1.29×10^{-8}	0.069	0.012	A/C	0.858	ASD	5.9×10^{-5}	0.082	SLC30A9, BEND4, TMEM33, DCAF4L1

a, Loci reaching genome-wide significance in analysis of the ASD phenotype alone. The 'analysis' column indicates the minimum P value arising from the original scan (ASD) and the combined analysis with the follow-up sample (Comb ASD). The column 'support from other scans' lists the other analyses (including MTAG) that further support the locus at genome-wide significance. For the ASD scan results, genome-wide-significant results in the locus from the other scans are shown; for Comb ASD, the results from ASD are displayed. **b**, Additional genome-wide-significant loci identified in the three MTAG analyses. The three analyses are ASD with schizophrenia (SCZ)¹⁵, educational attainment (Edu)²¹, and major depression (MD)²⁵. Here the 'analysis' column indicates which MTAG analysis gave the results (ASD-Edu or ASD-MD), and the columns 'support from other scans' provide the corresponding scan results in ASD alone. In both **a** and **b**, independent loci are defined to have $r^2 < 0.1$ and distance > 400 kb, and the index variant is displayed in the column 'index var'. Chr, chromosome; BP, chromosomal position; A1/A2, alleles; FRQ, allele frequency of A1; β , estimate of effect with respect to A1; s.e., standard error of β ; P, association P value of the index variant (P). 'Nearest genes' lists nearest genes from within 50 kb of the region spanned by all SNPs with $r^2 \geq 0.6$ to the index variant. Asterisks indicate a different lead SNP from the index variant.

(Supplementary Fig. 84). Interestingly, all case-control groups with or without ID showed significantly different loading for the two cognitive phenotypes: controls with ID had the lowest score, followed by ASD cases with ID, and ASD cases without ID again had significantly higher scores than those of any other group (educational attainment, $P = 2.6 \times 10^{-12}$; IQ, $P = 8.2 \times 10^{-12}$).

With respect to the diagnostic subcategories constructed hierarchically from ASD subtypes (Supplementary Table 13), the cognitive phenotypes again showed the strongest heterogeneity across the diagnostic classes (educational attainment, $P = 2.6 \times 10^{-11}$; IQ, $P = 3.4 \times 10^{-8}$), whereas neuroticism²³ ($P = 0.0015$), chronotype⁴² ($P = 0.011$), and subjective well-being²³ ($P = 0.029$) showed a weaker but nominally significant degree of heterogeneity, and schizophrenia, major depressive disorder, and BMI²⁹ were nonsignificant across the groups ($P > 0.19$) (Fig. 3). This pattern weakened only slightly when we excluded subjects with ID (Supplementary Fig. 85). For neuroticism, there was a clear split, with atypical and other/unspecified PDD cases having significantly higher PRSs than childhood autism and Asperger's syndrome, $P = 0.00013$. Given the genetic overlap of each subcategory with each phenotype, the hypothesis of homogeneity across subphenotypes was strongly rejected ($P = 1.6 \times 10^{-11}$), thereby establishing that these subcategories indeed have differences in their genetic architectures.

Focusing on educational attainment, we found a significant enrichment of PRSs for Asperger's syndrome ($P = 2.0 \times 10^{-17}$) in particular, and for childhood autism ($P = 1.5 \times 10^{-5}$), but not for the group of other/unspecified PDD ($P = 0.36$) or for atypical autism ($P = 0.13$) (Fig. 3). Excluding individuals with ID only marginally changed this result: atypical autism became nominally significant

($P = 0.020$) (Supplementary Fig. 85). These results show that the genetic architecture underlying educational attainment is indeed shared with ASD but to a variable degree across the disorder spectrum. We found that the observed excess in ASD subjects of alleles positively associated with education attainment^{43,44} was confined to Asperger's syndrome and childhood autism, and it was not seen here in atypical autism nor in other/unspecified PDD.

Finally, we evaluated the predictive ability of ASD PRSs by using five different sets of target and training samples within the combined iPSYCH-PGC sample. The observed mean variance explained by PRSs (Nagelkerke's R^2) was 2.45% ($P = 5.58 \times 10^{-140}$) with a pooled PRS-based case-control odds ratio (OR) = 1.33 (95% CI 1.30–1.36) (Supplementary Figs. 89 and 91). Dividing the target samples into PRS decile groups revealed an increase in ORs with increasing PRSs. The ORs for subjects with the highest PRSs increased to OR = 2.80 (95% CI 2.53–3.10) relative to the lowest decile (Fig. 4a and Supplementary Fig. 92). By leveraging correlated phenotypes in an attempt to improve prediction of ASD, we generated a multiphenotype PRS as a weighted sum of phenotype-specific PRSs (Methods). As expected, Nagelkerke's R^2 increased for each PRS included, attaining its maximum at the full model at 3.77% ($P = 2.03 \times 10^{-215}$) for the pooled analysis with an OR = 3.57 (95% CI 3.22–3.96) for the highest decile (Fig. 4b and Supplementary Figs. 93 and 94). These results demonstrate that an individual's ASD risk depends on the level of polygenic burden of thousands of common variants in a dose-dependent manner, which can be reinforced by adding SNP weights from ASD-correlated traits.

Functional annotation. To obtain information on the possible biological underpinnings of our GWAS results, we conducted several

Box 1 | Selected loci and candidates (ordered by chromosome)

Gene	Locus ^a and supporting evidence	Gene function
<i>NEGR1</i>	Chr 1:72729142 Shared ASD-MDD locus This locus is also significant in depression ^{25,57} , educational attainment ²¹ , intelligence ⁴¹ , obesity, and BMI ^{29,58–61} , and in an ASD-schizophrenia meta-analysis ⁵ . <i>NEGR1</i> is the only protein-coding gene in the locus. <i>NEGR1</i> is supported by brain Hi-C and eQTL analyses ²⁵ .	Neuronal growth regulator 1 (<i>NEGR1</i>) is an adhesion molecule modulating synapse formation in hippocampal neurons ^{62,63} and neurite outgrowth ^{64,65} . It is a member of the IgLON protein family, which is implicated in synaptic plasticity and axon extension ^{66–68} . <i>NEGR1</i> is predominantly expressed (and developmentally upregulated) in the hippocampus and cortex ⁶⁹ , as well as the hypothalamus ⁷⁰ .
<i>PTBP2</i>	Chr 1:96561801 ASD locus This locus is also significant in BMI ^{29,58,60} , weight ⁵⁸ , and educational attainment ²¹ . In schizophrenia, the locus shows a <i>P</i> value of 6.5×10^{-6} (ref. ¹⁵). <i>PTBP2</i> is the nearest protein-coding gene, ~625 kb from the index SNP. De novo and rare variants in <i>PTBP2</i> have been reported in ASD cases ^{1,3,71} . <i>PTBP2</i> is supported by Hi-C results in this study (Fig. 5d).	<i>PTBP2</i> , also known as nPTB (neuronal PTB) or brPTB (brain PTB), is a splicing regulator. <i>PTBP1</i> and its paralog <i>PTBP2</i> bind intronic polypyrimidine tracts in precursor mRNAs and target large sets of exons, thereby coordinating alternative-splicing programs during development ⁷² . Several switches in the expression of <i>PTBP1</i> and <i>PTBP2</i> regulate alternative splicing during neurogenesis and neuronal differentiation ^{73–76} .
<i>CADPS</i>	Chr 3:62481063 Shared ASD-Edu locus This locus is also significant in a study of cognitive-decline rate ⁷⁷ . <i>CADPS</i> is supported by Hi-C results in this study (Fig. 5a).	<i>CADPS</i> encodes a calcium-binding protein involved in exocytosis of neurotransmitters and neuropeptides. In line with <i>CADPS</i> mRNA being mainly expressed in the brain and pituitary (GTEx portal; see URLs), immunoreactive CAPS-1 is localized in neural and various endocrine tissues ⁷⁸ . In hippocampal synapses, <i>CADPS</i> regulates the pool of readily releasable vesicles at presynaptic terminals ^{79,80} .
<i>KCNN2</i>	Chr 5:113801423 ASD locus (gene-wise analysis) This locus is also significant in educational attainment ^{21,81} . <i>KCNN2</i> synaptic levels are regulated by the E3 ubiquitin ligase UBE3A ⁸² , whose overexpression has been linked to ASD risk ^{82,83} .	<i>KCNN2</i> is a voltage-independent Ca^{2+} -activated K^{+} channel that responds to changes in intracellular calcium concentration and couples calcium metabolism to potassium flux and membrane excitability. In central-nervous-system neurons, activation of <i>KCNN2</i> modulates neuronal excitability by causing membrane hyperpolarization ⁸⁴ . Hippocampal <i>KCNN2</i> has roles in the formation of new memory ⁸⁵ , encoding and consolidation of contextual fear ⁸⁶ , and in drug-induced plasticity ⁸⁷ .
<i>KMT2E</i>	Chr 7:104744219 ASD locus This locus is also significant in schizophrenia ^{15,88} and in ASD-schizophrenia meta-analysis ⁵ . <i>KMT2E</i> de novo mutations are associated with ASD at FDR <0.1 (ref. ³⁵). A <i>KMT2E</i> credible SNP is a loss-of-function variant (Supplementary Table 16).	<i>KMT2E</i> encodes histone-lysine N-methyltransferase 2E and forms a family together with <i>SETD5</i> (refs. ^{89,90}). Evidence suggests that recognition of the histone H3K4me3 mark by the <i>KMT2E</i> PHD finger can facilitate recruitment of <i>KMT2E</i> to transcription-active chromatin regions ^{91,92} . <i>KMT2E</i> has been implicated in chromatin regulation, control of cell-cycle progression, and maintaining genomic stability ⁹³ .
<i>MACROD2</i>	Chr 20:14836243 ASD locus This locus is significant in previous ASD GWAS ⁹⁴ but not supported in larger study ⁹⁵ . <i>MACROD2</i> is the only protein-coding gene in the locus.	<i>MACROD2</i> is a nuclear enzyme that binds mono-ADP-ribosylated (MARylated) proteins and functions as an eraser of mono-ADP-ribosylation ⁹⁶ . Intracellular MARylated histones and GSK3 β are substrates of <i>MACROD2</i> , and the removal of MAR from GSK3 β is responsible for reactivation of its kinase activity ⁹⁶ . This gene is expressed in the lung and multiple regions of the brain but has low or no expression across most other tissues (GTEx portal; see URLs).

^aPosition of index SNP is listed. Chr, chromosome.

analyses. First, we examined how the $\text{ASD } h_G^2$ partitioned on functional genomic categories as well as on cell-type-specific regulatory elements, by using stratified LD-score regression⁴⁵. This analysis identified significant enrichment of heritability in conserved DNA regions and monomethyl histone H3 Lys4 (H3K4me1) histone marks⁴⁶, as well as in genes expressed in central-nervous-system cell types as a group (Supplementary Figs. 95 and 96), in line with observations in schizophrenia¹⁵, major depression²⁵, and bipolar disorder²². Analyzing the enhancer-associated mark H3K4me1 in individual cells/tissues⁴⁶, we found significant enrichment in brain and neuronal cell lines (Supplementary Fig. 97). The highest enrichment was observed in the developing brain, germinal matrix, cortex-

derived neurospheres, and embryonic-stem-cell-derived neurons, results consistent with ASD as a neurodevelopmental disorder with largely prenatal origins, as supported by data from analysis of rare de novo variants³¹.

Common variation in ASD is located in regions that are highly enriched with regulatory elements predicted to be active in human corticogenesis (Supplementary Figs. 95–97). Because most gene regulatory events occur at a distance via chromosome looping, we leveraged Hi-C data from the germinal zone (GZ) and postmitotic-zone cortical plate (CP) in the developing fetal brain to identify potential target genes for these variants⁴⁷. We performed fine-mapping of 28 loci to identify the set of credible variants with likely

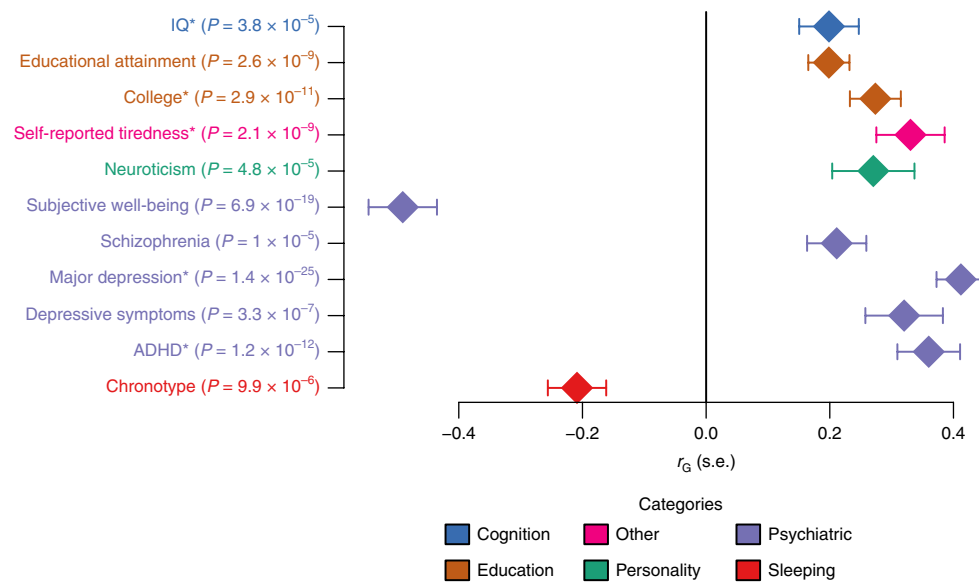


Fig. 2 | Genetic correlation with other traits. Significant genetic correlations between ASD ($n = 46,350$) and other traits after Bonferroni correction for testing a total of 234 traits available at LD Hub with the addition of several new phenotypes. Estimates and tests were performed with LDSC¹⁹. The results shown correspond to the following GWAS analyses: IQ⁴¹ ($n = 78,308$), educational attainment²¹ ($n = 328,917$), college⁵⁵ ($n = 111,114$), self-reported tiredness⁵⁶ ($n = 108,976$), neuroticism²³ ($n = 170,911$), subjective well-being²³ ($n = 298,420$), schizophrenia¹⁵ ($n = 82,315$), major depression²⁵ ($n = 480,359$), depressive symptoms²³ ($n = 161,460$), attention deficit/hyperactivity disorder (ADHD)²⁶ ($n = 53,293$), and chronotype⁴² ($n = 128,266$). Supplementary Table 5 shows the full output of this analysis. Asterisks indicate values are from in-house analyses of new summary statistics not yet included in LD Hub.

causal genetic risk⁴⁸ (Methods). Credible SNPs were significantly enriched in enhancer marks in the fetal brain (Supplementary Fig. 98), thus again confirming the likely regulatory role of these SNPs during brain development.

On the basis of location or evidence of physical contact from Hi-C, the 380 credible SNPs (28 loci) were assigned to 95 genes (40 protein coding), including 39 SNPs within promoters assigned to 9 genes, and 16 SNPs within the protein coding sequence of 8 genes (Supplementary Table 16 and Supplementary Fig. 98). Hi-C identified 86 genes, which interacted with credible SNPs in either the CP or GZ during brain development. Among these genes, 34 interacted with credible SNPs in both CP and GZ, thus representing a high-confidence gene list. Notable examples are illustrated in Fig. 5 and highlighted in Box 1. By analyzing their mean expression trajectory, we observed that the identified ASD-candidate genes (Supplementary Table 16) showed the highest expression during fetal corticogenesis, a finding in line with the enrichment of heritability in the regulatory elements in developing brain (Fig. 5e–g). Interestingly, both common and rare variation in ASD preferentially affects genes expressed during corticogenesis³¹, thus highlighting a potential spatiotemporal convergence of genetic risk on this specific developmental epoch, despite the disorder's profound genetic heterogeneity.

Discussion

The high heritability of ASD has been recognized for decades and remains among the highest for any complex disease despite many clinical diagnostic changes over the past 30–40 years resulting in a broader phenotype that characterizes more than 1% of the population. Although early GWAS permitted estimates that common polygenic variation should explain a substantial fraction of the heritability of ASD, individually significant loci remained elusive. This lack of results was suspected to be due to limited sample size, because studies of schizophrenia—with similar prevalence and heritability, and lower fitness—and major depression achieved striking results only when sample sizes five to ten times

larger than those available in ASD were used. This study has finally borne out that expectation with definitively demonstrated significant ‘hits’.

Here we report what are, to our knowledge, the first reported common risk variants robustly associated with ASD, on the basis of unique Danish resources in conjunction with results of the earlier PGC data—more than tripling the previous largest discovery sample. Of these, five loci were defined in ASD alone, and seven additional suggested at a stricter threshold by using GWAS results from three correlated phenotypes (schizophrenia, depression, and educational attainment) and a recently introduced analytic approach, MTAG. Both genome-wide LD-score regression analysis and the finding that, even among the loci defined in ASD alone, additional evidence in these other trait scans indicated that the polygenic architecture of ASD is significantly shared with the risk of adult psychiatric illness and higher educational attainment and intelligence. Of note, the MTAG analyses were carried out as three pairwise analyses. Consequently, we avoided the complex interactions that might have arisen if we ran three or four correlated phenotypes at a time⁹. Indeed, despite the secondary summary statistics coming from large, high-powered studies, we obtained relatively modest weights of the contributions from these statistics, because the genetic correlations were modest. The largest weight was 0.27 for schizophrenia, followed by 0.24 for major depression, and 0.11 for educational attainment. Moreover, the estimated worst-case FDR was 0.021, just 0.001 higher than that of the ASD GWAS alone. Thus, all loci identified by MTAG were found with an acceptable degree of certainty and had substantial contributions from ASD alone (Table 1a,b and Supplementary Table 6). We expect that most or all such loci will probably be identified in future ASD-only GWAS as sample sizes are increased substantially; however, given how new these methods are, the precise phenotypic consequences of these particular variants await expansion of all these trait GWAS.

In most GWAS studies, there has been little evidence of heterogeneity of association across phenotypic subgroups. In this study, however, we observed strong heterogeneity of genetic overlap

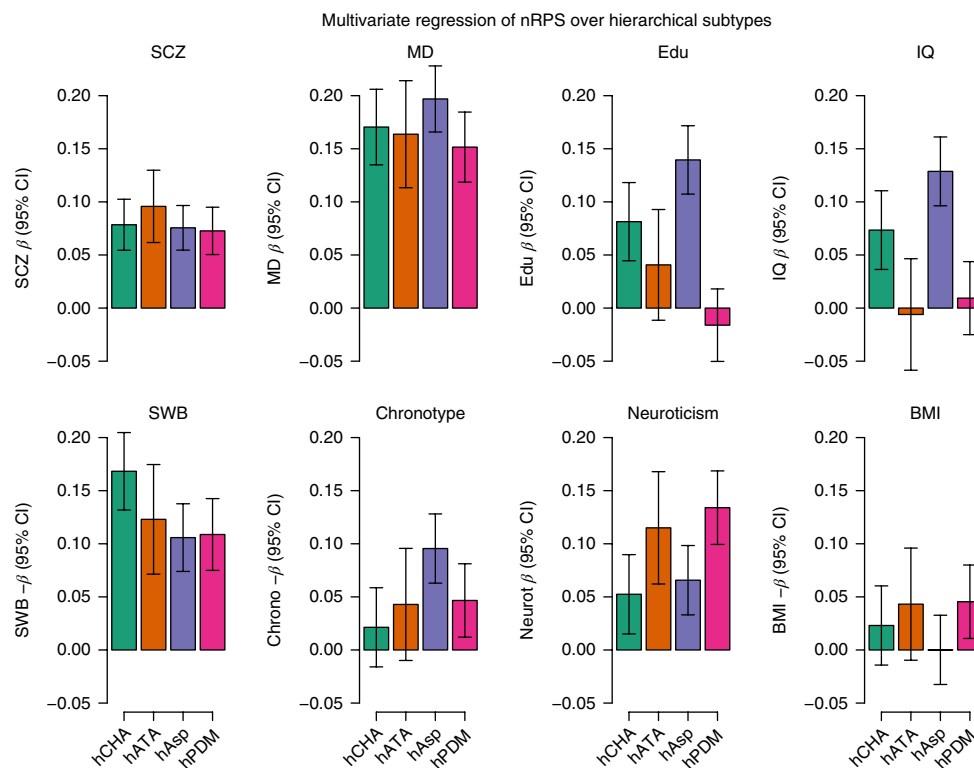


Fig. 3 | Profiling PRS load across distinct ASD subgroups. Results are shown for eight different phenotypes: schizophrenia (SCZ)¹⁵, major depression (MD)²⁵, educational attainment (Edu)²¹, human intelligence (IQ)⁴¹, subjective well-being (SWB)²³, chronotype⁴², neuroticism²³, and BMI²⁹. The bars show coefficients from multivariate multivariable regression of the eight normalized scores on the distinct ASD subtypes of 13,076 cases and 22,664 controls, adjusting for batches and PCs. The subtypes are the hierarchically defined subtypes for childhood autism (hCHA, $n = 3,310$), atypical autism (hATA, $n = 1,494$), Asperger's syndrome (hAsp, $n = 4,417$), and the lumped pervasive disorders developmental group (hPDM, $n = 3,855$). The orientations of the scores for subjective well-being, chronotype and BMI have been switched to improve graphical presentation. The corresponding plot where subjects with ID have been excluded is shown in Supplementary Fig. 85, and with ID as a subtype in Supplementary Fig. 84. Applying the same procedure to the internally trained ASD score did not display systematic heterogeneity ($P = 0.068$) except as expected for the ID groups ($P = 0.00027$) (Supplementary Fig. 88). Linear hypotheses were tested with the Pillai test.

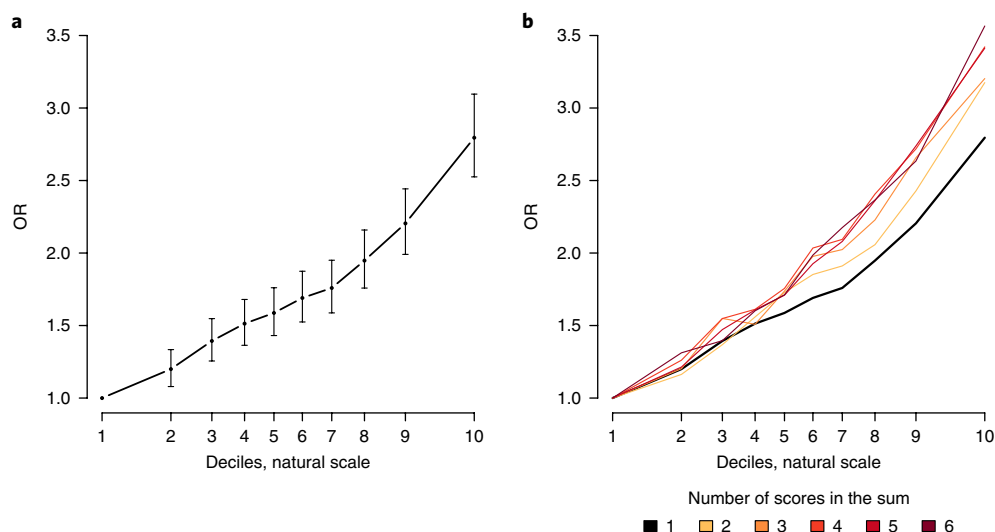


Fig. 4 | Decile plots (OR) by PRS within each decile for 13,076 cases and 22,664 controls. **a**, Decile plot with 95% CI for the internally trained ASD score (P -value threshold of 0.1). **b**, Decile plots on weighted sums of PRSs, starting with the ASD score of **a** and successively adding the scores for major depression²⁵, subjective well-being²³, schizophrenia¹⁵, educational attainment²¹, and chronotype⁴². In all instances, the P -value threshold for the score used was the one with the highest Nagelkerke's R^2 . Supplementary Figs. 92 and 94 show the stability across leave-one-out groups that was used to create these combined results.

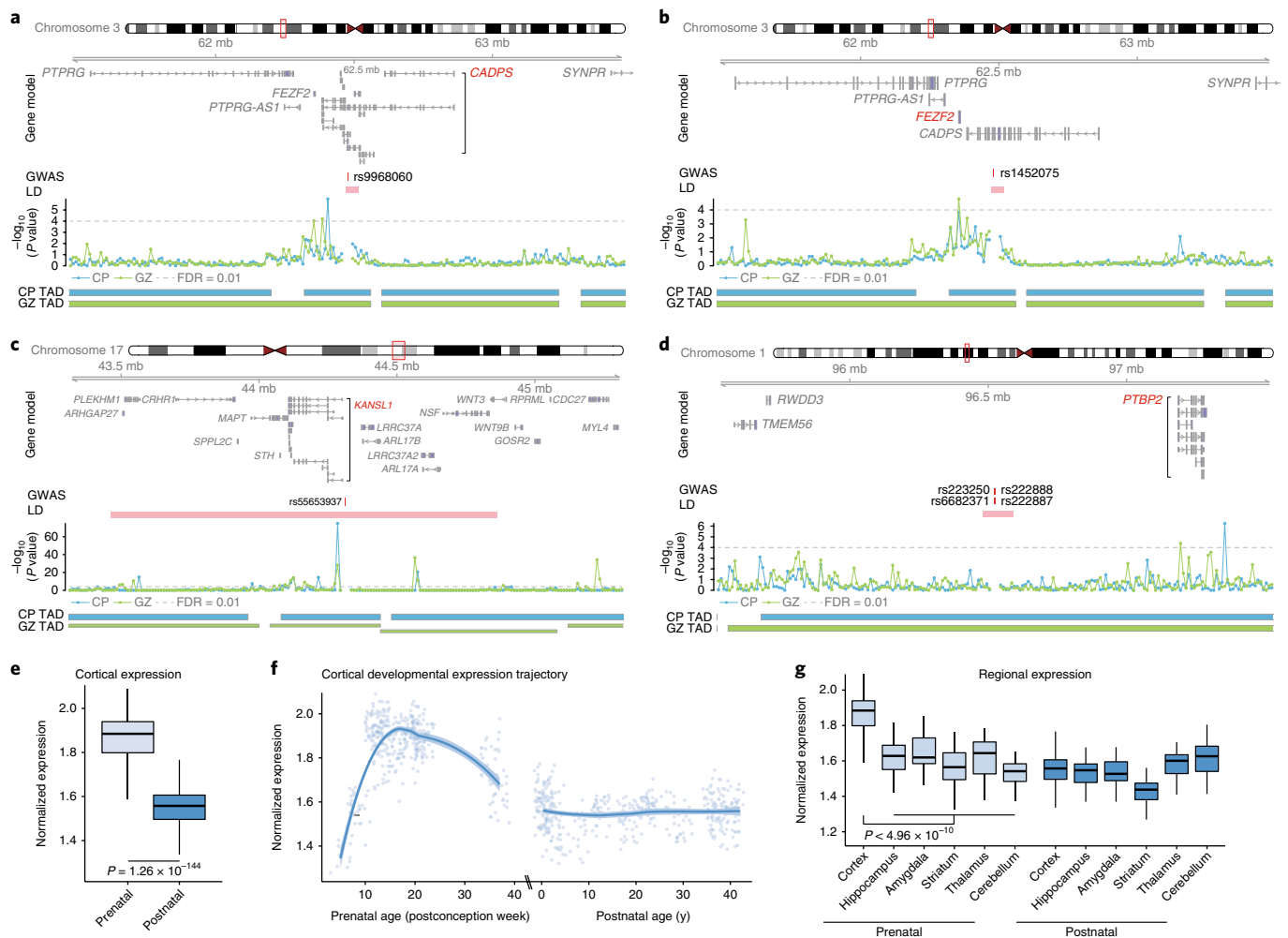


Fig. 5 | Chromatin interactions identify putative target genes of ASD loci. a–d. Chromatin-interaction maps of credible SNPs to the 1-Mb flanking region, providing putative candidate genes that physically interact with credible SNPs. The gene model is based on Gencode v19, and putative target genes are in red; the genomic coordinate for a credible SNP is labeled as GWAS; $-\log_{10}(P \text{ value})$, the significance of the interaction between a SNP and each 10-kb bin, is shown with a gray dotted line for FDR = 0.01 (one-sided significance test, calculated as the probability of observing a higher contact frequency under the fitted Weibull distribution matched by chromosome and distance); topologically associated domain (TAD) borders in the CP and GZ are shown. **e–g.** Developmental expression trajectories of ASD candidate genes show the highest expression in prenatal periods. Significance was determined by *t* test ($n = 410$ and 453 for prenatal and postnatal samples, respectively). Box plots showing median, interquartile range (IQR) with whiskers adding IQR to the first and third quartiles (**e,g**) and LOESS smooth curves plotted with actual data points (**f**). In **g**, ASD candidate genes are highly expressed in the developing cortex compared with other brain regions. Data were analyzed with one-way analysis of variance and post hoc Tukey test, FDR corrected ($n = 410/453$, $39/36$, $33/37$, $48/34$, $37/36$, and $32/39$ for prenatal/postnatal cortex, hippocampus, amygdala, striatum, thalamus, and cerebellum, respectively).

with other traits when our ASD samples were divided into distinct subsets. In particular, the excess of alleles associated with higher intelligence and educational attainment was observed only in the higher-functioning categories (particularly in individuals with Asperger's syndrome and individuals without comorbid ID) and not in the other/unspecified PDD and ID categories. These results are reminiscent of, and logically inverted relative to, the much greater role of spontaneous mutations in these latter categories, particularly in genes known to have an even larger effect in cohorts ascertained for ID/developmental delay⁴⁹. Interestingly, other/unspecified PDDs and atypical autism also have significantly higher PRSs for neuroticism than childhood autism and Asperger's syndrome. The different enrichment profiles observed provide evidence of a heterogeneous and qualitatively different genetic architecture among subtypes of ASD, which should inform future studies aiming at identifying etiologies and disease mechanisms in ASD.

The strong differences in estimated SNP heritability between ASD cases with versus without ID, and the highest values observed in Asperger's syndrome, provide genetic evidence of longstanding observations. In particular, the results align well with the observation that de novo variants are more frequently observed in ASD cases with ID than in cases without comorbid ID, that IQ correlates positively with family history of psychiatric disorders⁵⁰; and that severe ID (encompassing many syndromes that confer high risk of ASD) show far less heritability than that observed for mild ID⁵¹, intelligence in general⁵², and ASDs. Thus, it is perhaps unsurprising that our data suggest that the contribution of common variants may be more prominent in high-functioning ASD, such as Asperger's syndrome.

We further explored the functional implications of these results with complementary functional genomics data including Hi-C analyses of fetal brains and brain transcriptome data. Analyses at

genome-wide scale (partitioned h_G^2 (Supplementary Figs. 95–97) and brain transcriptome enrichment (Fig. 5e–g)) as well as at single loci (Fig. 5a–d and Box 1) highlighted the involvement of processes relating to brain development and neuronal function. Notably, several genes located in the identified loci have previously been linked to ASD risk in studies of de novo and rare variants (Box 1 and Supplementary Table 4), including *PTBP2*, *CADPS*, and *KMT2E*, which were found to interact with credible SNPs in the Hi-C analysis (*PTBP2* and *CADPS*) or to contain a loss-of-function credible SNP (*KMT2E*). Interestingly, aberrant splicing of the sister gene of *CADPS*, *CADPS2*, which has almost identical function, has been found in autism cases, and *Cadps2*-knockout mice display behavioral anomalies with translational relevance to autism⁵³. *PTBP2* encodes a neuronal splicing factor, and alterations in alternative splicing have been identified in brains from individuals diagnosed with ASD⁵⁴.

In summary, we established an initial robust set of common variant associations in ASD and have begun laying the groundwork through which the biology of ASD and related phenotypes will inevitably be better articulated.

URLs. GenomeDK high-performance-computing cluster in Denmark, <https://genome.au.dk/>; iPSYCH project, <http://ipsych.au.dk/>; iPSYCH download site, <http://ipsych.au.dk/downloads/>; NIMH Repository, https://www.nimhgenetics.org/available_data/autism/; PGC download site, <https://www.med.unc.edu/pgc/results-and-downloads/>; LISA cluster at SURFsara, <https://userinfo.surfsara.nl/systems/lisa/>; plink 1.9, <http://www.cog-genomics.org/plink/1.9/>; LDSC and associated files, <https://github.com/bulik/ldsc/>; LD Hub, <http://ldsc.broadinstitute.org/ldhub/>; GTEx portal, <https://gtexportal.org/home/>

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at <https://doi.org/10.1038/s41588-019-0344-8>.

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Competing interests

H.S., K.S., S. Steinberg, and G.B.W. are employees of deCODE genetics/Amgen. The 23andMe Research Team members are employed by 23andMe. D.H.G. is a scientific advisor for Ovid Therapeutic, Falcon Computing, and Axial Biotherapeutics. T.W. has acted as scientific advisor and lecturer for H. Lundbeck A/S.

Additional information

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Methods

Subjects. *iPSYCH sample.* The iPSYCH ASD sample is a part of a population based case-cohort sample extracted from a baseline cohort¹⁰ consisting of all children born in Denmark between 1 May 1981 and 31 December 2005. Singletons who were born to a known mother and were resident in Denmark on their first birthday were included. Cases were identified from the Danish Psychiatric Central Research Register (DPCRR)¹², which includes data on all individuals treated in Denmark at psychiatric hospitals (from 1969 onward) as well as at outpatient psychiatric clinics (from 1995 onward). Subjects were diagnosed with ASD in 2013 or earlier by a psychiatrist according to ICD10, including diagnoses of childhood autism (ICD10 code F84.0), atypical autism (F84.1), Asperger's syndrome (F84.5), other pervasive developmental disorders (F84.8), and pervasive developmental disorder, unspecified (F84.9). For controls, we selected a random sample from the set of eligible children excluding those with an ASD diagnosis by 2013.

The samples were linked by using the unique national personal identification number to the Danish Newborn Screening Biobank (DNSB) at Statens Serum Institute (SSI), where DNA was extracted from Guthrie cards, and whole-genome amplification was performed in triplicate, as described previously^{13,97}. Genotyping was performed at the Broad Institute of Harvard and MIT (Cambridge, MA, USA) with PsychChip arrays from Illumina according to the manufacturer's instructions. Genotype calling of markers with MAF >0.01 was performed by merging call sets from GenCall⁹⁸ and Birdseed⁹⁹, and less frequent variants were called with zCall¹⁰⁰. Genotyping and data processing were carried out in 23 waves.

All analyses of the iPSYCH sample and joint analyses with the PGC samples were performed at the secured national GenomeDK high-performance computing cluster in Denmark. The study was approved by the Regional Scientific Ethics Committee in Denmark and the Danish Data Protection Agency.

PGC samples. In brief, five cohorts provided genotypes to the sample (n denotes the number of trios for which genotypes were available): the Geschwied Autism Center of Excellence (ACE; $n = 391$), the Autism Genome Project¹⁰⁴ (AGP; $n = 2,272$), the Autism Genetic Resource Exchange^{101,102} (AGRE; $n = 974$), the NIMH Repository, the Montreal¹⁰³/Boston Collection (MONBOS; $n = 1,396$, and the Simons Simplex Collection^{104,105} (SSC; $n = 2,231$). The trios were analyzed as cases and pseudocontrols. A detailed description of the sample is available on the PGC website, and additional details are provided in Anney et al.⁵. Analyses of the PGC genotypes were conducted on the computer cluster LISA at the Dutch HPC center SURFsara.

Follow-up samples. As follow-up for the loci with P values < 10^{-6} , we asked for look-up in five samples of Nordic and Eastern European origin, including 2,119 cases and 142,379 controls in total: BUPGEN (Norway: 164 cases and 656 controls), PAGES (Sweden: 926 cases and 3,841 controls not part of the PGC sample above), the Finnish autism case-control study (Finland: 159 cases and 526 controls), and deCODE (Iceland: 574 cases and 136,968 controls; Eastern Europe: 296 cases and 388 controls) (details in Supplementary Note).

Statistical analyses. All statistical tests were two sided unless otherwise stated. Software versions and additional information can be found in the Nature Research Reporting Summary.

GWAS analysis. Ricopili¹⁵, the pipeline developed by the PGC Statistical Analysis Group was used for quality control, imputation, PCA, and primary association analysis (details in the Supplementary Note). The data were processed separately in the 23 genotyping batches in the case of iPSYCH and separately for each study in the PGC sample. Phasing was achieved with SHAPEIT¹⁰⁶, and imputation was done with IMPUTE2 (refs. ^{107,108}) with haplotypes from the 1000 Genomes Project, phase 3 (ref. ¹⁰⁹) as a reference.

After exclusion of regions of high LD¹¹⁰, the genotypes were pruned down to a set of approximately 30,000 markers (details in Supplementary Note). With PLINK's¹¹¹ identity by state analysis, pairs of subjects were identified with $\hat{\pi} > 0.2$, and one subject of each such pair was excluded at random (with a preference for keeping cases). PCA was carried out with smartPCA^{112,113}. In iPSYCH, a subsample of European ancestry was selected as an ellipsoid in the space of PC1–3 and centered and scaled by using the mean and eight s.d. of the subsample whose parents and grandparents were all known to have been born in Denmark ($n = 31,500$). In the PGC sample, the European (CEU) subset was chosen by using a Euclidean-distance measure weighted by the variance explained by each of the first three PCs. Individuals more distant than ten s.d. from the combined CEU and Toscani in Italy (TSI) HapMap reference populations were excluded. We conducted a secondary PCA on the remaining 13,076 cases and 22,664 controls to provide covariates for the association analyses. Numbers of subjects in the data-generation flow for the iPSYCH sample can be found in Supplementary Table 1.

We performed association analyses by applying PLINK 1.9 to the imputed dosage data (the sum of imputation probabilities $P(A1A2) + 2P(A1A1)$). In iPSYCH, we included the first four PCs as covariates as well as any PC beyond that, which were significantly associated with ASD in the sample, whereas the case-pseudocontrols from the PGC trios required no PC covariates. Combined results for iPSYCH and for iPSYCH with the PGC were achieved by meta-analysis

of batchwise and studywise results by using METAL¹¹⁴ (July 2010 version) with an inverse-variance-weighted fixed-effect model¹¹⁵. On chromosome X, males and females were analyzed separately and then meta-analyzed together. Subsequently, we applied a quality filter allowing only markers with an imputation info score 0.7, MAF of 0.01 and an effective sample size (Supplementary Note) of at least 70% of the study maximum. The degree to which the deviation in the test statistics could be ascribed to cryptic relatedness and population stratification rather than to polygenicity was measured from the intercept in LD-score regression¹⁹ (LDSC) as the ratio of (intercept – 1) and (mean $\chi^2 - 1$).

MTAG⁹ was applied with standard settings. The iPSYCH-PGC meta-analysis summary statistics were paired with the summary statistics for each of major depression²⁵ (excluding the Danish samples but including summary statistics from 23andMe⁹⁷; 111,902 cases, 312,113 controls, and mean $\chi^2 = 1.477$), schizophrenia¹⁵ (also excluding the Danish samples; 34,129 cases, 45,512 controls, and mean $\chi^2 = 1.804$) and educational attainment²¹ (328,917 samples and mean $\chi^2 = 1.648$). These are studies that have considerably more statistical power than the ASD scan, but because the genetic correlations are modest in the context of MTAG, the weights ascribed to the secondary phenotypes in the MTAG analyses remain relatively low (no higher than 0.27). The maximum FDR was estimated as recommended in the MTAG paper⁹ (details in the Supplementary Note).

The results were clumped, and we highlighted loci of interest by selecting those that were significant at 5×10^{-8} in the iPSYCH-PGC meta-analysis or the meta-analysis with the follow-up sample or were significant at 1.67×10^{-8} in any of the three MTAG analyses. The composite GWAS consisting of the minimal P values at each marker over these five analyses was used as a background when creating Manhattan plots for the different analyses showing both what was maximally achieved and what the individual analysis contributed to that.

Gene-based association and gene-set analyses. MAGMA 1.06 (ref. ³⁰) was applied to the ASD GWAS summary statistics to test for gene-based association. By using NCBI 37.3 gene definitions and restricting the analysis to SNPs located within the transcribed region, we tested mean SNP association with the sum of $-\log(\text{SNP } P \text{ value})$ as the test statistic. The resulting gene-based P values were further used in competitive gene-set enrichment analyses in MAGMA. One analysis explored the candidate sets M13, M16, and M17 from Parikshak et al.³¹, constrained, loss-of-function intolerant genes ($pLI > 0.9$; refs. ^{32,33}) derived from data from the Exome Aggregation Consortium (details in Supplementary Note), as well as gene sets found in studies of rare variants in autism by Sanders et al.³⁵ and the curated gene list from the SPARK consortium³⁶. Another was an agnostic analysis of the Gene Ontology sets^{37,38} for molecular function from MsigDB 6.0 (ref. ³⁹). We analyzed only genes outside the broad MHC region (hg19: Chr 6: 25–35 Mb) and included only gene sets with 10–1,000 genes. The gene sets from Sanders et al. and SPARK included only one gene in MHC and were exempt from the MHC exclusion to be as true to the set as possible. All gene sets with significant enrichment were inspected to ensure that the signal was not driven by one or a few associated loci with multiple genes in close LD.

SNP heritability. SNP heritability, h_G^2 , was estimated by using LDSC¹⁹ for the full ASD GWAS sample and GCTA^{40,116,117} for subsamples too small for LDSC. For LDSC, we used precomputed LD scores based on the European-ancestry samples of the 1000 Genomes Project¹¹⁸ restricted to HapMap3 (ref. ¹¹⁹) SNPs. The summary statistics with standard LDSC filtering were regressed onto these scores. For liability-scale estimates, we used a population prevalence for Denmark of 1.22% (ref. ¹⁸). Lacking proper prevalence estimates for subtypes, we scaled the full spectrum prevalence on the basis of the composition of the case sample.

For subsamples too small for LDSC, the GREML approach of GCTA^{40,116,117} was used. On best-guess genotypes (genotype probability > 0.8, missing rate < 0.01, and MAF > 0.05) with indels removed, a genetic relatedness matrix was fitted for the association sample (i.e., the subjects of European ancestry with $\hat{\pi} \leq 0.2$), thus providing a relatedness estimate for all pairwise combinations of individuals. Estimation of the phenotypic variance explained by the SNPs (REML) was performed by including PC1–4 as continuous covariates together with any other PC that was nominally significantly associated with the phenotype as well as batches as categorical indicator covariates. Testing equal heritability for nonoverlapping groups was performed with permutation tests (with 1,000 permutations), keeping the controls and randomly assigning the different case labels.

Following Finucane et al.⁴⁵, we conducted an enrichment analysis of the heritability for SNPs for functional annotation and for SNPs located in cell-type-specific regulatory elements. Using first the same 24 overlapping functional annotations (stripped down from 53), as in Finucane et al., we regressed the χ^2 from the ASD GWAS summary statistics on the cell-type-specific LD scores downloaded from the site mentioned above with baseline scores, regression weights, and allele frequencies based on European-ancestry 1000 Genome Project data. The enrichment of a category was defined as the proportion of SNP heritability in the category divided by the proportion of SNPs in that category. Still following Finucane et al., we performed a similar analysis using 220 cell-type-specific annotations divided into ten overlapping groups. In addition, we conducted an analysis based on annotations derived from data on

H3K4me1 imputed gapped peak data from the Roadmap Epigenomics Mapping Consortium¹²⁰, more specifically information excluding the broad MHC region (Chr 6: 25–35 Mb).

Genetic correlation. For the main ASD samples, SNP correlations, r_G , were estimated by using LDSC¹⁹, and for the analysis of ASD subtypes and subgroups in which the sample sizes were generally small, we used GCTA⁴⁰. In both cases, we followed the same procedures as those explained above. For all but a few phenotypes, LDSC estimates of correlation were achieved by upload to LD Hub²⁰ for comparison to 234 phenotypes in total.

Polygenic risk scores. For the PRSs, we clumped the summary statistics, applying standard Ricopili parameters (details in the Supplementary Note). To avoid potential strand conflicts, we excluded all ambiguous markers for summary statistics not generated by Ricopili by using the same imputation reference. PRSs were generated at the default P -value thresholds (5×10^{-8} , 1×10^{-6} , 1×10^{-4} , 0.001, 0.01, 0.05, 0.1, 0.2, 0.5, and 1) as a weighted sum of the allele dosages in the ASD GWAS sample, summing over the markers abiding by the P -value threshold in the training set and weighing by the additive scale effect measure of the marker ($\log(OR)$ or β) as estimated in the training set. Scores were normalized before analysis.

We evaluated the predictive power by using Nagelkerke's R^2 and plots of ORs and CIs over score deciles. Both R^2 and ORs were estimated in regression analyses including the relevant PCs and indicator variables for genotyping waves.

Lacking a large ASD sample outside of iPSYCH and PGC, we trained a set of PRSs for ASD internally as follows. We divided the sample into five subsamples of approximately equal size, respecting the division, into batches. We then ran five GWAS, leaving out each group in turn from the training set, then performed meta-analysis of these with the PGC results. This procedure produced a set of PRSs for each of the five subsamples trained on their complement. Before analyses, each score was normalized to the group in which it was defined. We evaluated the predictive power in each group and on the whole sample combined.

To exploit the genetic overlap with other phenotypes to improve prediction, we created a series of new PRSs by adding to the internally trained ASD score the PRSs of other highly correlated phenotypes in a weighted sum (details in the Supplementary Note).

To analyze ASD subtypes in relation to PRSs, we defined a hierarchical set of phenotypes in the following way: The first hierarchical subtypes were childhood autism; hierarchical atypical autism was defined as all individuals with atypical autism and no childhood autism diagnosis, and hierarchical Asperger's syndrome was defined as all individuals with an Asperger's syndrome diagnosis and neither childhood autism nor atypical autism. Finally, we lumped other pervasive developmental disorders and pervasive developmental disorder, unspecified into pervasive disorders developmental mixed, and the hierarchical version consisted of all subjects with such a diagnosis and none of the preceding diagnoses (Supplementary Table 13). We examined the distribution over the distinct ASD subtypes of PRSs for a number of phenotypes showing high r_G with ASD (as well as a few with low r_G as negative controls), by performing multivariate regression of the scores on the subtypes while adjusting for relevant PCs and wave-indicator variables in a linear regression (details in the Supplementary Note).

Hi-C analysis. The Hi-C data were generated from two major cortical laminae: the GZ, containing primarily mitotically active neural progenitors, and the cortical and subcortical plate, consisting primarily of postmitotic neurons⁴⁷. We first derived a set of credible SNPs (putative causal SNPs) from the identified top-ranking loci in the ASD GWAS by using CAVIAR⁴⁸. The 30 loci showing the strongest association were intersected with the Hi-C reference data, thus resulting in 28 loci for analysis. To test whether credible SNPs were enriched in active marks in the fetal brain¹²⁰, we used GREAT, as previously described^{47,121}. Credible SNPs were subgrouped into SNPs without known function (unannotated) and functionally annotated SNPs (SNPs in the gene promoters and SNPs causing nonsynonymous variants) (Supplementary Fig. 98). Then we integrated unannotated credible SNPs with chromatin-contact profiles during fetal corticogenesis⁴⁷, defining genes physically interacting with intergenic or intronic SNPs (Supplementary Fig. 98).

The spatiotemporal transcriptomic atlas of the human brain was obtained from Kang et al.¹²². We used transcriptomic profiles of multiple brain regions with developmental epochs spanning prenatal (6–37 weeks postconception) and postnatal (4 months to 42 years) periods. Expression values were log-transformed and centered to the mean expression level for each sample by using a scale(center = T , scale = F) + 1 function in R. ASD candidate genes identified by Hi-C analyses (Supplementary Fig. 98) were selected for each sample, and their average centered expression values were calculated and plotted.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary.

Data availability

The summary statistics are available for download the iPSYCH and at the PGC download sites (see URLs). For access to genotype data from the PGC samples and the iPSYCH sample, researchers should contact the lead principal investigators M.J.D. and A.D.B. for PGC-ASD and iPSYCH-ASD, respectively.

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Data collection

This is fully described in the Online Methods and associated Supplemental Note. In brief: GenCall, Birdseed and zCall were used for genotype calling and the genotypes merged.

Data analysis

This is fully described in the Online Methods and associated Supplemental Note. In brief: Genotype calling was done using GenCall (1.6.2.2), Birdseed (1.6) and zCall version 1 (Autocall, <https://github.com/jigold/zCall>). Quality control, imputation, association analyses, and polygenic risk scoring was done using the Ricopili pipeline: <https://github.com/Nealelab/ricopili>, which relies on the following software: SHAPEIT v2, IMPUTE2, Eigensoft 6.0.1 (incl. smartPCA), Plink 1.9, METAL 2011-03-25. For gene-based and gene-set analyses we used MAGMA 1.06. Estimation of credible SNPs were done using CAVIAR v1, and to test whether credible SNPs are enriched in active marks in the fetal brain and in-house implementation of the GREAT method was used (see Won et al. Nature. 2016;538(7626):523-527). Functional annotation of credible SNPs was done using Ensemble Variant Effect Predictor (VEP). SNP heritability, partitioning of the heritability and genetic correlations were estimated using LD score regression (<https://github.com/bulik/ldsc>) and LD hub (<http://ldsc.broadinstitute.org/>) for the large samples. Genetic correlation between ASD subtypes was estimated using GCTA v1.26.0. Multitrait association analyses were conducted using MTAG (<https://github.com/omeed-maghzian/mtag>). R v3.4 was used in general for statistical analyses and plotting (<https://www.Rproject.org>).

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As stated in the manuscript, summary statistics has already been made available at <http://ipsych.au.dk/downloads/> and <https://www.med.unc.edu/pgc/results-and-downloads>. For access to genotypes from the PGC samples and the iPSYCH sample, researchers should contact the lead PIs Mark J. Daly and Anders D. Børghlum for PGC-ASD and iPSYCH-ASD respectively.

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Life sciences study design

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Sample size	No sample size calculation was made. Previous studies of psychiatric disorders that are very polygenic (e.g. schizophrenia) have demonstrated that high numbers of cases and controls (in line with the sample size analyzed in this study) yield enough power to detect common risk variants with low effect sizes.
Data exclusions	Within each analyzed cohort we aimed at analyzing genetically homogeneous samples. Genetic outliers were excluded based on principal component analyses and related individuals were removed.
Replication	Consistency was checked internally between the 23 batches in iPSYCH and between iPSYCH and PGC. The 88 strongest signals were followed up in independent samples from other Nordic countries and Eastern Europe. We evaluated our results in three ways: sign test, genetic correlation, and metaanalysis of the combined samples, and these generally support the results from our primary GWAS. In addition support for the reported loci were obtained by MTAG analysis of correlated traits.
Randomization	It is an observational study comparing everybody in the selected birth cohorts with and ASD diagnosis as cases, and a random sample from the complement in said cohort as controls.
Blinding	In iPSYCH, diagnoses are drawn from registries. These are administrative data bases populated by data from the clinicians long before the current study. The blood samples are pulled from a biobank. Hence, the study participants and diagnosing clinicians are blinded with respect to this study. Genotyping is done on a massive scale on 85.000 individuals on 500.000 variables (which by imputation is expanded to ~10 million variables), and the data is generated without a specif goal or effect in mind except for an overall goal of investigating the genetic and environmental effects on psychiatric disorders. So although it is in principle possible for analysts in the lab to look up crude diagnostic data for a sample, it will not change the genotyping. - In the meta analysis we include data from the Psychiatric Genetics Consortium (PGC) which has been reported in an earlier publication. There design was different, but analyses analogous.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

In the meta-analysis we included samples from the Psychiatric Genetics Consortium (PGC) and the iPSYCH sample. The iPSYCH sample was processed in 23 batches (genotyping, qc and imputation was done separately for these batches) of approximately 3,500 individuals each. All analyzes were adjusted for batch, and principal components included to control for population stratification. The PGC samples are trio samples, hence there was no need to adjust for populations stratification there.

Recruitment

In iPSYCH, diagnoses are drawn from national registries and the blood samples are pulled from a the Danish Neonatal Screening Biobank. Hence, it is a population sample and bias from self-selection is impossible.

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