Identifying loci with different allele frequencies among cases of eight psychiatric disorders using CC-GWAS.

Wouter J. Peyrot[1,2,3], Alkes L. Price[1,2,4]

[1]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA. [2]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, USA. [3]Department of Psychiatry, Amsterdam UMC, Vrije Universiteit, Amsterdam 1081 HJ, Netherlands. [4]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA

Abstract

Psychiatric disorders are highly genetically correlated, and many studies have focused on their shared genetic components. However, little research has been conducted on the genetic differences between psychiatric disorders, because case-case comparisons currently require individual-level data from cases of both disorders. We developed a new method (CC-GWAS) to test for differences in allele frequency among cases of two different disorders using summary statistics from the respective case-control GWAS; CC-GWAS relies on analytical assessments of the genetic distance between cases and controls of each disorder. Simulations and analytical computations confirm that CC-GWAS is well-powered and attains effective control of type I error. We applied CC-GWAS to publicly available summary statistics for schizophrenia, bipolar disorder and major depressive disorder, and identified 116 independent genome-wide significant loci distinguishing these three disorders, including 21 CC-GWAS-specific loci that were not genome-wide significant in the input case-control summary statistics. Two of the CC-GWAS-specific loci implicate the genes *KLF6* and *KLF16* from the Kruppel-like family of transcription factors; these genes have been linked to neurite outgrowth and axon regeneration. We performed a broader set of case-case comparisons by additionally analyzing ADHD, anorexia nervosa, autism, obsessive-compulsive disorder and Tourette's Syndrome, yielding a total of 200 independent loci distinguishing eight psychiatric disorders, including 74 CC-GWAS-specific loci. We confirmed that loci identified by CC-GWAS replicated convincingly in applications to data sets for which independent replication data were available. In conclusion, CC-GWAS robustly identifies loci with different allele frequencies among cases of different disorders using results from the respective case-control GWAS, providing new insights into the genetic differences between eight psychiatric disorders.

Introduction

Psychiatric disorders are highly genetically correlated, and many studies have focused on their shared genetic components, including genetic correlation estimates of up to 0.7[1–3] and recent identification of 109 pleotropic loci across a broad set of eight psychiatric disorders[3]. However, much less research has been conducted on the genetic differences between psychiatric disorders, and biological differences between psychiatric disorders are poorly understood. Currently, differential diagnosis between disorders is often challenging and treatments are often non-disorder-specific, highlighting the importance of studying genetic differences between psychiatric disorders.

A recent study[4] progressed the research on genetic differences between disorders by comparing 24k SCZ cases vs. 15k BIP cases, yielding two significantly associated loci. However, ~25% of the cases were discarded compared to the respective case-control data (owing to non-matching ancestry and genotyping platform). Methods that analyse case-control summary statistics may be advantageous, because they make use of all genotyped samples and because summary statistics are often broadly publicly available[5]. Indeed, several methods have been developed to analyse GWAS summary statistics of two complex traits[3,6–13], but none of these methods can be used to conduct a case-case comparison (see Discussion). Currently, case-case comparisons of two disorders require individual-level data from cases of both disorders.

In this study, we propose a new method (CC-GWAS) to compare cases of two disorders based on the respective case-control GWAS summary statistics. CC-GWAS relies on a new genetic distance measure ($F_{ST,causal}$) quantifying the genetic distances between cases and controls of different disorders. We first apply CC-GWAS to publicly available GWAS summary statistics of the mood and psychotic disorders[3], schizophrenia (SCZ)[14,15], bipolar disorder (BIP)[16] and major depressive disorder (MDD)[17]. Subsequently, we analyse all comparisons of eight psychiatric disorders by additionally analysing attention deficit/hyperactivity disorder (ADHD)[18], anorexia nervosa (ANO)[19], autism spectrum disorder (ASD)[20], obsessive–compulsive disorder (OCD)[21], Tourette's Syndrome and Other

Tic Disorders (TS)[22]. Finally, we replicate CC-GWAS results of SCZ vs. MDD based on subsets of the data for which independent replication data were available.

Results

Overview of methods

CC-GWAS detects differences in allele frequencies among cases of disorders A and B by analysing case-control GWAS summary statistics for each disorder. CC-GWAS relies on the analytical variances and covariances of genetic effects of causal variants distinguishing caseA vs. controlA (A1A0), caseB vs. controlB (B1B0), and caseA vs. caseB (A1B1). We note that these variances are proportional to the average normalized squared difference in allele frequencies of causal variants; we call this quantity $F_{ST,causal}$ (defined separately for A1A0, B1B0, A1B1), as it is related to the quantity F_{ST} in population genetics (see Methods)[23]. $F_{ST,causal}$ is defined at the population level, i.e. assuming infinite sample size, and is derived based on estimates of the SNP-based heritabilities ($h^2_{l,A}$ and $h^2_{l,B}$), lifetime population prevalences (K_A and K_B), genetic correlation (r_g), and number of independent causal variants (m).

CC-GWAS weights the effect sizes from the respective case-control GWAS using weights that minimize the expected squared difference between estimated and true A1B1 effect sizes; we refer to these as ordinary least squares (OLS) weights (see Methods). The OLS weights are designed to optimize power to detect A1B1, and depend on sample size, sample overlap, and the expected variance of effect sizes reflected in $F_{ST,causal}$. The OLS weights may be susceptible to type I error for SNPs with nonzero A1A0 and B1B0 effect sizes but zero A1B1 effect size, which we refer to as "stress test" SNPs. To mitigate this, CC-GWAS also computes sample-size independent weights based on infinite sample size; we refer to these as Exact weights (see Methods). (At very large sample sizes, the OLS weights converge to the Exact weights.) CC-GWAS uses the OLS weights and Exact weights to compute *p*-values (see Methods). Specifically, CC-GWAS reports a SNP as statistically significant if it achieves $P<5\times10^{-8}$ using OLS weights *and* $P<10^{-4}$ using Exact weights, balancing power and type I error

(see Simulations). For statistically significant SNPs, CC-GWAS outputs OLS effect sizes reflecting direction and magnitude of effect. We note that the OLS weights assume that A1A0 and B1B0 effect sizes for causal variants follow a bivariate normal distribution. Violation of this assumption may result in increased type I error when using the OLS weights only; as noted above, the Exact weights protect against type I error in such scenarios. We further note that sample overlap of controls increases the power of CC-GWAS, by providing a more direct comparison of caseA vs. caseB. Further details of the CC-GWAS method are provided in the Methods section; we have released open-source software implementing the method (see URLs).

Quantifying genetic distances between cases and/or controls of each disorder

The CC-GWAS weights depend on a new population-level quantity $F_{ST,causal}$, the average normalized squared difference in allele frequencies of causal variants. $F_{ST,causal}$ is derived based on the SNP-based heritabilities ($h^2_{l,A}$ and $h^2_{l,B}$), lifetime population prevalences (K_A and K_B), genetic correlation (r_g), and number of independent causal variants (m). Estimates of $F_{ST,causal}$ for SCZ, BIP and MDD, based on parameters estimated in recent GWAS[15–17], are reported in Figure 1 and Table S1. $F_{ST,causal}$ allows for a direct comparison of cases and controls using $\sqrt{m * F_{ST,causal}}$ as a new genetic distance measure, where the square root facilitates 2-dimensional visualization (see Figure 1A and Methods). (CC-GWAS assumes that the set of causal variants is the same for both disorders, with causal effect sizes following a bivariate normal distribution, but $F_{ST,causal}$ does not rely on this assumption; see Methods). We note that $m * F_{ST,causal}$ is independent of m when other parameters are fixed, because the equation for $F_{ST,causal}$ has m in the denominator (see Methods). For SCZ and BIP, despite the large genetic correlation ($r_g = 0.70$), the genetic distance between SCZ cases and BIP cases is substantial ($\sqrt{m * F_{ST,causal}} = 0.49$, which is comparable to 0.66 for SCZ case-control and 0.60 for BIP case-control; Figure 1B). For SCZ and MDD ($r_g = 0.31$), the genetic distance between MDD cases and SCZ cases (0.63) is larger than for MDD case-control (0.29) (Figure 1C) owing to the larger prevalence and lower heritability of MDD, consistent with our empirical findings (see below). For MDD and BIP

($r_g = 0.33$), genetic distances are similar to MDD and SCZ (Figure 1D). To aid intuitive understanding of this new genetic distance measure, we note that the distances can be roughly interpreted as (i) the square root of the average squared difference in allele frequency at causal SNPs, (ii) proportional to the average power in GWAS (assuming equal sample sizes and numbers of causal SNPs), (iii) heritability on the observed scale based on 50/50 ascertainment (although the heritability has no clear interpretation when comparing overlapping small subsets of the population), and (iv) an indication of the accuracy of polygenic risk prediction. Furthermore, the cosine of the angle between the lines A1-A0 and B1-B0 is equal to the genetic correlation between disorder A and disorder B (see Methods). Figure S1 and Table S1 reports genetic distances across all eight psychiatric disorders, and for the autoimmune disorders (Crohn's disorder (CD)[24], ulcerative colitis (UC)[24] and rheumatoid arthritis (RA)[25]; analysed below to help validate the CC-GWAS method).

Simulations

We assessed the power and type I error of CC-GWAS using both simulations with individual-level data and analytical computations (see Methods). We compared four methods: CC-GWAS; the OLS component of CC-GWAS; the Exact component of CC-GWAS; and a naïve method that uses weight +1 for A1A0 and −1 for B1B0 (Delta method). All four methods are unpublished; we are not currently aware of any published method for performing case-case GWAS using case-control summary statistics (see Discussion). We assessed (i) power to detect causal SNPs with case-control effect sizes for both disorders drawn from a bivariate normal distribution (allele frequencies A0≠A1, B0≠B1, A1≠B1); (ii) type I error for "null-null" SNPs, defined as SNPs with no effect on either disorder (A0=A1, B0=B1, A1=B1); and (iii) type I error for "stress test" SNPs, defined as SNPs with A0≠A1, B0≠B1, A1=B1 (see above). Default parameter settings were loosely based on the genetic architectures of SCZ and MDD with liability-scale h^2=0.2, prevalence K=0.01, and sample size 100,000 cases + 100,000 controls for disorder A; liability-scale h^2=0.1, prevalence K=0.15, and sample size 100,000 cases + 100,000 controls for disorder B; genetic correlation r_g=0.5 between disorders; and m=5,000 causal SNPs affecting both

disorders with causal effect sizes following a bivariate normal distribution. For these parameter settings, the weights are 0.86 for A1A0 and –0.55 for B1B0 for the OLS approach, and 0.99 and –0.85 respectively for the Exact approach. The OLS approach assigns relatively more weight to A1A0 (0.86/0.55=1.56) than the Exact approach (0.99/0.85=1.16), because of the larger heritability and lower prevalence of A1A0 (implying higher signal to noise ratio at the same case-control sample size). The OLS-weights are shrunk in comparison to the Exact weights, accounting for the imperfect signal to noise ratio at finite sample size (however, CC-GWAS *p*-values are insensitive to rescaling the weights at a fixed ratio). Stress test SNPs were set to explain 0.10% of liability-scale variance in A and 0.29% of liability-scale variance in B (resulting in allele frequency A1=B1). Other parameter settings were also explored.

Results of analytical computations are reported in Figure 2 and Table S2; simulations with individual-level data produced identical results (Table S3), thus we focus primarily on results of analytical computations. We reached three main conclusions. First, CC-GWAS attains similar power as the OLS method, higher power than the Exact method, and much higher power than the Delta method (Figure 2A); we note that this is a best-case scenario for CC-GWAS, as the simulated bivariate genetic architecture follows the CC-GWAS assumptions. As expected, power increases with increasing sample size and decreases with increasing genetic correlation. The power of CC-GWAS to detect case-case differences lies in between the power of the input A1A0 and B1B0 summary statistics to detect case-control differences (Figure S2). Second, all methods perfectly control type I error at null-null SNPs (Figure 2B). Third, although the OLS method has a severe type I error problem at stress test SNPs (particularly when the genetic correlation is large), CC-GWAS attains effective control of type I error at stress test SNPs (type I error rate < 10^{-4}; Figure 2C), an extreme category of SNPs that is likely to occur rarely in empirical data. Notably, with increasing sample size the OLS weights converge towards the Exact weights (Figure S3), resulting in decreasing type I error rate for stress test SNPs. In conclusion, CC-GWAS balances the high power of the OLS method with effective control of type I error.

We performed seven secondary analyses, yielding the following conclusions. First, results were similar when varying K_B, $h^2_{l,B}$ and m (Figure S4); in particular, results were similar when increasing from *m*=5,000 causal SNPs to *m*=10,000 causal SNPs (Figure S5; we used *m*=5,000 causal SNPs in our main assessment because this setting corresponds to higher absolute power). Second, sample overlap in controls increases power, as this provides a more direct case-case comparisons (Figure S6). Third, the type I error rate of CC-GWAS is slightly less 1 in 10,000 for stress test SNPs explaining a large proportion of case-control variance (0.29% for disorder B in Figure 2C), but is much smaller for stress test SNPs explaining less variance (Figure S7). Fourth, when employing a more stringent *p*-value threshold for the Exact component of CC-GWAS than the default threshold of 10^{-4}, both the power for causal SNPs and the type I error rate for stress test SNPs decrease (Figure S8). We believe that the default threshold of 10^{-4} provides sufficient protection against type I error of stress test SNPs, which cannot be numerous (e.g. 100 independent stress test SNPs as defined in Figure 2C would explain 29% of liability-scale variance in disorder B). Fifth, we assessed CC-GWAS using the type S error rate, defined as the proportion of significantly identified loci (true positives) identified with the wrong sign[26,27]; this is an appealing metric for CC-GWAS, whose complexity precludes metrics based on distributions of test statistics. We determined that the type S error rate was negligible: less than 1 in 1,000,000 significantly identified variants for all parameter settings of Figure 2A (Table S4). Sixth, a direct case-case comparison (which requires individual-level data) may be more powerful than CC-GWAS when a great majority of cases can be included (Figure S9). Seventh, when case-case GWAS results are available, a method incorporating these results can be applied to further increase power (CC-GWAS+; Figure S10).

CC-GWAS identifies 116 loci with different allele frequencies among cases of SCZ, BIP and MDD

We applied CC-GWAS to publicly available summary statistics for SCZ,[15] BIP[16] and MDD[17] (Table 1; see URLs). To run CC-GWAS, we assumed 10,000 independent causal SNPs for each psychiatric disorder[28]. The underlying OLS weights and Exact weights used by CC-GWAS are reported in Table 1, along with

the disorder-specific parameters used to derive these weights. We defined *independent* genome-wide significant CC-GWAS loci by clumping correlated SNPs ($r^2 \geq 0.1$) in 3MB windows and collapsing remaining SNPs within 250kb[15] (Table S5). We defined *CC-GWAS-specific* loci as loci for which none of the genome-wide significant SNPs had $r^2 > 0.8$ with any of the genome-wide significant SNPs in the input case-control GWAS results (Table S5). We note that this definition of CC-GWAS-specific loci may include loci previously reported as genome-wide significant in analyses of other case-control data sets (see below). We further note that CC-GWAS loci that are not CC-GWAS-specific also contribute to our understanding of differences between different disorders.

For each pair of SCZ, BIP and MDD, the total number of independent CC-GWAS loci and number of independent CC-GWAS-specific loci are reported in Table 1. The CC-GWAS analysis identified 121 loci, summed across pairs of disorders: 12 for SCZ vs. BIP, 99 for SCZ vs. MDD, and 10 for BIP vs. MDD. CC-GWAS loci were considered shared between two pairs of disorders when at least one pair of genome-wide significant SNPs for the respective pairs of disorders had $r^2 > 0.8$ (Table S5). Thus, 4 of the CC-GWAS loci were shared between SCZ vs. BIP and SCZ vs. MDD and 1 was shared between SCZ vs. MDD and BIP vs. MDD, resulting in 116 independent CC-GWAS loci. 5 of the 12 SCZ vs. BIP loci were also significant in the SCZ case-control comparison while none were significant in the BIP case-control comparison (consistent with the larger SCZ case-control sample size); 89 of the 99 SCZ vs. MDD loci were also significant in SCZ case-control comparison while only 1 of those was also significant in the MDD case-control comparison (consistent with the relative genetic distances in Figure 1C); and 5 of the 10 BIP vs. MDD loci were also significant in the BIP case-control comparison while only 1 was significant in the MDD case-control comparison. The remaining 21 (7+10+4) loci were CC-GWAS-specific (and independent from each other); 8 of these loci have not been reported previously (conservatively defined as: no SNP in 1000 Genomes[29] with $r^2 > 0.8$ with a genome-wide significant CC-GWAS SNP in the locus reported for any trait in the NHGRI GWAS Catalog[30]; Table S5). Notably, the Exact approach did not filter out any variants identified using the OLS approach, i.e. all

SNPs with $P<5x10^{-8}$ using OLS weights also had $P<10^{-4}$ using Exact weights, because the OLS weights were relatively balanced.

For each CC-GWAS locus, the respective input case-control effect sizes for each disorder are reported in Figure 3 and Table S6. Details of the 21 CC-GWAS-specific loci are reported in Table 2, and details of the remaining 100 CC-GWAS loci are reported in Table S6 (the locus names reported in these tables incorporate results of our SMR analysis[31]; see below). For all 21 CC-GWAS-specific loci, the input case-control effect sizes were non-significant with opposing signs. For CC-GWAS-specific SCZ vs. BIP loci, the input case-control effect sizes had comparable magnitudes for SCZ and BIP, reflecting their similar SNP-heritabilities and prevalences (case-control effect sizes are on the standardized observed scale based on 50/50 case-control ascertainment). For CC-GWAS-specific SCZ vs. MDD and BIP vs. MDD loci, the input case-control effect sizes were smaller for MDD due to its lower SNP-heritability and higher prevalence, implying much lower observed-scale SNP-heritability and $F_{ST,causal}$ (Figure 1). For the remaining 100 loci, 4 of 5 had opposing case-control association signs in the respective input GWAS results for SCZ vs. BIP, 43 of 89 had opposing signs for SCZ vs. MDD, and 4 of 6 had opposing signs for BIP vs. MDD.

We performed five secondary analyses, yielding the following conclusions. First, when we employed a more stringent *p*-value threshold for the Exact component of CC-GWAS than the default threshold of 10^{-4}, significant loci were removed only when a threshold of 10^{-6} or below was employed (Table S7). Second, results were similar when applying a different clumping strategy to define independent loci (Table S8). Third, when conservatively correcting input summary statistics for their stratified LD score regression (S-LDSC) intercept[32–35] (similar to Turley et al.[6]), the number of significant CC-GWAS independent loci decreased (e.g. from 99 to 75 for SCZ vs. MDD; Table S9). However, we believe this correction is overly conservative, as S-LDSC intercept attenuation ratios[36] were relatively small, implying little evidence of confounding (Table S10); we note that any confounding, if present, would have a similar impact on CC-GWAS results as on the confounded input summary statistics (when applying no correction for S-LDSC intercept) (see Methods and Table S11). Fourth, results were little

changed when estimating heritabilities provided as input to CC-GWAS using LD score regression (LDSC)[32] instead of S-LDSC[33–35], despite systematically lower heritability estimates (Table S12). Fifth, when changing the number of causal SNPs m to 5,000 (resp. 20,000), slightly fewer (resp. slightly more) loci were detected (Table S13). Sixth, we extended the SCZ vs. BIP analysis by applying CC-GWAS+ (see above) to incorporate case-case summary statistics[4], but this did not increase the number of independent CC-GWAS-specific loci (Table S14).

CC-GWAS-specific loci implicate known and novel disorder genes

We used two approaches to link the 21 CC-GWAS-specific loci to genes (Table 2). First, we linked exonic lead SNPs to the corresponding genes. Second, we used the SMR test for colocalization[31] (see URLs) to identify CC-GWAS loci with significant associations between gene expression effect sizes in *cis* across 14 tissues (13 GTEx v7 brain tissues[37] and a meta-analysis of eQTL effects in brain tissues[38]; see URLs) and OLS case-case effect sizes (P<0.05 divided by the number of probes tested for each pair of disorders; see Methods), and used the HEIDI test for heterogeneity[31] to exclude loci with evidence of linkage effects (P<0.05) (see Methods and Table S15). Below, we highlight 4 CC-GWAS-specific loci from Table 2, representing both known and novel findings.

The CC-GWAS-specific SCZ vs. MDD CC-GWAS locus defined by lead SNP rs2563297 (chr5:140,097,072) produced significant SMR colocalization results for 11 gene-tissue pairs representing 7 unique genes (Table S15). The 7 unique genes included 5 protocadherin alpha (*PCDHA*) genes, which play a critical role in the establishment and function of specific cell-cell connections in the brain[39], and the *NDUFA2* gene, which has been associated with Leigh syndrome (an early-onset progressive neurodegenerative disorder)[40]. Significant CC-GWAS SNPs in this locus have previously been associated to schizophrenia[41–44] (in data sets distinct from our input schizophrenia GWAS[15], in which this locus was not significant due to sampling variance and/or ancestry differences), depressive symptoms[6], neuroticism[45], educational attainment[44,46], intelligence[47], blood pressure[48,49], and a meta-analyses of schizophrenia, education and cognition[44], implying that this is a highly pleiotropic locus.

This implies that CC-GWAS can increase power to detect associated loci in the input case-control GWAS data sets analyzed here.

The CC-GWAS-specific SCZ vs. MDD locus defined by lead SNP rs2944833 (chr7:71,774,496) produced a significant SMR colocalization result for one gene-tissue pair, involving the *CALN1* gene in meta-analyzed brain eQTL[38] (Table S15). *CALN1* plays a role in the physiology of neurons and is potentially important in memory and learning[50]. Indeed, SNPs in this locus have previously been associated to educational attainment[46,51], intelligence[47,52], cognitive function[53], and a meta-analysis of schizophrenia, education and cognition[44]. Again, this implies that CC-GWAS can increase power to detect associated loci in the input case-control GWAS data sets analyzed here.

Finally, two distinct CC-GWAS-specific loci implicated genes in the Kruppel-like family of transcription factors. The CC-GWAS-specific SCZ vs. BIP locus defined by lead SNP rs1054972 (chr19:1,852,582) lies within an exon of *KLF16*, and the CC-GWAS-specific SCZ vs. MDD locus defined by lead SNP rs17731 (chr10:3,821,561) lies within an exon of *KLF6*. The respective case-control effect sizes suggest that rs1054972 and rs17731 both have an impact on SCZ, but have not yet reached significance in the respective case-control analyses (P=1.3e–5 and P=2.9e–7 respectively; Table 2 and Table S16). *KLF16* and *KLF6* play a role in DNA-binding transcription factor activity and in neurite outgrowth and axon regeneration[54], and we hypothesize they may play a role in the previously described schizophrenia pathomechanism of synaptic pruning[55]. Furthermore, the *KLF5* gene from the same gene family has previously been reported to be downregulated in post-mortem brains of schizophrenia patients[56]. At the time of our analyses, *KLF16* and *KLF6* had not previously been associated to schizophrenia; *KLF6* has very recently been associated to schizophrenia in a meta-analysis of East Asian and European populations[43], but *KLF16* has still not been associated to schizophrenia. This implies that CC-GWAS can identify novel disorder genes.

CC-GWAS identifies 200 loci distinguishing cases of eight psychiatric disorders

We applied CC-GWAS to all 28 pairs of eight psychiatric disorders by analysing ADHD[18], ANO[19], ASD[20], OCD[21], and TS[22] in addition to SCZ[15], BIP[16] and MDD[17] (Table 3; see URLs). To run GG-CWAS, we assumed 10,000 independent causal SNPs for each psychiatric disorder[28]. The underlying OLS weights and Exact weights used by CC-GWAS are reported in Table S7.

For each pair of psychiatric disorders, the total number of independent CC-GWAS loci and number of independent CC-GWAS-specific loci are reported in Table 4. The CC-GWAS analysis identified 321 loci, summed across pairs of disorders (0 to 99 loci per pair of disorders). Many of the loci were shared between pairs of disorders, resulting in 200 independent loci; in particular, 49 SCZ case-control loci were shared across 10 pairs of disorders (SCZ and one other disorder), explaining 88 of the 121 overlapping loci. 126 of the 200 loci were also significant in one (or both) of the two input case-control comparisons. The remaining 74 loci were CC-GWAS-specific; 33 (45%) of these loci have not previously been reported in the NHGRI GWAS catalog[30]. The proportion of independent loci that were CC-GWAS-specific (74/200) was larger than in our above analysis of SCZ, BIP and MDD (21/116), but the proportions were more similar when summing across pairs of disorders (85/321 and 21/121, respectively); the difference between 74/200 and 85/321 reflects the fact that CC-GWAS-specific loci are less likely to be shared between pairs of disorders. Notably, the Exact approach filtered out variants identified using the OLS approach for three pairs of disorders: from 9 to 1 for SCZ vs. OCD, from 30 to 19 for SCZ vs. TS, and from 3 to 2 for ADHD vs. OCD, a consequence of highly imbalanced OLS weights for these specific pairs of disorders due to differences in sample sizes of the input case-control GWAS (Table S7). However, the Exact approach did not filter out any variants identified using the OLS approach for the remaining 25 pairs of disorders.

For each CC-GWAS locus, the respective input case-control effect sizes for each disorder are reported in Figure S11 and Table S6. Details of the 74 CC-GWAS-specific loci and of the remaining 126 CC-GWAS are reported in Table S6. For all 74 CC-GWAS-specific loci, the input case-control effect sizes were non-significant with opposing signs. For the remaining 126 CC-GWAS loci, 51 had opposite case-

control association signs in the respective input GWAS results. Results of SMR analyses[31] of the CC-GWAS-specific loci are reported in Table S15.

CC-GWAS loci replicate in independent data sets

We investigated whether case-case associations identified by CC-GWAS replicate in independent data sets. Of the eight psychiatric disorders, only SCZ and MDD had sufficient sample size to perform replication analyses of the SCZ vs. MDD results based on publicly available GWAS results of subsets of the data[14,57]. The other psychiatric disorders had much lower sample sizes (Table 3), precluding replication efforts based on subset data. For SCZ vs. MDD, we applied CC-GWAS to publicly available summary statistics for subsets of the SCZ data[14] and MDD data[57] (discovery data; Table S17; see URLs). We replicated these findings using independent summary statistics constructed by subtracting these summary statistics from the full SCZ data[15] and full MDD data[17] using MetaSubtract[58] (replication data; Table S17). If the discovery data are not exact subsets of the full data, we anticipate that replication results would be conservative, as independent signals from the discovery data would be subtracted from the full data when producing the replication data. To further validate the CC-GWAS method, we also analysed three case-case comparisons of three autoimmune disorders with publicly available GWAS results for independent discovery and replication data sets with substantial sample sizes (Crohn's disorder (CD)[24], ulcerative colitis (UC)[24] and rheumatoid arthritis (RA)[25]; Table S17; see URLs); we assumed $m = 1{,}000$ independent causal SNPs for each autoimmune disorder[28]. The genetic distances between the autoimmune disorders are displayed in Figure S1; the OLS weights, Exact weights and number of CC-GWAS loci and CC-GWAS-specific loci are reported in Table S17; and details of the CC-GWAS loci are reported in Table S18. We replicated the results of the CC-GWAS analysis of the autoimmune disorders (discovery data[24,25]) using independent Immunochip replication data[24,25], which was available for 63 loci (Table S17).

Results for these four pairs of disorders are reported in Figure 4, Table S17 and Table S18. For SCZ vs. MDD, the CC-GWAS discovery analysis identified 58 independent loci (less than the 99

independent loci in Table 1, due to smaller sample size), of which 54 (93%) had the same effect sign in the CC-GWAS replication analysis and 29 (50%) had same sign and P_{OLS}<0.05. The power of the replication sample for SCZ vs. MDD was considerable smaller than of the discovery sample (effect size SE 2 times larger, corresponding to 4 times smaller effective sample size). The replication slope (based on a regression of replication vs. discovery effect sizes[59]) was equal to 0.57 (SE 0.06) (Figure 4A), which was comparable to the replication slopes for SCZ case-control (0.62, SE 0.05) and MDD case-control (0.60, SE 0.11) genome-wide significant loci using the same discovery and replication data sets (Figure S12, Table S17 and Table S19); we hypothesize that all slopes were smaller than 1 owing to within-disorder heterogeneity[1]. For the autoimmune disorders, 63 independent CC-GWAS loci were available for replication, of which 63 (100%) had the same effect sign in the CC-GWAS replication analysis and 59 (94%) had same sign and P_{OLS}<0.05. For the autoimmune disorders, power for discovery and replication were similar (similar effect size SE and effective sample size). The replication slope for the three autoimmune disorders was equal to 0.83 (SE 0.03) (Figure 4B), comparable to the corresponding case-control replication slopes (Figure S12). We further investigated the replication of the subset of 22 CC-GWAS-specific loci (9 for SCZ vs. MDD and 13 for the 3 autoimmune disorders), pooling the 4 replication studies to overcome the limited number of CC-GWAS-specific loci. We obtained a replication slope of 0.70 (SE 0.07) for the 22 CC-GWAS-specific loci (Figure 4C), which was borderline significantly different (P=0.07) from the slope of 0.83 (0.02) for the 99 remaining loci (Figure 4D); we note that CC-GWAS-specific loci had smaller case-case effect sizes and are thus expected to be more susceptible to winner's curse[60] (and to attain a lower replication slope). We conclude that case-case associations identified by CC-GWAS replicate convincingly in independent data sets.

Discussion

We identified 200 independent loci with different allele frequencies among cases of eight psychiatric disorders by applying our CC-GWAS method to the respective case-control GWAS summary statistics. 116 of these loci had different allele frequencies among cases of the mood and psychotic disorders[3]

(SCZ, BIP and MDD). 74 of the 200 loci were CC-GWAS-specific, highlighting the potential of CC-GWAS to produce new biological insights. In particular, the lead SNPs of two distinct loci were located in exons of *KLF6* and *KLF16*, which have been linked to neurite outgrowth and axon regeneration[54]; we hypothesize that these genes may be involved in the role of synaptic pruning in SCZ[55]. We confirmed the robustness of CC-GWAS via simulations, analytical computations, and independent replication of empirical CC-GWAS results.

Although there exist other methods to combine GWAS results from two disorders or complex traits[3,6–13], CC-GWAS is the first method to compare cases of two disorders based on the respective case-control GWAS summary statistics – and also the first method to compute the genetic distance between cases and/or controls of two disorders (using $F_{ST,causal}$). For example, the methods of refs.[6–8] combine GWAS results of correlated disorders or traits to increase power; these methods can be applied to increase the power of case-control analyses, but not to perform case-case analyses. Our CC-GWAS-specific loci are inherently different from the loci identified by those methods, because CC-GWAS computes a weighted difference while those methods compute a weighted sum of the respective case-control GWAS results. The GWIS method[9] provides a general framework to derive GWAS results from a function of phenotypes, but does not naturally extend to case-case comparisons. The ASSET method[10] conducts subset-based meta-analyses to increase power and explore subsets of studies for effects that are in the same or possibly opposite directions; CC-GWAS differs from ASSET[10] by applying weights based on the genetic distance between cases and controls to specifically test difference in allele frequency among cases of two disorders. The method of ref.[11] investigates results from meta-analysis and provides a statistic representing the posterior predicted probability of association (*m-value*) for each study included in the meta-analysis. The method of ref.[11] can identify SNPs with predicted disorder-specific effects (i.e. SNPs with a large *m*-value for one disorder and low *m*-value for another disorder): these SNPs are expected to have different allele frequencies among cases, but CC-GWAS models the case-case comparison more directly while explicitly controlling for potential false positive detection of stress test SNPs and providing a formal test of significance. In

recent work from the Cross-Disorder Group of the Psychiatric Genomics Consortium[3], the same eight psychiatric disorders were analyzed with the ASSET method[10] and the method of ref.[11] yielding (i) 146 independent genome-wide loci based on the ASSET method[10], including 109 pleiotropic loci with *m*-values[11] larger than 0.9 for more than one disorder, and (ii) 11 loci with opposing directions of effect across disorders based on analyses of ASSET-loci with suggestive significance ($P<10^{-6}$) with subsequent false discovery rate (*FDR*) correction in *both* of the respective disorders. The 146 ASSET-loci analyzed in ref.[3] overlapped with 58/200 (29%) of CC-GWAS loci and 5/74 (7%) of CC-GWAS-specific loci (Table S6), confirming that CC-GWAS is different from ASSET (we note that CC-GWAS analyses were based on different input GWAS results for ANO, MDD and SCZ). The 11 loci with opposing effects from ref.[3] are expected to have different allele frequencies among cases, but CC-GWAS is more inclusive as it does not require controlling *FDR* in both of the respective case-control GWAS results and because loci with similar direction of case-control effects can still have different allele frequency among cases of both disorders. Of the 11 loci with opposing effects[3], 8 overlapped with CC-GWAS loci for the same set of disorders, confirming that CC-GWAS detects these loci as expected while being more inclusive. The *mtCOJO* method[12] estimates genetic effects conditional on other traits. Some mtCOJO loci and CC-GWAS loci may overlap, but CC-GWAS addresses a conceptually different question than conditional analyses. In ref.[13], *mtCOJO*[12] is applied on case-control GWAS results of SCZ, BIP, MDD, ADHD and ASD to identify putative disorder-specific SNPs by correcting GWAS results of one disorder for the causal relationships with the four other disorders. Of the 162 CC-GWAS loci detected in comparisons of these five disorders, 102/162 (63%) of CC-GWAS loci and 8/50 (16%) of CC-GWAS-specific loci overlapped with loci from ref.[13], confirming that CC-GWAS is different from *mtCOJO*[12] (we note that CC-GWAS analyses were based on different GWAS results for MDD only). In summary, although some of these previous methods[3,6–13] identify loci that are expected to have different allele frequencies among cases, none of these methods explicitly compares the allele frequency among cases of different disorders or explicitly controls for potential false positives at stress test SNPs. Indeed, the most natural method to compare CC-GWAS to is a case-case GWAS based on individual-level data, as performed in ref.[4] for

SCZ vs. BIP based on individual level data from ref.[14] and ref.[16] respectively. CC-GWAS identified 12 SCZ vs. BIP loci (or 10 when applied to data from ref.[14] and ref.[16], as in ref.[4]) compared to 2 SCZ vs. BIP loci in ref.[4], which discarded ~25% of the cases compared to the respective case-control data (owing to non-matching ancestry and genotyping platform); we note that CC-GWAS is much less sensitive to subtle differences in ancestry and genotyping platform (Table S20) because the case-case comparison accounts for the allele frequency in matched controls by comparing case-control effects.

The CC-GWAS method has several limitations. First, the OLS case-case effect size estimates depend on the sample sizes of the input case-control GWAS summary statistics, because the OLS weights depend on these sample sizes. This bias-variance tradeoff can be avoided by using Exact effect size estimates (which are independent of sample size), e.g. in genetic correlation analyses[2]. Second, the choice of the threshold for the Exact *p*-values in CC-GWAS is somewhat arbitrary, but we believe 10^{-4} is a reasonable choice as it (i) effectively protects against false positives due to stress test SNPs (Figure 2 and Figure S7), which cannot be numerous (e.g. 100 independent stress test SNPs as defined in Figure 2C would explain 29% of liability-scale variance in disorder B), and (ii) has only limited impact on the power of CC-GWAS (Figure 2); other choices of this threshold produced identical results in most of our empirical analyses (25 of 28 pairs of disorders; Table S7). Third, for significant CC-GWAS-specific loci ($P<5\times10^{-8}$) with input case-control *p*-values > 5×10^{-8}, CC-GWAS does not provide a formal assessment of which case-control effect(s) the locus is associated to. However, the case-control *p*-values at the locus can provide suggestive evidence. In some instances (e.g. SCZ vs. MDD), CC-GWAS-specific loci are likely to largely derive from one of the disorders (SCZ) due to differential power (Figure 1 and Table 2), but this does not limit the value of identifying novel loci distinguishing these disorders. Fourth, we have analyzed 28 pairs of disorders using the significance threshold of 5×10^{-8}, which may raise concerns about multiple testing. However, we note that the threshold of 5×10^{-8} is much more conservative than false discovery rate[61] (FDR) approaches using either a per-pair of disorders FDR of 0.05 or a global FDR of 0.05 (Table S21), and that our use of the 5×10^{-8} threshold is analogous to the use of this threshold in GWAS studies that analyze many traits (e.g. 58 traits in ref.[62]). Fifth, when

comparing cases of more than two disorders, CC-GWAS must be applied in pairwise fashion. Extending CC-GWAS to more than two disorders is a direction for future research. Sixth, CC-GWAS could in principle be susceptible to confounding due to subtle ancestry differences between the two input case-control GWAS. However, for null-null SNPs, we verified that type I error rate is not inflated because there are no case-control allele frequency differences (other than due to sampling variance) in the input case-control GWAS (Table S20). For stress-test SNPs (which cannot be numerous; see above), there is a somewhat increased risk of false positives if the allele frequency difference between populations is on the order of 0.20 for a common SNP (which is typical for differences between continental populations[23]), but no increased risk of false positives if the allele frequency differences between populations is on the order of 0.05 for a common SNP (which is typical of differences within a continental population[63]) (Table S20); although SNPs can be highly differentiated within a continental population in rare instances[63], the existence of a SNP that is both a stress-test SNP and highly differentiated within a continental population is very unlikely. Seventh, CC-GWAS was designed to compare two disorders (with different definitions of controls and potential overlap of cases), but it is also of interest to compare subtypes within a disorder[64] (with same definitions of controls and no overlap of cases). However, we confirmed via simulation that CC-GWAS can be applied to subtypes (replacing the Exact approach with the Delta method; Table S22).

In conclusion, we have shown that CC-GWAS can reliably identify loci with different allele frequencies among cases (including both case-control loci and CC-GWAS-specific loci), providing novel biological insights into the genetic differences between cases of eight psychiatric disorders. Thus, CC-GWAS helps promote the ambitious but important goal of better clinical diagnoses and more disorder-specific treatment of psychiatric disorders.

URLs

CC-GWAS software: https://github.com/wouterpeyrot/CC-GWAS;

CC-GWAS results for 8 psychiatric disorders: https://data.broadinstitute.org/alkesgroup/CC-GWAS/;

LDSC software: https://github.com/bulik/ldsc;

SMR software: https://cnsgenomics.com/software/smr/;

PLINK1.9 software: www.cog-genomics.org/plink/1.9/;

GWAS results for ADHD, ANO, ASD, BIP, BIP vs. SCZ, MDD (Wray 2018), OCD, SCZ (Ripke 2014), and TS: https://www.med.unc.edu/pgc/results-and-downloads/;

GWAS results for MDD (Howard 2019): https://datashare.is.ed.ac.uk/handle/10283/3203;

GWAS results for SCZ (Pardinas 2018): https://walters.psycm.cf.ac.uk/;

GWAS results for CD and UC: https://www.ibdgenetics.org/downloads.html;

GWAS results for RA: http://www.sg.med.osaka-u.ac.jp/tools.html;

eQTL data of 13 GTEx v7 brain tissues and meta-analysis of eQTL effects in brain tissues: https://cnsgenomics.com/software/smr/#DataResource.

Methods

Quantifying genetic distances between cases and/or controls of each disorder

We derive $F_{ST,causal}$ for the comparisons $A1A0$, $B1B0$, $A1B1$, $A1B0$, $A0B1$, $A0B0$ as follows. Consider two disorders A and B with lifetime prevalences K_A and K_B, liability-scale heritabilities h^2_{lA} and h^2_{lB}, and genetic correlation r_g. Assume the heritabilities and genetic correlation have been assessed on data of m independent SNPs, and assume these SNPs impact both traits with effects following a bivariate normal distribution. First, the heritabilities are transposed to the observed scales, h^2_{oA} and h^2_{oB}, with proportions of cases of 0.5 in line with refs.[65,66]

$$h^2_o = h^2_l \frac{0.5^2(1-0.5)^2 z^2}{K^2(1-K)^2} \qquad \text{Eq 1}$$

where z is the height of the standard normal distribution at threshold T defined as $K = P(x > T \mid x \sim N(0,1))$. The coheritability is also expressed on this scale as: $coh_{oA,oB} = r_g\sqrt{h^2_{oA}\, h^2_{oB}}$. The average variance explained per SNP in A is h^2_{oA}/m and in B h^2_{oB}/m, and the average genetic covariance per SNP is $coh_{oA,oB}/m$. For SNP i, the allele frequencies of the reference allele in cases and controls are represented as $p_{i,A1}$, $p_{i,A0}$, $p_{i,B1}$, and $p_{i,B0}$.

Throughout the paper, the effect-sizes β are on the standardized observed scale (i.e. with standardized genotype and standardized phenotype) based on 50/50 case-control ascertainment. When assuming Hardy-Weinberg equilibrium and assuming small effect sizes (typical for polygenic disorders), the β of linear regression of standardized case-control status $A1A0$ on standardized genotype G_i can be approximated in terms of allele frequencies as

$$\beta_{i,A1A0} = cov(s(A), s(G_i)) \approx \frac{E[s(A)G_i]}{\sqrt{2p_i(1-p_i)}} \approx \frac{p_{i,A1}-p_{i,A0}}{\sqrt{2p_i(1-p_i)}} \qquad \text{Eq 2}$$

Simulations confirm that these approximations are justified for loci with $0.5 < OR < 2$ (Table S23). For comparison, all estimated effect sizes for SCZ[15], BIP[16] and MDD[17] have $0.7 < OR < 1.4$.

The derivations of $F_{ST,causal,A1A0}$ and $F_{ST,causal,B1B0}$ follow from Eq 2. On the standardized scale the variance explained equals the square of the beta. Because the loci are assumed independent, the average variance explained gives

$$F_{ST,causal,A1A0} = \frac{E[(p_{i,A1}-p_{i,A0})^2]}{E[2p_i(1-p_i)]} \approx E\left[\frac{(p_{i,A1}-p_{i,A0})^2}{2p_i(1-p_i)}\right] \approx \frac{h^2_{oA}}{m}$$

$$F_{ST,causal,B1B0} \approx \frac{h^2_{oB}}{m} \qquad \text{Eq 3}$$

The first approximations has been proposed[23] to obtain stable estimates of F_{ST} and assumes the standardized SNP effects are equally distributed across the allele frequency spectrum.

Deriving $F_{ST,causal}$ for the comparisons $A1B1$, $A1B0$, $A0B1$ and $A0B0$ requires some additional steps. First note the covariance of $\beta_{i,A1A0}$ and $\beta_{i,B1B0}$ equals

$$\frac{E[(p_{i,A1}-p_{i,A0})(p_{i,B1}-p_{i,B0})]}{E[2p_i(1-p_i)]} \approx \frac{coh_{oA,oB}}{m} \qquad \text{Eq 4}$$

Second, note that allele frequencies and difference in allele frequencies can be rewritten as

$$\begin{aligned}
&p_{i,A1} = \{p_i - (1-K_A)p_{i,A0}\}/K_A \\
&p_{i,A0} = \{p_i - K_A p_{i,A1}\}/(1-K_A) \\
&p_{i,A1} - p_{i,A0} = p_{i,A1} - \{p_i - K_A p_{i,A1}\}/(1-K_A) = \{p_{i,A1} - p_i\}/(1-K_A) \\
&p_{i,A1} - p_{i,A0} = \{p_i - (1-K_A)p_{i,A0}\}/K_A - p_{i,A0} = \{p_i - p_{i,A0}\}/K_A \qquad \text{Eq 5}
\end{aligned}$$

Substituting Eq 5 in Eq 3 and Eq 4, gives

$$\begin{aligned}
&E\left[(p_{i,A1}-p_i)^2\right] \approx \frac{h_{oA}^2}{m}\,(1-K_A)^2 E[2p_i(1-p_i)] \\
&E\left[(p_{i,A0}-p_i)^2\right] \approx (-1^2)\frac{h_{oA}^2}{m}\,{K_A}^2 E[2p_i(1-p_i)] \\
&E\left[(p_{i,B1}-p_i)^2\right] \approx \frac{h_{oB}^2}{m}\,(1-K_B)^2 E[2p_i(1-p_i)] \\
&E\left[(p_{i,B0}-p_i)^2\right] \approx (-1^2)\frac{h_{oA}^2}{m}\,{K_B}^2 E[2p_i(1-p_i)] \\
&E\left[(p_{i,A1}-p_i)(p_{i,B1}-p_i)\right] \approx \frac{coh_{oA,oB}}{m}\,(1-K_A)(1-K_B)E[2p_i(1-p_i)] \\
&E\left[(p_{i,A1}-p_i)(p_{i,B0}-p_i)\right] \approx -1 * \frac{coh_{oA,oB}}{m}\,(1-K_A)K_B E[2p_i(1-p_i)] \\
&E\left[(p_{i,A0}-p_i)(p_{i,B1}-p_i)\right] \approx -1 * \frac{coh_{oA,oB}}{m}\,K_A(1-K_B)E[2p_i(1-p_i)] \\
&E\left[(p_{i,A0}-p_i)(p_{i,B0}-p_i)\right] \approx -1 * -1 * \frac{coh_{oA,oB}}{m}\,K_A K_B E[2p_i(1-p_i)] \\
&E\left[(p_{i,A1}-p_i)(p_{i,A0}-p_i)\right] \approx -1 * \frac{h_{oA}^2}{m}(1-K_A)K_A E[2p_i(1-p_i)] \\
&E\left[(p_{i,B1}-p_i)(p_{i,B0}-p_i)\right] \approx -1 * \frac{h_{oB}^2}{m}(1-K_B)K_B E[2p_i(1-p_i)] \qquad \text{Eq 6}
\end{aligned}$$

While noting that that the $F_{ST,causal,XY}$ is estimated as

$$F_{ST,causal,XY} = \frac{E[(p_{i,X}-p_{i,Y})^2]}{E[2p_i(1-p_i)]} = \frac{E[((p_{i,X}-p_i)-(p_{i,Y}-p_i))^2]}{E[2p_i(1-p_i)]} = \frac{E[(p_{i,X}-p_i)^2]-2E[(p_{i,X}-p_i)(p_{i,Y}-p_i)]+E[(p_{i,Y}-p_i)^2]}{E[2p_i(1-p_i)]}, \quad \text{Eq 7}$$

we find

$$\begin{aligned}
F_{ST,causal,A1B1} &\approx \frac{h_{oA}^2}{m}(1-K_A)^2 - 2\frac{coh_{oA,oB}}{m}(1-K_A)(1-K_B) + \frac{h_{oB}^2}{m}(1-K_B)^2 \\
F_{ST,causal,A1B0} &\approx \frac{h_{oA}^2}{m}(1-K_A)^2 + 2\frac{coh_{oA,oB}}{m}(1-K_A)K_B + \frac{h_{oB}^2}{m}{K_B}^2 \\
F_{ST,causal,A0B1} &\approx \frac{h_{oA}^2}{m}{K_A}^2 + 2\frac{coh_{oA,oB}}{m}K_A(1-K_B) + \frac{h_{oB}^2}{m}(1-K_B)^2 \\
F_{ST,causal,A0B0} &\approx \frac{h_{oA}^2}{m}{K_A}^2 - 2\frac{coh_{oA,oB}}{m}K_AK_B + \frac{h_{oB}^2}{m}{K_B}^2 \qquad \text{Eq 8}
\end{aligned}$$

These approximations of $F_{ST,causal}$ were confirmed with simulations (Table S24). (CC-GWAS depends on the assumption that all m SNPs have an impact on both traits and that effects sizes follow a bivariate normal distribution (see below). However, we note that the equations of $F_{ST,causal}$ require less stringent assumptions. To illustrate, the equations also hold when simulating data (see below) of 1,000 independent SNPs of which 500 have no impact on either disorder, 154 have uniform and similar sized effects on both disorders, 282 have uniform effects on disorder A only, and 64 have uniform effects on disorder B only (Table S24 Panel B).)

We now proceed with using $F_{ST,causal}$ to display $A1$, $A0$, $B1$ and $B0$ in a 2-dimensional plot (Figure 1 and Figure S1). Because the loci are assumed independent, $\sqrt{F_{ST,causal,XY}} = \sqrt{\left(\frac{E[(p_{i,X}-p_{i,Y})^2]}{E[2p_i(1-p_i)]}\right)}$ can be interpreted as a Euclidian distance measure in an m-dimensional space, where the 4 points $A1$, $A0$, $B1$ and $B0$ are defined by their m allele frequencies (e.g. $(p_{i,A1}-p_i)/\sqrt{2p_i(1-p_i)}$ represents the coordinate of point $A1$ on the axis corresponding to SNP i). The allele frequency in the full population represents the origin (population mean). While realizing that the lines ($A1$-$A0$) and ($B1$-$B0$) must both go through the population mean, one can see that $A1$, $A0$, $B1$, $B0$ and the population mean can be represented in a 2-dimensional plot. From Eq 3 and Eq 6, it follows that the distance between the population mean and $A1$ (resp. $B1$) equals $(1-K_A)\sqrt{Fst_{causal,A1A0}}$ (resp. $(1-K_B)\sqrt{Fst_{causal,B1B0}}$). While noting that the distance between $A1$ and $B1$ equals $\sqrt{Fst_{A1B1}}$, we know the lengths of the three sides of triangle defined by the population

mean, $A1$ an $B1$. The law of cosines gives the angle of the lines (population mean - $A1$) and (population mean - $B1$) as

$$arccos\left(\frac{(1-K_A)^2 F_{ST,causal,A1A0} + (1-K_B)^2 F_{ST,causal,B1B0} - F_{ST,causal,A1B1}}{2(1-K_A)\sqrt{F_{ST,causal,A1A0}}(1-K_B)\sqrt{F_{ST,causal,B1B0}}}\right) =$$

$$arccos\left(\frac{2\,(1-K_A)(1-K_B)\frac{coh_{oA,oB}}{m}}{2(1-K_A)(1-K_B)\sqrt{\frac{h^2_{oA}}{m}\frac{h^2_{oB}}{m}}}\right) = arccos(r_g) \qquad \text{Eq 9}$$

Thus, the genetic correlation r_g is equal to the cosines of the angle of the lines (population mean - $A1$) and (population mean - $B1$). It can analogously be shown that the angle between (population mean - $A0$) and (population mean -$B0$) is the same, and that the angle between (population mean - $A1$) and (population mean - $B0$) equals 180 minus the angle between (population mean - $A1$) and (population mean - $B1$), which confirms the use of $F_{ST,causal}$ to display $A1$, $A0$, $B1$, $B0$ and the population mean in a 2-domensional plot. To aid further interpretation, the perpendicular projection of line ($A1 - A0$) on line ($B1 - B0$) has a length equal to r_g times length ($A0$-$A1$) (i.e. $r_g\sqrt{Fst_{causal,A1A0}}$), because r_g equals the cosines between these lines.

In application, we derive $F_{ST,causal}$ analytically based on the heritabilities, population prevalences and genetic correlation. We note two important differences between $F_{ST,causal}$ and the F_{ST} from population genetics[23]. First, we restrict our definition of $F_{ST,causal}$ to independent SNPs, while F_{ST} from population genetics is based on all genome-wide SNPs. If one where to extend $F_{ST,causal}$ to genome-wide SNPs, $F_{ST,causal}$ at loci with large LD-scores would be larger than at SNPs with low LD-scores due to tagging. In contrast, the F_{ST} from population genetics is mainly attributable to drift and more or less evenly distributed over the genome (except for small effects of selection). Second, $F_{ST,causal}$ between cases and controls is of the order of magnitude of 10^{-6} depending on the number of SNPs m considered. In contrast, the F_{ST} between European and East Asian has been estimated[23] at 0.11. Because of the low magnitude of $F_{ST,causal}$, we report $m * F_{ST,causal}$ in Figure 1 and Figure S1 (note that $m * F_{ST,causal}$ is independent of m when other parameters are fixed, because the equations for $F_{ST,causal}$ has m in the denominator (see Eq 3 and Eq 8)).

The purpose of $F_{ST,causal}$ is to aid intuition to the bivariate genetic architecture of two disorders and to develop the CC-GWAS method (see further), and we do not provide formal standard errors of $F_{ST,causal}$. However, an approximation of the standard errors of $F_{ST,causal}$ can be obtained from the standard errors of the estimates of the heritabilities and co-heritability (typically assessed with methods like LD score regression). For Fst_{A1A0} and Fst_{B1B0}, this follows directly from Eq 3. For Fst_{A1B1}, we assume that the error of the three terms in Eq 8 are independent. This assumption is

likely violated, but it serves in obtaining approximations of the standard errors of $F_{ST,causal}$ (given in the legend of Figure 1).

CC-GWAS method

The CC-GWAS method relies on $F_{ST,causal}$, and assumes that all m SNPs impact both disorders with effect sizes following a bivariate normal distribution. CC-GWAS weights the effect sizes from the respective case-control GWAS using weights that minimize the expected squared difference between estimated and true A1B1 effect sizes; we refer to these as ordinary least squares (OLS) weights. To obtain the OLS weights, we analytically derive the expected coefficients of regressing the causal effect sizes $A1B1$ on the GWAS results of $A1A0$ and $B1B1$

$$\beta_{A1B1} \sim \omega_{A1A0}^{OLS}\hat{\beta}_{A1A0}^{GWAS} + \omega_{B1B0}^{OLS}\hat{\beta}_{B1B0}^{GWAS} \qquad \text{Eq 10}$$

Ordinary least square (OLS) regression gives

$$\begin{pmatrix} \omega_{A1A0}^{OLS} \\ \omega_{B1B0}^{OLS} \end{pmatrix} = \left(m \begin{pmatrix} 1 & 0 & 0 \\ 0 & var(\hat{\beta}_{A1A0}^{GWAS}) & cov(\hat{\beta}_{A1A0}^{GWAS}, \hat{\beta}_{B1B0}^{GWAS}) \\ 0 & cov(\hat{\beta}_{A1A0}^{GWAS}, \hat{\beta}_{B1B0}^{GWAS}) & var(\hat{\beta}_{B1B0}^{GWAS}) \end{pmatrix} \right)^{-1} \left(m \begin{pmatrix} 0 \\ cov(\hat{\beta}_{A1A0}^{GWAS}, \beta_{A1B1}) \\ cov(\hat{\beta}_{B1B0}^{GWAS}, \beta_{A1B1}) \end{pmatrix} \right) \qquad \text{Eq 11}$$

When assuming error terms are independent from effect sizes, we find

$$var(\hat{\beta}_{A1A0}^{GWAS}) = var(\beta_{A1A0}) + var(\varepsilon_{A1A0}) \approx \frac{h_{oA}^2}{m} + var(\varepsilon_{A1A0}) = F_{ST,causal,A1A0} + var(\varepsilon_{A1A0}) \qquad \text{Eq 12}$$

and the analogue for $var(\hat{\beta}_{B1B0}^{GWAS})$. For the covariance of the GWAS results, we find based on Eq 4

$$cov(\hat{\beta}_{A1A0}^{GWAS}, \hat{\beta}_{B1B0}^{GWAS}) \approx \frac{coh_{oA,oB}}{m} + cov(\varepsilon_{A1A0}, \varepsilon_{B1B0}) \qquad \text{Eq 13}$$

(At the end of this section, we discuss the expectation and estimation of the variance and covariance of error terms as well as scaling of odds ratios to standardized observed scale based on 50/50 case-

control ascertainment.) The expectation of the covariance between the GWAS results and β_{A1B1} follow from Eq 6 as

$$cov\left(\hat{\beta}_{A1A0}^{GWAS}, \beta_{A1B1}\right) = cov(\beta_{A1A0}, \beta_{A1B1}) + cov(\varepsilon_{A1A0}, \beta_{A1B1}) = E[\beta_{A1A0} * \beta_{A1B1}] = (1 - K_A)F_{ST,causal,A1A0} - (1 - K_B)\frac{coh_{oA,oB}}{m}$$

$$cov\left(\hat{\beta}_{B1B0}^{GWAS}, \beta_{A1B1}\right) = -(1 - K_B)F_{ST,causal,B1B0} + (1 - K_A)\frac{coh_{oA,oB}}{m} \qquad \text{Eq 14}$$

Thus, the OLS weights are defined to minimize the expected squared distance between estimated and causal effect sizes β_{A1B1}.

$$\hat{\beta}_{A1B1}^{OLS} = \omega_{A1A0}^{OLS}\hat{\beta}_{A1A0}^{GWAS} + \omega_{B1B0}^{OLS}\hat{\beta}_{B1B0}^{GWAS} \qquad \text{Eq 15}$$

To summarize, the OLS weights depend on the number of independent causal SNPs, the heritabilities, population prevalences, the genetic correlation, and the variance and covariance of error terms of the betas (depending on sample sizes N_{A1}, N_{A0}, N_{B1}, N_{B0} and the overlap between $A0$ and $B0$).

The OLS weights may be susceptible to type I error for SNPs with nonzero A1A0 and B1B0 effect sizes but zero A1B1 effect size, which we refer to as "stress test" SNPs (see further). To mitigate this, CC-GWAS also computes sample-size independent weights based on infinite sample size; we refer to these as Exact weights. The Exact weights depend only on the population prevalences K_A and K_B. From Eq 2 it follows that

$$\beta_{i,A1A0} \approx \frac{p_{i,A1} - p_i}{\sqrt{2p_i(1-p_i)}} - \frac{p_{i,A0} - p_i}{\sqrt{2p_i(1-p_i)}} = scaled\left(p_{i,A1}\right) - scaled\left(p_{i,A0}\right) \qquad \text{Eq 16}$$

Multiplying with $(1 - K_A)$ and substituting $p_{i,A0}$ based on Eq 5, gives

$$(1 - K_A)\beta_{i,A1A0} \approx \frac{(1-K_A)p_{i,A1} - \{p_i - K_A p_{i,A1}\}}{\sqrt{2p_i(1-p_i)}} = scaled\left(p_{i,A1}\right) \qquad \text{Eq 17}$$

From this, $\beta_{i,A1B1}$ follows as

$$\beta_{i,A1B1} \approx scaled\left(p_{i,A1}\right) - scaled\left(p_{i,B1}\right) \approx (1 - K_A)\beta_{i,A1A0} - (1 - K_B)\beta_{i,B1B0} \qquad \text{Eq 18}$$

We note that simulations confirm that this approximation is justified for loci with $0.5 < OR < 2$ for both A and B (Table S23). The Exact weights thus follow as

$$\omega_{A1A0}^{Exact} = (1 - K_A)$$
$$\omega_{B1B0}^{Exact} = -(1 - K_B) \qquad \text{Eq 19}$$

and

$$\hat{\beta}_{A1B1}^{Exact} = (1 - K_A)\hat{\beta}_{A1A0}^{GWAS} - (1 - K_B)\hat{\beta}_{B1B0}^{GWAS} \qquad \text{Eq 20}$$

The p-values of the OLS- and Exact approach are estimated as follows. First note that the standard error of $\hat{\beta}_{i,A1B1}$ of the OLS- and Exact approach at SNP i follow as

$$SE(\hat{\beta}_{i,A1B1}) = \sqrt{\omega_{A1A0}^2 var(\varepsilon_{i,A1A0}) + \omega_{B1B0}^2 var(\varepsilon_{i,B1B0}) + 2\omega_{A1A0}\omega_{B1B0} cov(\varepsilon_{i,A1A0}, \varepsilon_{i,B1B0})} \qquad \text{Eq 21}$$

While noting that $\omega_{A1A0} > 0$ and $\omega_{B1B0} < 0$, this indicates that sample overlap of controls (introducing positive covariance between $\varepsilon_{i,A1A0}$ and $\varepsilon_{i,B1B0}$) will increase power of CC-GWAS. Betas and their standard errors give z-values, from which p-values follow (assuming normally distributed error terms). CC-GWAS reports a SNP as statistically significant if it achieves $P < 5 * 10^{-8}$ using OLS weights *and* $P < 10^{-4}$ using Exact weights, balancing power and type I error.

When GWAS results are available for a direct case-case comparison, $\hat{\beta}_{A1B1}^{GWAS}$, CC-GWAS can be extended to CC-GWAS+. The OLS+ weights are defined as

$$\hat{\beta}_{A1B1}^{OLS+} = \omega_{A1A0}^{OLS+}\hat{\beta}_{A1A0}^{GWAS} + \omega_{B1B0}^{OLS+}\hat{\beta}_{B1B0}^{GWAS} + \omega_{A1B1}^{OLS+}\hat{\beta}_{A1B1}^{GWAS} \qquad \text{Eq 22}$$

The OLS+ weights follow analogue to Eq 11 for the OLS weights while noting that

$$var(\hat{\beta}_{A1B1}^{GWAS}) \approx F_{ST,causal,A1B1} + var(\varepsilon_{A1B1})$$
$$cov(\hat{\beta}_{A1A0}^{GWAS}, \hat{\beta}_{A1B1}^{GWAS}) \approx (1 - K_A)F_{ST,causal,A1A0} - (1 - K_B)\frac{coh_{oA,oB}}{m} + cov(\varepsilon_{A1A0}, \varepsilon_{A1B1})$$
$$cov(\hat{\beta}_{B1B0}^{GWAS}, \hat{\beta}_{A1B1}^{GWAS}) \approx -(1 - K_B)F_{ST,causal,B1B0} + (1 - K_A)\frac{coh_{oA,oB}}{m} + cov(\varepsilon_{B1B0}, \varepsilon_{A1B1})$$

$$cov\left(\hat{\beta}_{A1B1}^{GWAS}, \beta_{A1B1}\right) \approx F_{ST,causal,A1B1} \qquad \text{Eq 23}$$

The standard error $SE\left(\hat{\beta}_{i,A1B1}^{OLS+}\right)$ follows as

$$\sqrt{\left(\left(\omega_{A1A0}^{OLS+}\right)^2 var\left(\varepsilon_{i,A1A0}\right) + \left(\omega_{B1B0}^{OLS+}\right)^2 var\left(\varepsilon_{i,B1B0}\right) + \left(\omega_{A1B1}^{OLS+}\right)^2 var\left(\varepsilon_{i,A1B1}\right) + 2\omega_{A1A0}^{OLS+}\omega_{B1B0}^{OLS+} cov\left(\varepsilon_{i,A1A0}, \varepsilon_{i,B1B0}\right) + 2\omega_{A1A0}^{OLS+}\omega_{A1B1}^{OLS+} cov\left(\varepsilon_{i,A1A0}, \varepsilon_{i,A1B1}\right) + 2\omega_{B1B0}^{OLS+}\omega_{A1B1}^{OLS+} cov\left(\varepsilon_{i,B1B0}, \varepsilon_{i,A1B1}\right)\right)} \qquad \text{Eq 24}$$

The Exact+ approach is simply defined as $\hat{\beta}_{A1B1}^{GWAS}$ (i.e. $\omega_{A1A0}^{Exact+} = 0$, $\omega_{B1B0}^{Exact+} = 0$ and $\omega_{A1B1}^{Exact+} = 1$). CC-GWAS+ reports a SNP as statistically significant if it achieves $P < 5 * 10^{-8}$ using OLS+ weights *and* $P < 10^{-4}$ using Exact+ weights.

We now derive the expectation of the variance and covariance of the error terms of the betas. Assume the GWAS results are based on N_{A1} (resp. N_{B1}) cases and N_{A0} (resp. N_{B0}) controls of disorder A (resp. disorder B). First, while assuming small effect loci (typical for polygenic disorders), note that the variance of the allele frequency of SNP i in X (with X representing one of $A1$, $A0$, $B1$, $B0$) can be approximated by

$$var\left(\varepsilon_{i,X}\right) = \frac{p_{i,X}(1-p_{i,X})}{2N_X} \approx \frac{p_i(1-p_i)}{2N_X} \qquad \text{Eq 25}$$

From Eq 2, we find

$$var\left(\varepsilon_{i,A1A0}\right) \approx var\left(\frac{\hat{p}_{i,A1}-\hat{p}_{i,A0}}{\sqrt{2\hat{p}_i(1-\hat{p}_i)}}\right) \approx \frac{\frac{p_i(1-p_i)}{2N_{A1}}+\frac{p_i(1-p_i)}{2N_{A0}}}{2\hat{p}_i(1-\hat{p}_i)} \approx \frac{1}{4N_{A1}} + \frac{1}{4N_{A0}} = \frac{1}{N_{Eff,A1A0}}$$

$$var\left(\varepsilon_{i,B1B0}\right) \approx \frac{1}{4N_{B1}} + \frac{1}{4N_{B0}} = \frac{1}{N_{Eff,B1B0}}$$

$$var\left(\varepsilon_{i,A1B1}\right) \approx \frac{1}{4N_{A1}} + \frac{1}{4N_{B1}} = \frac{1}{N_{Eff,A1B1}} \qquad \text{Eq 26}$$

When assuming an overlap of $N_{overlap,A0B0}$ of controls, the expectation of the covariance of the error can be derived as follows. First, note that the error term of the allele frequency in all controls $A0$ can be expressed in terms of the error terms of $(A0, in\ overlap)$ and $(A0, not\ in\ overlap)$ as

$$\varepsilon_{i,A0} = \frac{N_{overlap,A0B0}}{N_{A0}}\varepsilon_{i,A0,in\ overlap} + \frac{N_{A0,not\ in\ overlap}}{N_{A0}}\varepsilon_{i,A0,not\ in\ overlap} \qquad \text{Eq 27}$$

Thus, the covariance of error terms follows as

$$cov\left(\varepsilon_{i,A1A0}, \varepsilon_{i,B1B0}\right) = E\left[\frac{(\varepsilon_{i,A1}-\varepsilon_{i,A0})}{\sqrt{2\hat{p}_i(1-\hat{p}_i)}} * \frac{(\varepsilon_{i,B1}-\varepsilon_{i,B0})}{\sqrt{2\hat{p}_i(1-\hat{p}_i)}}\right] \approx$$

$$\frac{E\left[\left(\frac{N_{overlap,A0B0}}{N_{A0}}\right)\varepsilon_{i,A0,in\ overlap} * \left(\frac{N_{overlap,A0B0}}{N_{B0}}\right)\varepsilon_{i,B0,in\ overlap}\right]}{E[2\hat{p}_i(1-\hat{p}_i)]} \approx \frac{N_{overlap,A0B0}}{4N_{A0}N_{B0}} \qquad \text{Eq 28}$$

Now, assume of number of $N_{A1\ in\ A1B1}$ and $N_{B1\ in\ A1B1}$ are available for a direct case-case comparison included in CC-GWAS+ (typically $N_{A1\ in\ A1B1} < N_{A1}$ and $N_{B1\ in\ A1B1} < N_{B1}$). This gives the following expected covariance of error terms

$$cov\left(\varepsilon_{i,A1A0}, \varepsilon_{i,A1B1}\right) \approx E\left[\frac{(\varepsilon_{i,A1}-\varepsilon_{i,A0})}{\sqrt{2\hat{p}_i(1-\hat{p}_i)}} * \frac{(\varepsilon_{i,A1\ in\ A1B1}-\varepsilon_{i,B1\ in\ A1B1})}{\sqrt{2\hat{p}_i(1-\hat{p}_i)}}\right] \approx$$

$$\frac{E\left[\left(\frac{N_{A1\ in\ A1B1}}{N_{A1}}\right)\varepsilon_{i,A1\ in\ A1B1}\varepsilon_{i,A1\ in\ A1B1}\right]}{E[2\hat{p}_i(1-\hat{p}_i)]} \approx \frac{\left(\frac{N_{A1\ in\ A1B1}}{N_{A1}}\right)\frac{p_i(1-p_i)}{2N_{A1\ in\ A1B1}}}{2p_i(1-p_i)} = \frac{1}{4N_{A1}}$$

$$cov\left(\varepsilon_{i,B1B0}, \varepsilon_{i,A1B1}\right) \approx -\frac{1}{4N_{B1}} \qquad \text{Eq 29}$$

Thus, the expectations of the variance and the covariance of the error terms are given. We note that in practical application of CC-GWAS, the covariances of error terms are based on analytical computation and the intercept of cross-trait LD score regression (described in the section '*Application of CC-GWAS to empirical data sets*').

In practice, case-control GWAS results are not presented on the standardized observed scale based on 50/50 case-control ascertainment (β_i) but as odd ratios (OR_i) from logistic regression. We apply two approaches to transpose results from logistic regression to β_i. First, we assume for large sample sizes that the z-value from logistic regression is equal to the z-value from linear regression on the observed scale. With the expected variance of error-terms derived as $\frac{1}{N_{Eff}}$ (Eq 26), we find

$$\beta_i \approx z_{i,logistic\ regression}\sqrt{\frac{1}{N_{Eff}}} \qquad \text{Eq 30}$$

The second approach is based on equation 5 in the paper from from Lloyd-Jones et al.[67], which reads for 50% cases as

$$OR_i = \frac{[0.5+\beta_i(1-p_i)][1-0.5+\beta_i p_i]}{[0.5-\beta_i p_i][1-0.5-\beta_i(1-p_i)]} \qquad \text{Eq 31}$$

Rewriting gives

$$(1-p_i)p_i(1-OR_i)\beta_i^2 + 0.5(1+OR_i)\beta_i + 0.25(1-OR_i) = 0 \qquad \text{Eq 32}$$

The solution from this quadratic equation with $0 < \beta_i < 2$ for $OR_i > 1$, and $-2 < \beta_i < 0$ for $OR_i < 1$, gives an approximation of β_i. We confirm in simulation (see below) that both approaches approximate β_i well, but the first approach is slightly less noisy. In the CC-GWAS software, we therefore transform the betas with the first approach, and compare these to transformations with the second approach to provide a rough double-check of whether N_{Eff} has been defined accurately.

CC-GWAS simulations to assess power and type I error

We simulated individual level data of m independent SNPs in line with ref.[66] for disorders A and B as follows. Liability-scale effect sizes ($\beta_{i,lA}$ and $\beta_{i,lB}$) were drawn from a bivariate normal distribution with variances h_{lA}^2/m and h_{lB}^2/m and covariance $\frac{1}{m} r_g \sqrt{(h_{oA}^2\, h_{oB}^2)}$. Effective allele frequencies (EAF) of m SNPs were drawn from a uniform distribution $[0.05,0.5]$. Individuals were simulated one-by-one by

1. Randomly assigning m genotypes G_i (i.e. 0, 1 or 2 effective alleles) with the probabilities given by the $EAFs$ while assuming Hardy-Weinberg equilibrium
2. Defining genetic liabilities as $g_{lA} = \sum \beta_{i,lA}(G_i - 2EAF_i)/\sqrt{2EAF_i(1-EAF_i)}$, and $g_{lB} = \sum \beta_{i,lB}(G_i - 2EAF_i)/\sqrt{2EAF_i(1-EAF_i)}$
3. Defining liabilities as $l_A = g_{lA} + e_{lA}$ and $l_B = g_{lB} + e_{lB}$, with e_{lA} drawn from a standard normal distribution with variance $1 - h_{lA}^2$, and e_{lB} drawn from a standard normal distribution with variance $1 - h_{lB}^2$. (Here we simulate e_{lA} and e_{lB} to be uncorrelated, but note that correlation of e_{lA} and e_{lB} does not impact simulation results.)
4. Defining disorder status as $A = 1$ (resp. $B = 1$) when $l_A > T_A$ (resp. $l_B > T_B$) with T_A (resp. T_B) corresponding to a population prevalence of K_A (resp. K_B)

Individuals were simulated until the required number of nonoverlapping cases and controls (N_{A1}, N_{A0}, N_{B1}, N_{B0}) were obtained. Subsequently, GWASs $A1A0$ and $B1B0$ were performed with logistic regression in Plink 1.9[68], CC-GWAS was applied as described above, and the power of CC-GWAS, the OLS approach, the Exact approach and the delta method were recorded. A second set om $3 * m$ SNPs with no effect on lA and lB were included in the simulation to estimate the respective

type I error rates of null-null SNPs. Then, we simulated $3 * m$ stress test SNPs as follows. We defined a stress test SNP to explain a proportion of α_{lA} of variance on the liability scale of A, and a proportion of α_{oA} on the observed scale via the standard transformarion[65,66]. The effect of the stress test SNP on the observed scale follows as $\beta_{stress\ test\ SNP,oA} = \sqrt{\alpha_{oA}}$, and the allele frequency in cases of A is approximated by $(1 - K_A)\beta_{stress\ test\ SNP,oA}$ (Eq 17) and the allele frequency in controls of A by $-K_A\beta_{stress\ test\ SNP,oA}$. The allele frequency in cases of B is per definition equal to $(1 - K_A)\beta_{stress\ test\ SNP,oA}$, thus giving $\beta_{stress\ test\ SNP,oB} = (1 - K_A)\beta_{stress\ test\ SNP,oA}/(1 - K_B)$ (Eq 17). The allele frequency in controls is approximated by $-K_B\beta_{stress\ test\ SNP,oB}$. We simulated cases and controls in line with these allele frequencies. Thus, simulating $3 * m$ stress test SNPs allowed recording of type I error of stress test SNPs. Simulations were repeated 50 times. We note that simulations using real LD patterns are essential for methods impacted by LD between SNPs, but the CC-GWAS method is not impacted by LD between SNPs and simulations were therefore based on independent SNPs.

The parameters of simulation were largely in line with those used in Figure 2, but in order to reduce computational time, sample sizes were reduced to $N_{A1} = N_{A0} = N_{B1} = N_{B0} = 4{,}000$, number of causal SNPs to $m = 1{,}000$, and required levels of significance were reduced to $p < 0.01$ for the OLS weights and $p < 0.05$ for the Exact weights. Three values of genetic correlation were simulated (0.2, 0.5 and 0.8). Simulation results are displayed in Table S3 and match analytical computations (described below). The confirmation of analytical computations, allows increasing parameters in Figure 2 to more realistic values.

CC-GWAS analytical computations to assess power and type I error

For analytical computations, first consider one sets of weights to derive expected results for the OLS approach, Exact approach and the delta method

$$\hat{\beta}_{A1B1} = \omega_{A1A0}\hat{\beta}_{A1A0}^{GWAS} + \omega_{B1B0}\hat{\beta}_{B1B0}^{GWAS} \qquad \text{Eq 33}$$

The variance of betas and error terms follow as

$$var(\hat{\beta}_{A1B1}) = \omega_{A1A0}^2 var(\hat{\beta}_{A1A0}^{GWAS}) + \omega_{B1B0}^2 var(\hat{\beta}_{B1B0}^{GWAS}) + 2\omega_{A1A0}\omega_{B1B0} cov(\hat{\beta}_{A1A0}^{GWAS}, \hat{\beta}_{B1B0}^{GWAS})$$
$$var(\hat{\varepsilon}_{A1B1}) = \omega_{A1A0}^2 var(\varepsilon_{A1A0}^{GWAS}) + \omega_{B1B0}^2 var(\varepsilon_{B1B0}^{GWAS}) + 2\omega_{A1A0}\omega_{B1B0} cov(\varepsilon_{A1A0}^{GWAS}, \varepsilon_{B1B0}^{GWAS})$$

Eq 34

the analytical expectations of which have been derived above. The variance of z-values at causal loci follows as $var(z_{A1B1}) = var(\hat{\beta}_{A1B1})/SE^2 = var(\hat{\beta}_{A1B1})/var(\hat{\varepsilon}_{A1B1})$, and the power as

$P\left(|z_{A1B1}| > z_{th} \mid z_{A1B1} \sim N(0, var(z^{X}_{A1B1})\right)$ with $z_{th} = \phi^{-1}(1 - p_{threshold}/2)$ and ϕ^{-1} the standard normal quantile function (with $p_{threshold} = 0.01$ to compare to simulation and $p_{threshold} = 5 * 10^{-8}$ in Figure 2). Type I error rate at null-null loci is well controlled (i.e. equals p-value) while noting that $var(z^{X}_{A1B1|null-null\ SNPs}) = 1$. Type I error at stress test SNPs follows as $P(|z_{A1B1}| > z_{th} \mid z_{A1B1} \sim N(\omega_{A1A0}\beta_{stress\ test\ SNP,oA} + \omega_{B1B0}\beta_{stress\ test\ SNP,oB}, 1)$.

Now consider CC-GWAS combining the estimates of $\hat{\beta}^{OLS}_{A1B1}$ and $\hat{\beta}^{Exact}_{A1B1}$ requiring $p^{OLS} < p^{OLS}_{threshold}$ and $p^{Exact} < p^{Exact}_{threshold}$ (with corresponding z^{OLS}_{th} and z^{Exact}_{th}) for significance (with thresholds set at 0.01 and 0.05 to compare to simulation, and $5 * 10^{-8}$ and 10^{-4} in Figure 2). The covariance of $\hat{\beta}^{OLS}_{A1B1}$ and $\hat{\beta}^{Exact}_{A1B1}$ follows as

$$cov(\hat{\beta}^{OLS}_{A1B1}, \hat{\beta}^{Exact}_{A1B1}) = \omega^{OLS}_{A1A0}\omega^{Exact}_{A1A0} var(\hat{\beta}^{GWAS}_{A1A0}) + \omega^{OLS}_{A1A0}\omega^{Exact}_{B1B0} cov(\hat{\beta}^{GWAS}_{A1A0}, \hat{\beta}^{GWAS}_{B1B0}) + \omega^{OLS}_{B1B0}\omega^{Exact}_{A1A0} cov(\hat{\beta}^{GWAS}_{B1B0}, \hat{\beta}^{GWAS}_{A1A0}) + \omega^{OLS}_{B1B0}\omega^{Exact}_{B1B0} var(\hat{\beta}^{GWAS}_{B1B0}) \quad \text{Eq 35}$$

the analytical expectations of which are given in the above. The covariance of the error terms $cov(\varepsilon^{OLS}_{A1B1}, \varepsilon^{Exact}_{A1B1})$ follow analogously, and the covariance of z-values follows at causal SNPs as $cov(z^{OLS}_{A1B1}, z^{Exact}_{A1B1}) = cov(\hat{\beta}^{OLS}_{A1B1}, \hat{\beta}^{Exact}_{A1B1})\sqrt{(var(\varepsilon^{OLS}_{A1B1})var(\varepsilon^{Exact}_{A1B1})}$. In combination with the expectations of $var(z^{OLS}_{A1B1})$ and $var(z^{Exact}_{A1B1})$, this defines the variances and covariance of the bivariate normal distribution $f(x = z^{OLS}_{A1B1}, y = z^{Exact}_{A1B1})$ around mean (0,0) at causal SNPs. The power follows as

$$\int_{z^{Exact}_{th}}^{\infty}\int_{z^{OLS}_{th}}^{\infty} f\,dxdy + \int_{-\infty}^{-z^{Exact}_{th}}\int_{-\infty}^{-z^{OLS}_{th}} f\,dxdy + \int_{-\infty}^{-z^{Exact}_{th}}\int_{z^{OLS}_{th}}^{\infty} f\,dxdy + \int_{z^{Exact}_{th}}^{\infty}\int_{-\infty}^{-z^{OLS}_{th}} f\,dxdy \quad \text{Eq 36}$$

We note that the last two terms (i.e. with OLS and Exact approach meeting the required level of significance at opposite sign) have negligible magnitudes. The type I error at null-null SNPs is found by substituting in the equation above the bivariate normal distribution f' with mean (0,0) and variance covariance of z-values at null-null SNPs, i.e. $var(z^{OLS}_{A1B1|null-null\ SNPs}) = var(z^{Exact}_{A1B1|null-null\ SNPs}) = 1$ and $cov(z^{OLS}_{A1B1|null-null\ SNPs}, z^{Exact}_{A1B1|null-null\ SNPs}) = cov(\varepsilon^{OLS}_{A1B1}, \varepsilon^{Exact}_{A1B1})\sqrt{var(\varepsilon^{OLS}_{A1B1})var(\varepsilon^{Exact}_{A1B1})}$. The type I error at stress test SNPs is found by substituting in the equation above the bivariate normal distribution f'' with mean $(\alpha_{A1A0}\beta_{stress\ test\ SNP,oA} + \alpha_{B1B0}\beta_{stress\ test\ SNP,oB}, (1 -$

$K_A)\beta_{stress\ test\ SNP,oA} + (1 - K_B)\beta_{stress\ test\ SNP,oB})$ and the same variance covariance as of z-values at null-null SNPs.

In secondary analyses, we investigate the impact of overlap in controls. For simulation, we therefore exchanged double controls ($A = 0$ and $B = 0$) between those selected as $A0$ and $B0$ selected by chance (thereby preventing the impact of double screening of controls[69]). For analytical computations, we simply adjusted the covariance of error terms in line with the equation above. We also assessed CC-GWAS using the type S error rate, defined as the proportion of significantly identified loci (true positives) identified with the wrong sign[26,27]. We therefore extended the bivariate normal distribution f at causal loci to $f'''\left(x = z_{A1B1}^{OLS}, y = z_{A1B1}^{Exact}, v = \beta_{A1B1}\right)$ with an additional dimension covering the causal effects β_{A1B1}, while noting that $var(\beta_{A1B1}) = Fst_{A1B1}$ and $cov(z_{A1B1}, \beta_{A1B1}) = \left[\omega_{A1A0} cov\left(\hat{\beta}_{A1A0}^{GWAS}, \beta_{A1B1}\right) + \omega_{B1B0} cov\left(\hat{\beta}_{B1B0}^{GWAS}, \beta_{A1B1}\right)\right] / \sqrt{var(\varepsilon_{A1B1}^{X})}$ and mean $(0,0,0)$. The type S error follows as

$$\int_{-\infty}^{0} \int_{z_{th}^{Exact}}^{\infty} \int_{z_{th}^{OLS}}^{\infty} f'''\, dxdydv + \int_{0}^{\infty} \int_{-\infty}^{-z_{th}^{Exact}} \int_{-\infty}^{-z_{th}^{OLS}} f'''\, dxdydv$$

Eq 37

When a direct case-case comparison is available, CC-GWAS can be extended to CC-GWAS+, the derivations of which follow analogue to the above and are not shown here. These analytical computations were confirmed with simulation (Table S3).

Empirical data sets

We compared cases from SCZ[15], BIP[16], MDD[17], ADHD[18], ANO[19], ASD[20], OCD[21], and TS[22] based on publicly available case-control GWAS results (see URLs). To further validate CC-GWAS we also compare cases from CD[24], UC[24] and RA[25], based on the case-control GWAS results from samples genotyped on chips with genome-wide coverage (see URLs). Numbers of cases and controls are listed in Table 1 and Table S7. The transformation of odds ratios to the standardize betas on the observed scale (Eq 30) requires N_{eff} (Eq 26). For some of the disorders (BIP, MDD, ANO and RA), N_{eff} was provided on a SNP-by-SNP basis in publicly available GWAS results. For other disorders (SCZ, ADHD, ASD, OCD, TS, CD and UC), we approximated a genome-wide fixed N_{eff} by summing the N_{eff} of the contributing cohorts as $\sum_{cohorts} \frac{4}{1/N_{case,cohort\ i} + 1/N_{control,cohort\ i}}$. In quality control SNPs were removed with $MAF < 0.01$, $INFO < 0.6$, $N_{eff} < 0.67 * max(N_{eff})$, duplicate SNP names, strand-ambiguous SNPs, and the MHC region (chr6:25,000,000-34,000,000) was excluded due to its compilated LD structure. All reported

SNP names and chromosome positions are based on GRCh37/hg19. (In principle, applying a fixed N_{eff} for cohorts without SNP-by-SNP N_{eff} information could lead to inaccurate transformation of beta for some SNPs. Therefore, we reran CC-GWAS analyses for SCZ, BIP and MDD with fixed N_{eff} yielding nearly identical results to the primary analyses (with fixed N_{eff} for SCZ and SNP-by-SNP N_{eff} for BIP and MDD). This confirms that using fixed N_{eff} is appropriate.)

Application of CC-GWAS to empirical data sets

Input parameters of CC-GWAS are the population prevalences (K), liability-scale heritabilities (h_l^2), genetic correlation (r_g), the intercept from cross-trait LD score regression[2] (used to model covariance of the error-terms), the sample-sizes including overlap of controls (also used to model covariance of the error-terms; see below), and expectation of the number of independent causal SNPs (m). Prevalences are displayed in Table 1 and Table S7 and were based on ref.[70] for the eight psychiatric disorders, on ref.[71] for UC and CD, and ref.[25] for RA. Heritabilities were assessed with stratified LD score regression based on the baseline LD v2.0 model[33–35], and transposed to liability-scale[65,66]. Genetic correlations were estimated with cross-trait LD score regression[2]. The number of causal SNPs was set at $m = 10{,}000$ for the psychiatric disorders, and $m = 1{,}000$ for CD, UC and RA based on ref.[28].

We note that the intercept of cross-trait LD score regression (representing covariance of z-values at null-null SNPs[6]) can analytically be derived as

$$intercept \approx \frac{cov(\varepsilon_{A1A0}^{GWAS}, \varepsilon_{B1B0}^{GWAS})}{\sqrt{var(\varepsilon_{A1A0}^{GWAS}) var(\varepsilon_{B1B0}^{GWAS})}} \approx \frac{N_{overlap}}{4N_{A0} * N_{B0} \sqrt{\frac{1}{4N_{A1}} + \frac{1}{4N_{A0}}} \sqrt{\frac{1}{4N_{B1}} + \frac{1}{4N_{B0}}}} \qquad \text{Eq 38}$$

The intercepts estimated with cross-trait LD score regression[2] were typically larger than those expected analytically based on Eq 38 (Table S25) based on sample-overlap (Table S26). This could roughly be attributable to (i) underestimation of sample-overlap (ii) factors increasing the cross-trait LD score regression intercept other than covariance of error terms based on sample-overlap. For example, Yengo et al. showed that shared population stratification may increase the intercept[72]. In addition, it is known that attenuation bias of univariate LD score regression can increase the univariate LD score intercept[36], and we hypothesize this phenomenon may also increase the cross-trait LD score regression intercept. Importantly, we note that overestimation of the covariance of error terms will underestimate the standard error of CC-GWAS results (Eq 21) thereby risking increased false positive rate. Therefore, in CC-GWAS we model the covariance of error terms based on the minimum of the intercept from cross-trait LD score regression and the expected intercept based on Eq 38.

Based on the listed input parameters, CC-GWAS (software provided in R[73]; see URL section) was applied one disorder pair at a time. CC-GWAS first estimates $F_{ST,causal}$ and plots $A1$, $A0$, $B1$, and $B0$ as described above (and displayed in Figure 1 and Figure S1). Second, CC-GWAS transforms case-control input GWAS results (on the per-allele OR scale) to the standardized observed scale based on 50/50 case-control ascertainment in two ways as described above (via N_{eff} and via the equation from Lloyd-Jones et al.[67]). The transformations were in concordance with correlation between betas of both approaches > 0.985 and only slight differences in magnitude with relative differences > 0.9 and < 1.1. Third, OLS weights and Exact weights (displayed in Table 1 and Table S7) were computed as described. Fourth, based on these weights, OLS estimates ($\hat{\beta}_{A1B1}^{OLS}$) and Exact estimates ($\hat{\beta}_{A1B1}^{Exact}$) were computed with accompanying standard errors and p-values as described above. Fifths and finally, CC-GWAS reports SNPs as statistically significant that achieve $P<5x10^{-8}$ using OLS weights *and* $P<10^{-4}$ using Exact weights.

CC-GWAS results were clumped in line with ref.[15] based using 1000 Genomes data[29] as LD reference panel with Plink 1.9[68] (--clump-p1 5e-8 --clump-p2 5e-8 --clump-r2 0.1 --clump-kb 3000; see URLs) (Table S5). Loci within 250kb of each other after the first clumping step were collapsed. We defined *CC-GWAS-specific* loci as loci for which none of the genome-wide significant SNPs have an $r^2>0.8$ with any of the genome-wide significant SNPs in the input case-control GWAS results (Table S5). An overview of the CC-GWAS loci is given in Table 1 and Table S7, and details are provided in Table 2 and Table S6.

Secondary analyses included a different clumping strategy in line with ref.[36] with Plink 1.9 (--clump-p1 5e-8 --clump-p2 5e-8 --clump-r2 0.01 --clump-kb 5000) while subsequently collapsing loci within 100kb of each other. In another set of secondary analyses input case-control summary statistics were corrected for the respective intercepts of stratified LD score regression (similar to Turley et al.[6]) by dividing the input case-control standard errors by $\sqrt{intercept}$ and adjusting the z-values and p-values accordingly. We believe this correction may be overly conservative, because some increase of the intercept may be expected due to attenuation bias of imperfect matching of LD patterns[36]. Nevertheless, we verified as follows that CC-GWAS provides proportionally biased results when applied on biased input GWAS results (i.e. CC-GWAS does not introduce an additional layer of bias). First, in simulation we multiplied the standard errors witch $c_{A1A0} = c_{B1B0} = 0.9$ and verified that the increase in $var\left(z_{A1B1|null-null\ SNPs}^{OLS}\right)$ was proportional to the increase in $var\left(z_{A1A0|null-null\ SNPs}^{GWAS}\right) = var\left(z_{B1B0|null-null\ SNPs}^{GWAS}\right)$ under various scenarios (Table S11). Second, we note this bias can also be analytically derived as

$$var\left(z^{OLS}_{A1B1|null-loci}\right) = \frac{\alpha^2_{A1A0}var(\varepsilon_{A1A0}) + \alpha^2_{B1B0}var(\varepsilon_{B1B0}) + 2\alpha_{A1A0}\alpha_{B1B0}cov(\varepsilon_{A1A0}, \varepsilon_{B1B0})}{\alpha^2_{A1A0}c^2_{A1A0}var(\varepsilon_{A1A0}) + \alpha^2_{B1B0}c^2_{B1B0}var(\varepsilon_{B1B0}) + 2\alpha_{A1A0}\alpha_{B1B0}cov(\varepsilon_{A1A0}, \varepsilon_{B1B0})} \quad \text{Eq 39}$$

as confirmed with simulation (Table S11); note that for unbiased ($c_{A1A0} = c_{B1B0} = 1$) case-control GWAS results $var\left(z^{OLS}_{A1B1|null-loci}\right) = 1$ as expected. In another set of secondary analyses, CC-GWAS of SCZ vs. MDD was extended to include the direct case-case comparison from ref.[4] including 23,585 SCZ cases and 15,270 BIP cases (see URL section).

SMR and HEIDI analyses

We used the SMR test for colocalization[31] to identify CC-GWAS loci with significant associations between gene expression effect sizes in *cis* and OLS case-case effect sizes. We tested cis-eQTL effects in 13 GTEx v7 brain tissues[37] (Amygdala, Anterior cingulate cortex, Caudate basal ganglia, Cerebellar Hemisphere, Cerebellum, Cortex, Frontal Cortex, Hippocampus, Hypothalamus, Nucleus accumbens basal ganglia, Putamen basal ganglia, Spinal cord cervical c-1, and Substantia nigra; see URLs), and a meta-analysis of eQTL effects in brain tissues[38] (see URLs). In line with standard application of SMR[31], we tested probes of genes with significant eQTL associations, with the lead eQTL SNP within 1MB of the lead CC-GWAS SNP. SMR analyses were performed on 2MB cis windows around the tested probe. The threshold of significance was adjusted per tested disorder-pair by dividing 0.05 by the respective number of probes tested (Table S15). We used the HEIDI test for heterogeneity[31] to exclude loci with evidence of linkage effects ($P < 0.05$).

Replication data sets

For replication analyses, we used CC-GWAS discovery results of additional analyses of SCZ vs. MDD based on GWAS results from Ripke et al.[14] and Wray et al.[57] (Table S17; see URLs). To obtain independent replication data, we applied MetaSubtract[58] separately for SCZ (results (i) Pardinas et al.[15] – results (ii) Ripke et al.[14]) and for MDD (results (i) Howard et al.[17] – results (ii) Wray et al.[57]). MetaSubtract[58] recovers these results by subtracting results (ii) from results (i) as follows:

$$\beta_{(ii)-(i)} = \frac{\left(\frac{1}{SE^2_{(i)}}\beta_{(i)}\right) - \frac{1}{SE^2_{(ii)}}\beta_{(ii)}}{\frac{1}{SE^2_{(i)}} - \frac{1}{SE^2_{(i)-(ii)}}}, \text{ with } SE^2_{(i)-(ii)} = \frac{1}{\frac{1}{SE^2_{(i)}} - \frac{1}{SE^2_{(ii)}}} \quad \text{Eq 40}$$

Replication GWAS results were thus obtained for $7{,}035$ cases and $21{,}187$ controls ($N_{eff} = 22{,}369$) for SCZ and $111{,}175$ cases and $216{,}289$ controls ($N_{eff} = 242{,}275$) for MDD, with information on $4{,}105{,}296$ SNPs for SCZ and MDD. Independence of replication results and discovery results were confirmed with cross-trait LD score regression intercepts of 0.037 for SCZ and -0.004 for MDD. We corrected the replication results for the univariate LD score regression intercepts (by multiplying SE with $\sqrt{intercept}$), as the intercepts appeared slightly inflated (1.25 (attenuation ratio 0.58) for SCZ and 1.12 (attenuation ratio 0.25) for MDD). We note this correction may have been overly conservative, because if the discovery data are not exact subsets of the full data, we anticipate that MetaSubtract replication results would be conservative, as independent signals from the discovery data would be subtracted from the full data when producing the replication data.

For further replication analyses, we used CC-GWAS discovery results from the three comparisons of CD[24], UC[24] and RA[25] (Table S17). For CD[24] and UC[24], two sets of GWAS results are publicly available (see URLs): (a) results of 1000 genomes Phase 1 imputed SNPs from individuals genotyped on genotyping chips with genome-wide coverage, and (b) meta-analyses results of (a) with additional samples genotyped with the Immunochip covering 196,524 SNPs without genome-wide coverage[24]. Our CC-GWAS discovery results were based on GWAS results (a). For replication, we needed the Immunochip only results, which we obtained by subtracting (a) from (b) with MetaSubtract[58]. These Immonochip only results were thus obtained for $101{,}482$ SNPs after QC for CD and $101{,}792$ SNPs for UC, and were based on $14{,}594$ cases and $26{,}715$ controls ($N_{eff} = 37{,}752$) for CD and $10{,}679$ cases and $26{,}715$ controls ($N_{eff} = 30{,}517$) for UC. For RA, the genome-wide GWAS results and Immunochip results are separately available (see URLs) providing replication results of $830{,}956$ SNPs (note that Okada et al.[25] imputed Immunochip SNP data) of $5{,}486$ cases and $14{,}556$ controls ($N_{eff} = 15{,}274$). (Note that LD score regression could not be applied on the Immunochip results from CD, UC and RA, because the Immunochip does not provide the genome-wide coverage required for LD score regression[33]. Further note that scaling of the SE based on the univariate LD score regression intercept only impacts the number of significant replicated loci, but not the regression slope in Figure 4.)

Application of CC-GWAS to replication data sets

For replication of SCZ vs. MDD, CD vs. UC, CD vs. RA and UC vs. RA we computed OLS $A1B1$ effect based on the OLS weights from the respective discovery results (Table S17). (We applied OLS weights from the discovery analyses rather than re-estimating the OLS weights based on the replication GWAS results, because OLS weights are sample-size dependent.) For SCZ vs. MDD, the covariance of the error terms was negligible (cross-trait LD score regression intercept of 0.012) while the covariance could

not be estimated for the autoimmune disease pairs (as LD score regression cannot be applied on Immunochip data) and was thus set to 0. Because covariance of the error terms decreases the standard error of OLS estimates (Eq 21), setting the covariance to 0 may lead to conservative bias with respect to significance in replication, but it does not impact the magnitude of the OLS estimates themselves nor the regression slopes displayed in Figure 4.

References

1. Lee, S. H. *et al.* Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat. Genet.* **45**, 984–94 (2013).
2. Bulik-Sullivan, B. *et al.* An atlas of genetic correlations across human diseases and traits. *Nat. Genet.* (2015). doi:10.1038/ng.3406
3. Lee, P. H. *et al.* Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric Disorders. *Cell* **179**, 1469-1482.e11 (2019).
4. Ruderfer, D. M. *et al.* Genomic Dissection of Bipolar Disorder and Schizophrenia, Including 28 Subphenotypes. *Cell* **173**, 1705-1715.e16 (2018).
5. Pasaniuc, B. & Price, A. L. Dissecting the genetics of complex traits using summary association statistics. *Nat. Rev. Genet.* **18**, 117–127 (2017).
6. Turley, P. *et al.* Multi-trait analysis of genome-wide association summary statistics using MTAG. *Nat. Genet.* **50**, 229–237 (2018).
7. Qi, G. & Chatterjee, N. Heritability informed power optimization (HIPO) leads to enhanced detection of genetic associations across multiple traits. *PLoS Genet.* **14**, e1007549 (2018).
8. Baselmans, B. M. L. *et al.* Multivariate genome-wide analyses of the well-being spectrum. *Nat. Genet.* **51**, 445–451 (2019).
9. Nieuwboer, H. A., Pool, R., Dolan, C. V., Boomsma, D. I. & Nivard, M. G. GWIS: Genome-Wide Inferred Statistics for Functions of Multiple Phenotypes. *Am. J. Hum. Genet.* **99**, 917–927 (2016).
10. Bhattacharjee, S. *et al.* A subset-based approach improves power and interpretation for the combined analysis of genetic association studies of heterogeneous traits. *Am. J. Hum. Genet.* **90**, 821–35 (2012).
11. Han, B. & Eskin, E. Interpreting meta-analyses of genome-wide association studies. *PLoS Genet.* **8**, e1002555 (2012).
12. Zhu, Z. *et al.* Causal associations between risk factors and common diseases inferred from

GWAS summary data. *Nat. Commun.* **9**, 224 (2018).

13. Byrne, E. M. *et al.* Conditional GWAS analysis identifies putative disorder-specific SNPs for psychiatric disorders. *bioRxiv* 592899 (2019). doi:10.1101/592899

14. Ripke, S. *et al.* Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421–7 (2014).

15. Pardiñas, A. F. *et al.* Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. *Nat. Genet.* **50**, 381–389 (2018).

16. Stahl, E. A. *et al.* Genome-wide association study identifies 30 loci associated with bipolar disorder. *Nat. Genet.* **51**, 793–803 (2019).

17. Howard, D. M. *et al.* Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. *Nat. Neurosci.* **22**, 343–352 (2019).

18. Demontis, D. *et al.* Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. *Nat. Genet.* **51**, 63–75 (2019).

19. Watson, H. J. *et al.* Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa. *Nat. Genet.* **51**, 1207–1214 (2019).

20. Grove, J. *et al.* Identification of common genetic risk variants for autism spectrum disorder. *Nat. Genet.* **51**, 431–444 (2019).

21. International Obsessive Compulsive Disorder Foundation Genetics Collaborative (IOCDF-GC) and OCD Collaborative Genetics Association Studies (OCGAS). Revealing the complex genetic architecture of obsessive-compulsive disorder using meta-analysis. *Mol. Psychiatry* **23**, 1181–1188 (2018).

22. Yu, D. *et al.* Interrogating the Genetic Determinants of Tourette's Syndrome and Other Tic Disorders Through Genome-Wide Association Studies. *Am. J. Psychiatry* **176**, 217–227 (2019).

23. Bhatia, G., Patterson, N., Sankararaman, S. & Price, A. L. Estimating and interpreting FST: The impact of rare variants. *Genome Res.* **23**, 1514–1521 (2013).

24. Liu, J. Z. *et al.* Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat. Genet.* **47**, 979–986 (2015).

25. Okada, Y. *et al.* Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* **506**, 376–81 (2014).

26. Gelman, A. & Tuerlinckx, F. Type S error rates for classical and Bayesian single and multiple comparison procedures. *Comput. Stat.* **15**, 373–390 (2000).

27. Stephens, M. False discovery rates: a new deal. *Biostatistics* **18**, 275–294 (2017).

28. O'Connor, L. J. *et al.* Extreme Polygenicity of Complex Traits Is Explained by Negative Selection. *Am. J. Hum. Genet.* **105**, 456–476 (2019).

29. 1000 Genomes Project Consortium *et al.* A global reference for human genetic variation. *Nature* **526**, 68–74 (2015).

30. Buniello, A. *et al.* The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res.* **47**, D1005–D1012 (2019).

31. Zhu, Z. *et al.* Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat. Genet.* **48**, (2016).

32. Bulik-Sullivan, B. K. *et al.* LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat. Genet.* **47**, 291–5 (2015).

33. Finucane, H. K. *et al.* Partitioning heritability by functional annotation using genome-wide association summary statistics. *Nat. Genet.* (2015). doi:10.1038/ng.3404

34. Gazal, S. *et al.* Linkage disequilibrium–dependent architecture of human complex traits shows action of negative selection. *Nat. Genet.* **49**, 1421–1427 (2017).

35. Gazal, S., Marquez-Luna, C., Finucane, H. K. & Price, A. L. Reconciling S-LDSC and LDAK functional enrichment estimates. *Nat. Genet.* **51**, 1202–1204 (2019).

36. Loh, P.-R., Kichaev, G., Gazal, S., Schoech, A. P. & Price, A. L. Mixed-model association for biobank-scale datasets. *Nat. Genet.* **50**, 906–908 (2018).

37. GTEx Consortium *et al.* Genetic effects on gene expression across human tissues. *Nature* **550**, 204–213 (2017).

38. Qi, T. *et al.* Identifying gene targets for brain-related traits using transcriptomic and methylomic data from blood. *Nat. Commun.* **9**, 2282 (2018).

39. O'Leary, N. A. *et al.* Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res.* **44**, D733-45 (2016).

40. Hoefs, S. J. G. *et al.* NDUFA2 complex I mutation leads to Leigh disease. *Am. J. Hum. Genet.* **82**, 1306–15 (2008).

41. Li, Z. *et al.* Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. *Nat. Genet.* **49**, 1576–1583 (2017).

42. Ikeda, M. *et al.* Genome-Wide Association Study Detected Novel Susceptibility Genes for Schizophrenia and Shared Trans-Populations/Diseases Genetic Effect. *Schizophr. Bull.* **45**, 824–834 (2019).

43. Lam, M. *et al.* Comparative genetic architectures of schizophrenia in East Asian and European populations. *Nat. Genet.* **51**, 1670–1678 (2019).

44. Lam, M. *et al.* Pleiotropic Meta-Analysis of Cognition, Education, and Schizophrenia Differentiates Roles of Early Neurodevelopmental and Adult Synaptic Pathways. *Am. J. Hum. Genet.* **105**, 334–350 (2019).

45. Nagel, M. *et al.* Meta-analysis of genome-wide association studies for neuroticism in 449,484 individuals identifies novel genetic loci and pathways. *Nat. Genet.* **50**, 920–927 (2018).

46. Lee, J. J. *et al.* Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nat. Genet.* **50**, 1112–1121 (2018).

47. Savage, J. E. *et al.* Genome-wide association meta-analysis in 269,867 individuals identifies new genetic and functional links to intelligence. *Nat. Genet.* **50**, 912–919 (2018).

48. Giri, A. *et al.* Trans-ethnic association study of blood pressure determinants in over 750,000 individuals. *Nat. Genet.* **51**, 51–62 (2019).

49. Evangelou, E. *et al.* Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat. Genet.* **50**, 1412–1425 (2018).

50. The UniProt Consortium. UniProt: the universal protein knowledgebase. *Nucleic Acids Res.* **45**, D158–D169 (2017).

51. Okbay, A. *et al.* Genome-wide association study identifies 74 loci associated with educational attainment. *Nature* **533**, 539–542 (2016).

52. Hill, W. D. *et al.* A combined analysis of genetically correlated traits identifies 187 loci and a role for neurogenesis and myelination in intelligence. *Mol. Psychiatry* **24**, 169–181 (2019).

53. Davies, G. *et al.* Study of 300,486 individuals identifies 148 independent genetic loci influencing general cognitive function. *Nat. Commun.* **9**, 2098 (2018).

54. Moore, D. L., Apara, A. & Goldberg, J. L. Krüppel-like transcription factors in the nervous system: novel players in neurite outgrowth and axon regeneration. *Mol. Cell. Neurosci.* **47**, 233–43 (2011).

55. Sekar, A. *et al.* Schizophrenia risk from complex variation of complement component 4. *Nature* **530**, 177–83 (2016).

56. Yanagi, M. *et al.* Expression of Kruppel-like factor 5 gene in human brain and association of the gene with the susceptibility to schizophrenia. *Schizophr. Res.* **100**, 291–301 (2008).

57. Wray, N. R. *et al.* Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nat. Genet.* **50**, 668–681 (2018).

58. Nolte, I. M. *et al.* Missing heritability: is the gap closing? An analysis of 32 complex traits in the Lifelines Cohort Study. *Eur. J. Hum. Genet.* **25**, 877–885 (2017).

59. Marigorta, U. M. & Navarro, A. High trans-ethnic replicability of GWAS results implies common causal variants. *PLoS Genet.* **9**, e1003566 (2013).

60. Palmer, C. & Pe'er, I. Statistical correction of the Winner's Curse explains replication variability in quantitative trait genome-wide association studies. *PLOS Genet.* **13**, e1006916 (2017).

61. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B* **57**, 289–300 (1995).

62. Kanai, M. *et al.* Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nat. Genet.* **50**, 390–400 (2018).

63. Price, A. L. *et al.* Discerning the ancestry of European Americans in genetic association studies. *PLoS Genet.* **4**, e236 (2008).

64. Milne, R. L. *et al.* Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. *Nat. Genet.* **49**, 1767–1778 (2017).

65. Lee, S. H., Wray, N. R., Goddard, M. E. & Visscher, P. M. Estimating Missing Heritability for Disease from Genome-wide Association Studies. *Am. J. Hum. Genet.* **88**, 294–305 (2011).

66. Golan, D., Lander, E. S. & Rosset, S. Measuring missing heritability: Inferring the contribution of common variants. *Proc. Natl. Acad. Sci. U. S. A.* **111**, E5272-81 (2014).

67. Lloyd-Jones, L. R., Robinson, M. R., Yang, J. & Visscher, P. M. Transformation of Summary Statistics from Linear Mixed Model Association on All-or-None Traits to Odds Ratio. *Genetics* **208**, 1397–1408 (2018).

68. Chang, C. C. *et al.* Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**, 7 (2015).

69. van Rheenen, W., Peyrot, W. J., Schork, A. J., Lee, S. H. & Wray, N. R. Genetic correlations of polygenic disease traits: from theory to practice. *Nat. Rev. Genet.* **20**, 567–581 (2019).

70. Sullivan, P. F. & Geschwind, D. H. Defining the Genetic, Genomic, Cellular, and Diagnostic Architectures of Psychiatric Disorders. *Cell* **177**, 162–183 (2019).

71. Molodecky, N. A. *et al.* Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* **142**, 46-54.e42; quiz e30 (2012).

72. Yengo, L., Yang, J. & Visscher, P. M. Expectation of the intercept from bivariate LD score regression in the presence of population stratification. *bioRxiv* 310565 (2018).

doi:10.1101/310565

73. R Core Team. R: A Language and Environment for Statistical Computing. (2018).

Acknowledgements

We thank A. Schoech, L. O'Connor, O. Weissbrod, S. Gazal, D. Ruderfer, B.W.J.H. Penninx, N.R. Wray, K. Kendler, J. Smoller, W. van Rheenen, and the members of the Cross-Disorder Group of the Psychiatric Genomics Consortium for helpful discussions. We thank S. Ripke and V. Trubetskoy for providing information about sample-overlap. This research was funded by NIH grants R01 HG006399, R01 MH107649, and R01 MH101244, and NWO Veni grant (91619152) to W.J.P.

Author contribution

W.J.P. and A.L.P. designed experiments, W.J.P. performed experiments and analysed data. W.J.P. and A.L.P. wrote the manuscript.

Competing interests

The authors declare no competing interests.

									Number of significant independent loci			
											CC-GWAS	
			A1A0		B1B0							**CC-GWAS**
A1A0 (N case/ N control)	B1B0 (N case/ N control)	# SNPs	*K (%)*	h^2	*K (%)*	h^2	r_g	OLS weights	A1A0	B1B0	**all**	**specific**
SCZ (40,675/64,643)	BIP (20,352/31,358)	4,548,414	0.40	0.20	1.00	0.20	0.70	0.55/-0.43	139	15	**12**	**7**
SCZ (40,675/64,643)	MDD (170,756/329,443)	4,483,387	0.40	0.20	16.00	0.10	0.31	0.77/-0.51	139	50	**99**	**10**
BIP (20,352/31,358)	MDD (170,756/329,443)	6,265,453	1.00	0.20	16.00	0.10	0.33	0.58/-0.43	14	53	**10**	**4**

Table 1. Summary of CC-GWAS results for schizophrenia, bipolar disorder and major depressive disorder.

For each pair of schizophrenia (SCZ)[15], bipolar disorder (BIP)[16] and major depressive disorder (MDD)[17], we report the case-control sample sizes, #SNPs, prevalence (K)[70], liability-scale heritability estimated using stratified LD score regression[33–35] (h^2), genetic correlation estimated using cross-trait LD score regression[2] (r_g), OLS weights, number of independent genome-wide significant loci for each case-control comparison, number of independent genome-significant CC-GWAS loci, and number of independent genome-significant CC-GWAS loci that are CC-GWAS-specific. Exact weights are equal to $(1 - K_A)$ for disorder A and – $(1 - K_B)$ for disorder B.

Disorder							A1A0		B1B0		A1B1 (OLS)	
A	B	SNP	Chr	Position	Freq	Locus name	Beta	P	Beta	P	Beta	P
SCZ	BIP	rs9866687^a	3	94,828,190	0.44	LINC00879	1.24e-02	1.38e-04	-1.45e-02	1.70e-03	1.30e-02	4.05e-08
SCZ	BIP	rs7790864^a	7	28,478,625	0.38	CREB5	1.46e-02	7.18e-06	-1.23e-02	7.93e-03	1.32e-02	2.18e-08
SCZ	BIP	rs12554512	9	23,352,293	0.43	ELAVL2	-6.22e-03	5.54e-02	2.25e-02	1.28e-06	-1.30e-02	4.06e-08
SCZ	BIP	rs3764002	12	108,618,630	0.26	WSCD2^c	1.62e-02	6.05e-07	-1.54e-02	9.04e-04	1.55e-02	6.33e-11
SCZ	BIP	rs9319540^a	16	79,458,022	0.58	MAF	1.22e-02	1.84e-04	-1.49e-02	1.26e-03	1.30e-02	3.67e-08
SCZ	BIP	rs1054972	19	1,852,582	0.2	KLF16^c	-1.42e-02	1.32e-05	1.31e-02	4.74e-03	-1.33e-02	1.75e-08
SCZ	BIP	rs11696888	20	47,753,265	0.43	CSE1L^b	-1.21e-02	1.94e-04	1.80e-02	1.05e-04	-1.43e-02	1.39e-09
SCZ	MDD	rs2471403	2	48,490,508	0.48	FOXN2^b	-1.70e-02	1.78e-07	3.08e-03	6.21e-02	-1.47e-02	2.34e-08
SCZ	MDD	rs16846133^a	2	212,289,728	0.31	ERBB4	-1.63e-02	5.68e-07	4.43e-03	5.84e-03	-1.48e-02	1.71e-08
SCZ	MDD	rs2563297	5	140,097,072	0.44	PCDHA7^b	1.60e-02	8.76e-07	-6.00e-03	3.73e-04	1.54e-02	5.25e-09
SCZ	MDD	rs113113059	6	43,160,375	0.19	CUL9^b	-1.68e-02	2.37e-07	4.84e-03	2.93e-03	-1.55e-02	4.11e-09
SCZ	MDD	rs2944833	7	71,774,496	0.57	CALN1^b	-1.70e-02	1.80e-07	2.52e-03	1.21e-01	-1.44e-02	4.22e-08
SCZ	MDD	rs71523422^a	8	31,445,336	0.08	NRG1	-1.57e-02	1.41e-06	4.69e-03	3.82e-03	-1.45e-02	3.38e-08
SCZ	MDD	rs10967586^a	9	26,895,808	0.13	CAAP1	1.67e-02	2.87e-07	-4.57e-03	4.60e-03	1.52e-02	6.94e-09
SCZ	MDD	rs17731	10	3,821,561	0.35	KLF6^c	1.67e-02	2.86e-07	-3.39e-03	3.89e-02	1.46e-02	2.64e-08
SCZ	MDD	rs34232444	19	4,965,404	0.3	UHRF1	-1.45e-02	8.70e-06	7.66e-03	2.56e-06	-1.51e-02	9.92e-09
SCZ	MDD	rs8137258^a	22	20,135,961	0.22	ZDHHC8	1.59e-02	9.87e-07	-5.65e-03	4.50e-04	1.52e-02	7.82e-09
BIP	MDD	rs28565152	5	7,542,911	0.25	ADCY2	2.35e-02	3.83e-07	-3.77e-03	2.23e-02	1.53e-02	2.79e-08
BIP	MDD	rs12538191^a	7	44,980,824	0.24	SNHG15^b	-2.44e-02	1.46e-07	2.81e-03	8.21e-02	-1.54e-02	2.36e-08
BIP	MDD	rs4447398	15	42,904,904	0.88	LRRC57^b	-2.46e-02	1.10e-07	2.54e-03	1.22e-01	-1.54e-02	2.28e-08
BIP	MDD	rs11908600	20	43,633,418	0.3	STK4^b	-2.34e-02	4.26e-07	3.53e-03	2.90e-02	-1.51e-02	4.16e-08

Table 2. List of 21 CC-GWAS-specific loci for SCZ, BIP and MDD.

For each CC-GWAS-specific locus, we report the lead CC-GWAS SNP and its chromosome, physical position, and reference allele frequency, the locus name, the respective case-control effect sizes and *p*-values, and the OLS case-case effect size and *p*-value. Effect sizes are reported on the standardized observed scale based on 50/50 case-control ascertainment. [a]denotes loci that have not been reported previously[30]. [b]denotes locus names based on (most) significant SMR results. [c]denotes locus names based on exonic lead SNPs. Remaining locus names are based on nearest gene, and do not refer to any inferred biological function. Case-case effect sizes and *p*-values for the Exact approach are reported in Table S6. SCZ, schizophrenia; BIP, bipolar disorder; MDD, major depressive disorder.

Disorder	N case	N control	*K (%)*	h^2	# loci
SCZ	40,675	64,643	0.40	0.20	139
BIP	20,352	31,358	1.00	0.20	15
MDD	170,756	329,443	16.00	0.10	50
ADHD	19,099	34,194	5.30	0.25	9
ANO	16,992	55,525	0.90	0.15	7
ASD	18,381	27,969	1.70	0.11	2
OCD	2,688	7,037	1.10	0.25	0
TS	4,819	9,488	0.50	0.18	1

Table 3. List of eight psychiatric disorders.

For each of schizophrenia (SCZ)[15], bipolar disorder (BIP)[16], major depressive disorder (MDD)[17], attention deficit/hyperactivity disorder (ADHD)[18], anorexia nervosa (ANO)[19], autism spectrum disorder (ASD)[20], obsessive–compulsive disorder (OCD)[21], Tourette's Syndrome and Other Tic Disorders (TS)[22], we report the case-control sample size, prevalence (K)[70], liability-scale heritability estimated using stratified LD score regression[33–35] (h^2), and number of independent genome-wide significant case-control loci.

rg\# loci	SCZ	BIP	MDD	ADHD	ANO	ASD	OCD	TS
SCZ	-	12 (7)	99 (10)	43 (14)	41 (5)	41 (9)	1 (1)	19 (6)
BIP	0.70	-	10 (4)	8 (6)	5 (2)	3 (0)	1 (1)	5 (3)
MDD	0.31	0.33	-	9 (2)	6 (1)	3 (2)	0 (0)	0 (0)
ADHD	0.16	0.18	0.44	-	4 (3)	1 (0)	2 (2)	2 (2)
ANO	0.26	0.10	0.28	0.01	-	1 (1)	0 (0)	2 (1)
ASD	0.25	0.17	0.34	0.37	0.11	-	1 (1)	1 (1)
OCD	0.32	0.27	0.25	-0.20	0.42	0.10	-	1 (1)
TS	0.11	0.08	0.23	0.19	0.08	0.16	0.50	-

Table 4. Summary of CC-GWAS results for eight psychiatric disorders.
For each pair of disorders, we report the genetic correlation estimated using cross-trait LD score regression[2] (r_g) (lower left) and the number of independent genome-significant CC-GWAS loci (number of CC-GWAS-specific loci in parentheses) (upper right). The OLS weights and number of SNPs tested are reported in Table S7. SCZ, schizophrenia; BIP, bipolar disorder; MDD, major depressive disorder; ADHD, attention deficit/hyperactivity disorder; ANO, anorexia nervosa; ASD, autism spectrum disorder; OCD, obsessive–compulsive disorder; TS, Tourette's Syndrome and Other Tic Disorders.

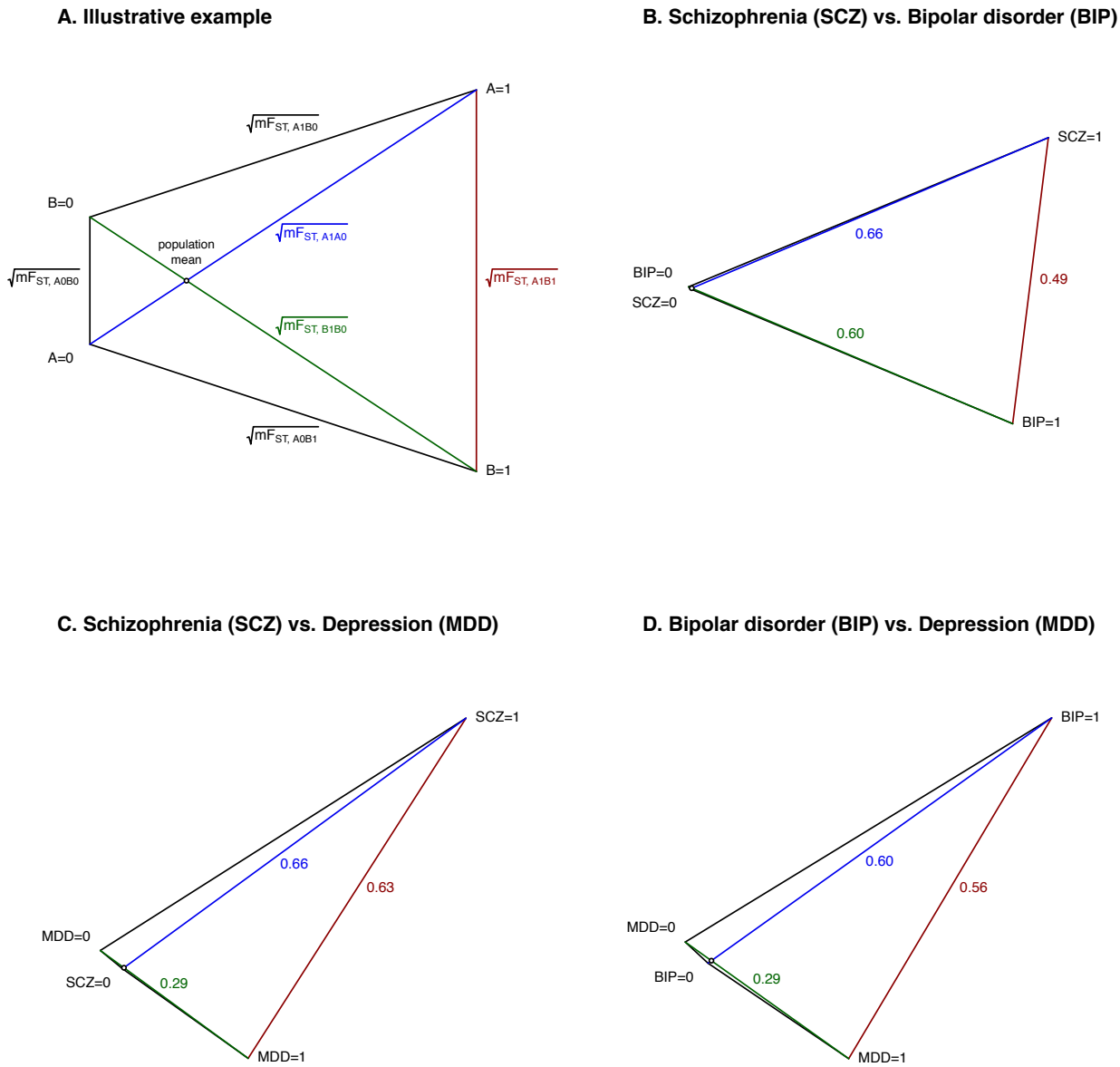

Figure 1. Genetic distance between cases and/or controls of SCZ, BIP and MDD.
We report genetic distances for (A) an illustrative example, (B) SCZ vs. BIP, (C) SCZ vs. MDD and (D) SCZ vs. BIP. Genetic distances are displayed as $\sqrt{m * F_{ST,causal}}$, where m is the number of independent causal variants and the square root facilitates 2-dimensional visualization. The quantity $m * F_{ST,causal}$ is derived based on the respective population prevalences, SNP-based heritabilities and genetic correlations (reported in Table 1). Approximate standard errors of $m * F_{ST,causal,A1B1}$ are 0.04 for SCZ vs. BIP, 0.02 for SCZ vs. MDD and 0.03 for BIP vs. MDD (see Methods). The cosine of the angle between the lines A1-A0 and B1-B0 is equal to the genetic correlation between disorder A and disorder B (see Methods). Numerical results are reported in Table S1.

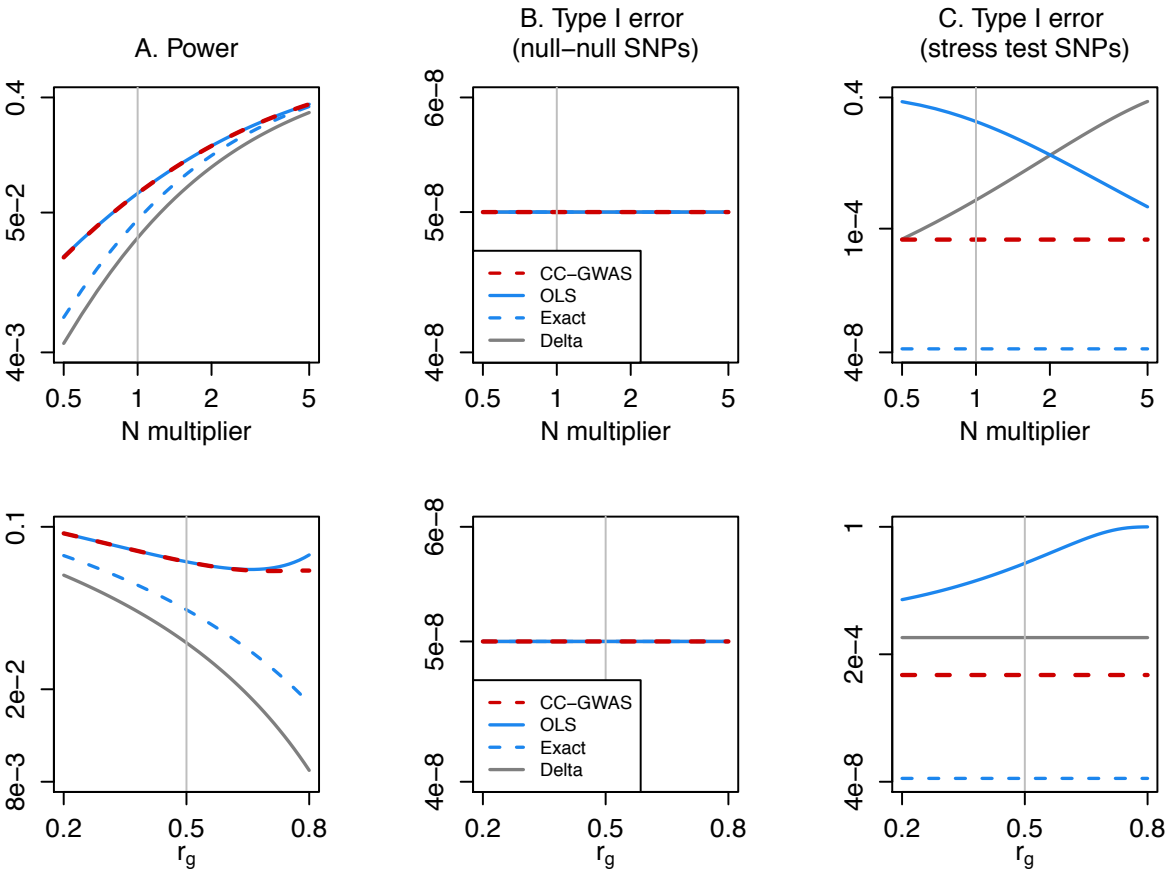

Figure 2. Power and type I error of CC-GWAS.
We report (A) the power to detect SNPs with effect sizes following a bivariate normal distribution, (B) the type I error rate for loci with no effect on A1A0 or B1B0 ("null-null" SNPs) and (C) the type I error rate for SNPs with the same allele frequency in A1 vs. B1 that explain 0.10% of variance in A1 vs. A0 and 0.29% of variance in B1 vs. B0 ("stress test" SNPs), for each of four methods: CC-GWAS, the OLS approach, the Exact approach, and a naïve Delta method (see text). Default parameter settings are: h^2=0.2, prevalence K=0.01, and sample size 100,000 cases + 100,000 controls for disorder A; liability-scale h^2=0.1, prevalence K=0.15, and sample size 100,000 cases + 100,000 controls for disorder B; m=5,000 causal SNPs for each disorder; and genetic correlation r_g=0.5 between disorders. Numerical results of these analytical computations are reported in Table S2, and confirmed with simulation results in Table S3.

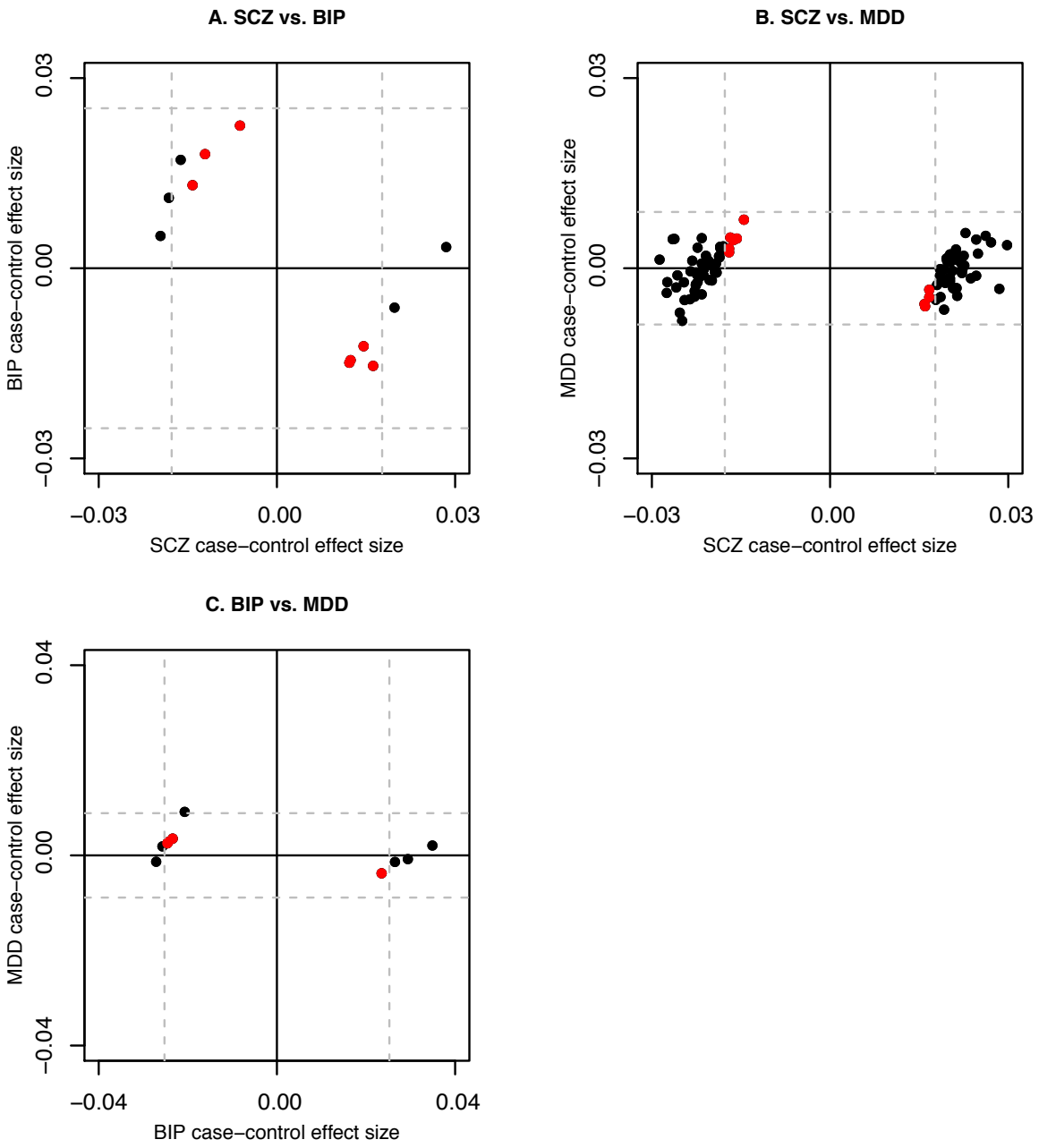

Figure 3. Case-control effect sizes at CC-GWAS loci for SCZ, BIP and MDD.
We report the respective case-control effect sizes for lead SNPs at CC-GWAS loci for (A) SCZ vs. BIP, (B) SCZ vs. MDD and (C) BIP vs. MDD. Effect sizes are reported on the standardized observed scale based on 50/50 case-control ascertainment. Red points denote CC-GWAS-specific loci, and black points denote remaining loci. Dashed lines denote effect-size thresholds for genome-wide significance. All red points (denoting lead SNPs for CC-GWAS-specific loci) lie inside the dashed lines for both disorders; in panel A, one black point (denoting the lead SNP for a CC-GWAS locus that is not CC-GWAS-specific) lies inside the dashed lines for both SCZ and BIP, because the lead SNP is not genome-wide significant for SCZ but is in LD with a SNP that is genome-wide significant for SCZ. Numerical results are reported in Table S6. SCZ, schizophrenia; BIP, bipolar disorder; MDD, major depressive disorder.

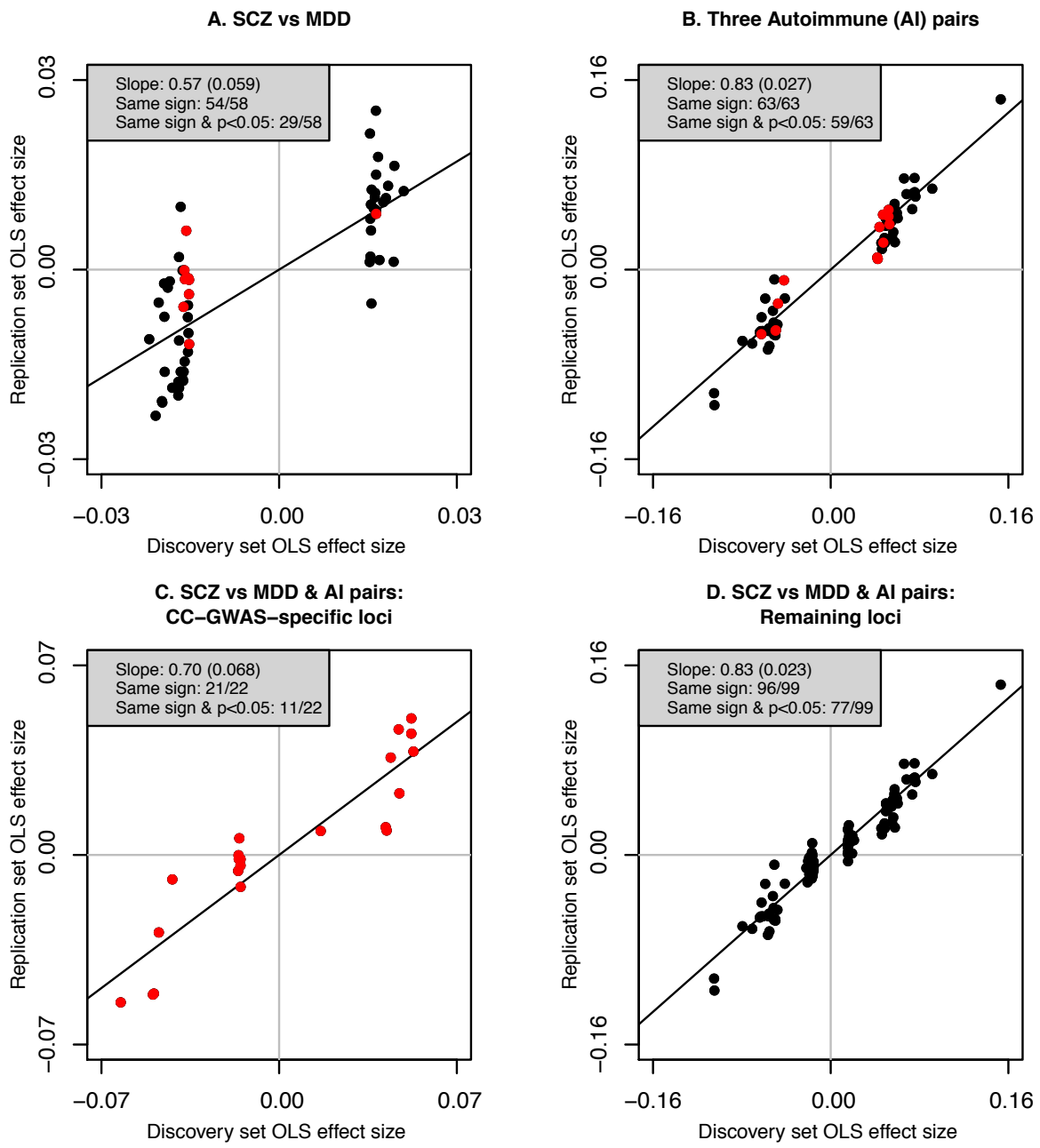

Figure 4. Independent replication of CC-GWAS results.
We report replication case-case OLS effect sizes vs. discovery case-case OLS effect sizes for (A) schizophrenia (SCZ) vs. major depressive disorder (MDD), (B) three autoimmune disorders, (C) SCZ vs. MDD and three autoimmune disorders, restricting to CC-GWAS-specific loci, and (D) SCZ vs. MDD and three autoimmune disorders, restricting to remaining loci. We also report regression slopes (SE in parentheses), effect sign concordance, and effect sign concordance together with replication P_{OLS}<0.05. Red points denote CC-GWAS-specific loci, and black points denote remaining loci. Numerical results are reported in Table S18, and corresponding case-control replication results are reported in Figure S12 and Table S19.