# S1 Text

## Quality control of NCMH genetic data

DNA samples were collected from venous blood or saliva, where possible. The samples were genotyped using a customised version of the PsychChip in several batches, with rigorous quality control (QC) procedures. QC was performed within batch, as follows: SNPs were aligned to the Haplotype Reference Consortium1 data using GenomeHarmoniser2, SNPs were removed if they had MAF<0.01, genotyping rate<0.95, or HWE p<10-6, and individuals were removed if they had missingness>0.05, sex discrepancy, or were duplicate samples. The data were merged with other samples on the same or equivalent platform and the QC parameters were reapplied, then the samples were imputed using the Michigan Imputation Server (using Eagle v2.4 for phasing, Minimac4 for imputation, and the HRC V1.1 imputation reference panel)3. After imputation, dosage data were converted to best guess genotype data using Plink24.

Post-imputation QC filters were genotype probability >0.9 per individual, missingness <0.03, MAF>0.01, HWE>10-4, and INFO/r2>0.8. Batches were merged, including only overlapping SNPs and excluding ambiguous (CT/AG) variants and SNPs with inconsistent alleles. Family relationships were confirmed using identity-by-descent (IBD) in PLINK. Control individuals were excluded if they had a relative amongst the cases. PCAiR5, a package that robustly estimates population structure while taking into account kinship information in the sample, was used to run a principal components analysis (PCA), on an LD-pruned set of common (MAF>0.05) markers and non-European samples were excluded, given that the discovery genome-wide association studies (GWAS) available were of primarily European ancestries6. A GWAS of batch was run on unrelated samples and SNPs associated with batch (p<0.01) were excluded. PCAiR was run again on the final set of markers to extract the top 5 PCs to use as covariates. The cumulative variance explained by the top 5 PCs was 0.27% and no PC beyond that explained more than 0.0517% of the genotypic variance.

## PRS calculation

Polygenic risk scores were derived using PLINK version 1.97 based on 6 large psychiatric disorder discovery GWAS of primarily European ancestries, with no overlap with the target sample. Data for ADHD PRS for 1 individual from the target sample was excluded as they were included in the published ADHD GWAS. For each discovery GWAS, we selected common (MAF>0.05) variants that overlapped with the target data and performed LD-clumping in PLINK (--clump-kb 500 --clump-r2 0.2) to obtain an independent set of SNPs, while retaining the most significant SNP in each LD (linkage disequilibrium) block. For schizophrenia PRS, we additionally excluded all variants in the extended major histocompatibility complex (MHC), region (chromosome 6, base positions 25–35Mb) to avoid potential bias by extensive LD in this region. PRS were calculated for each individual by summing the number of alleles (weighted by the log of the odds ratio) across the set of SNPs in PLINK (using the command --score). We calculated PRS using 7 different p-value thresholds to select SNPs (pT<1, pT<0.5, pT<0.1, pT<0.05, pT<0.01, pT<0.001, pT<0.00001).

For each discovery phenotype, we then performed PCA of the correlation matrix of these 7 PRS and extracted the first PC for analyses, following the PRS-PCA method, an approach that reduces overfitting and has been shown to maintain good power8. The sign of the loadings of the PRS variables on the first PCs is arbitrary and therefore PRS-PCs that were negatively correlated with the raw PRS variables were inverted. For each of the 6 discovery phenotypes, the first PRS-PCs explained between 64.2–78.3% of the variation in the different p-value threshold PRS in NCMH. The PRS-PCs were standardised using z-score transformations for each analysis.

## Copy number variant (CNV) calling and quality control

CNVs were called in NCMH using PennCNV following standard protocol9. CNV calls were merged if the distance separating two CNVs was less than 50% of their combined length. CNV QC was restricted to samples passing SNP-based QC, which included removal of ancestry outliers; see above for details. Individuals were also excluded if they were outliers on any of the following QC metrics generated by PennCNV: Log R Ratio standard deviation (LRR SD) >0.2, waviness factor (WF) >0.03 or <-0.03 and total number of CNVs >100. Following exclusion of these poorly performing samples, CNV QC was performed. CNV calls were filtered out based on the following criteria: size (<100kb), coverage (<20 probes), and PennCNV confidence score (<10).

We identified 54 CNVs impacting on 34 well-defined regions (including duplications and deletions at same loci) implicated in neurodevelopmental disorders (NDs), based on previously published criteria10. To be called as genuine ND CNVs, called CNVs underwent further checks that are specific to each locus. These checks included one or more of the following: a) the CNV is required to hit specific genes at a locus, b) exons of a gene may be required to be hit, c) the CNV may be required to be a certain length (e.g. greater than 1MB), and d) the CNV may be required to be longer than a certain percentage of the “critical region”. See published work10 for the full list of criteria. Visual inspection of B allele frequency (BAF) and LRR at these loci was performed to confirm these CNVs.

Additional QC steps were then performed on the full set of called CNVs to obtain information on burden of large rare CNVs in each individual. CNVs were further filtered to those that are >500kb in size, and have good probe density (>20kb/probe, calculated as size/probes per CNV). CNVs spanning more than 50% of any of the following regions were excluded: centromeres, telomeres (100kb from ends of chromosomes), known segmental duplications, and immunoglobulin or T cell receptor loci. CNV loci occurring at >1% frequency in the remaining set of samples were removed.

Dichotomous variables were derived for presence of a neurodevelopmental CNV >100kb, any large (>500kb) CNV, as well as large duplications and deletions separately.

## PGC clinical MDD sample

Age-at-onset of MDD was defined as the age at which individuals self-reported first having symptoms meeting MDD (10,30). Age-at-onset was recoded as missing if it was greater than the age of the participants at interview. Individuals missing information on age-at-onset who were younger than 26 years old at assessment (N=41) were also included in the early onset group. Due to small sample sizes in some of the sub-groups, analyses were restricted to studies with N≥50 total samples and ≥10 individuals on the smaller stratum (2 studies were excluded from the early-onset analysis and 4 studies were excluded from the later onset analysis).

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# Supplementary Tables

## S1 Table: Clinical and socioeconomic characteristics of males and females with anxiety and depression in NCMH

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Phenotype (continuous)** | **Males, Mean(SE)** | **Females, Mean(SE)** | **OR(95% CI)** | **P** |
| **Age at assessment\*** | 42.6 (0.4) | 41.0 (0.3) | 0.99 (0.99-1.00) | 3.5 x 10-4 |
| **Age of onset (any psychiatric problems)** | 22.6(0.4) | 20.6(0.2) | 0.99 (0.98-1.00) | 1.4 x 10-3 |
| **Age of access to services** | 29.9(0.4) | 26.8(0.3) | 0.98 (0.97-0.99) | 1.2 x 10-7 |
| **Age of access to treatment** | 30.1(0.7) | 26.7(0.4) | 0.97 (0.95-0.98) | 1.1 x 10-7 |
| **HADS current anxiety symptoms** | 11.6(0.2) | 11.2(0.2) | 0.97 (0.95-1.00) | 0.027 |
| **HADS current depression symptoms** | 9.5(0.3) | 7.7(0.2) | 0.94 (0.92-0.96) | 5.9 x 10-9 |
| **Phenotype (dichotomous)** | **Males, N(%)** | **Females, N(%)** | **OR(95% CI)** | **P** |
| **NDs** | 429(29.7) | 424(15.5) | 0.37 (0.32-0.44) | 9.7 x 10-33 |
| **OCD** | 146(10.1) | 249(9.1) | 0.87 (0.70-1.07) | 0.19 |
| **PTSD** | 373(25.8) | 528(19.3) | 0.70 (0.60-0.81) | 2.9 x 10-6 |
| **Eating disorders** | 44(3.0) | 340(12.4) | 4.41 (3.19-6.08) | 1.7 x 10-19 |
| **Substance misuse** | 222(15.4) | 214(7.8) | 0.48 (0.39-0.58) | 3.6 x 10-13 |
| **Personality disorder** | 74(5.1) | 215(7.9) | 1.52 (1.16-2.00) | 2.6 x 10-3 |
| **Low income** | 667(58.2) | 1270(59.2) | 1.05 (0.91-1.21) | 0.51 |
| **Low education** | 154(12.1) | 209(8.5) | 0.70 (0.56-0.88) | 2.2 x 10-3 |
| **NEET** | 437(39.3) | 619(30.0) | 0.67 (0.58-0.79) | 5.5 x 10-7 |

\* Age at assessment is included as a covariate in all other analyses.

HADS: Hospital Anxiety and Depression Scale; NDs: neurodevelopmental disorders; OCD: obsessive compulsive disorder; PTSD: post-traumatic stress disorder; NEET: not in education, employment or training. Males are coded as 0, females are coded as 1; therefore OR>1 indicates females have a higher likelihood for a given phenotype.

## S2 Table: Association of psychiatric polygenic risk scores with anxiety/depression case-control status in NCMH, in the full sample and split by sex

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **PRS** | **Full sample** | | | **Males** | | | | **Females** | | |
| **OR(95% CI)** | **P** | **R2** | **OR(95% CI)** | **P** | **R2** | **OR(95% CI)** | | **P** | **R2** | |
| **ADHD** | 1.28 (1.09–1.51) | 2.7x10-3 | 0.011 | 1.43 (1.13-1.81) | 2.9x10-3 | 0.025 | 1.18 (0.94-1.46) | | 0.15 | 4.6x10-3 | |
| **ANX** | 1.36 (1.15–1.61) | 4.0x10-4 | 0.018 | 1.42 (1.10-1.84) | 7.7x10-3 | 0.024 | 1.33 (1.05-1.67) | | 0.016 | 0.014 | |
| **ASD** | 1.20 (1.02–1.40) | 0.026 | 5.8x10-3 | 1.26 (1.02-1.56) | 0.034 | 0.010 | 1.16 (0.93-1.45) | | 0.20 | 3.8x10-3 | |
| **BD** | 1.17 (0.97–1.41) | 0.10 | 4.5x10-3 | 1.22 (0.92-1.60) | 0.17 | 7.0x10-3 | 1.16 (0.90-1.50) | | 0.24 | 4.0x10-3 | |
| **MDD** | 1.49 (1.25–1.79) | 1.3x10-5 | 0.029 | 1.37 (1.04-1.79) | 0.023 | 0.019 | 1.62 (1.28-2.05) | | 5.7x10-5 | 0.039 | |
| **SCZ** | 1.12 (0.93–1.35) | 0.22 | 2.4x10-3 | 1.18 (0.92-1.52) | 0.20 | 5.2x10-3 | 1.11 (0.86-1.44) | | 0.43 | 1.9x10-3 | |

ADHD: attention deficit hyperactivity disorder; ANX: anxiety disorders; ASD: autism spectrum disorder; BD: bipolar disorder; MDD: major depressive disorder; PRS: polygenic risk score; SCZ: schizophrenia. Controls are coded as 0, cases with anxiety/depression are coded as 1; therefore OR>1 indicates a higher PRS in cases and indicates the effect size per 1 SD of the PRS.

## S3 Table: Association of polygenic risk scores for ADHD (primary analysis) and other psychiatric disorders (exploratory analysis) with sex of individuals with anxiety and depression in NCMH, after adjusting for comorbid neurodevelopmental disorders

|  |  |  |  |
| --- | --- | --- | --- |
| **PRS** | **OR(95% CI)** | **P** | **R2** |
| **ADHD** | 1.03 (0.92-1.16) | 0.57 | 3.2 x 10-4 |
| **ANX** | 0.93 (0.83-1.04) | 0.21 | 1.6 x 10-3 |
| **ASD** | 1.07 (0.95-1.20) | 0.24 | 1.4 x 10-3 |
| **BD** | 1.02 (0.91-1.15) | 0.68 | 1.7 x 10-4 |
| **MDD** | 1.01 (0.90-1.13) | 0.88 | 2.5 x 10-5 |
| **SCZ** | 0.95 (0.84-1.06) | 0.34 | 9.3 x 10-4 |

ADHD: attention deficit hyperactivity disorder; ANX: anxiety disorders; ASD: autism spectrum disorder; BD: bipolar disorder; MDD: major depressive disorder; PRS: polygenic risk score; SCZ: schizophrenia. Males are coded as 0, females are coded as 1; therefore OR>1 indicates a higher PRS in females and indicates the effect size per 1 SD of the PRS.

## S4 Table: Association of psychiatric polygenic risk scores with sex of individuals diagnosed with MDD in the PGC replication sample, stratified by age-at-onset

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Age-at-onset** | **PRS** | **Males** | **Females** | **OR(95% CI)** | **P** | **R2** |
| **Young (<26 years old)** | **ANX** | 1804 | 4411 | 1.01 (0.96-1.08) | 0.63 | 4.8 x 10-3 |
| **SCZ** | 1804 | 4411 | 1.00 (0.94-1.06) | 1.00 | 4.9 x 10-3 |
| **Older (>25 years old)** | **ANX** | 2100 | 3752 | 0.93 (0.88-0.99) | 0.017 | 4.7 x 10-3 |
| **SCZ** | 2100 | 3752 | 1.00 (0.94-1.05) | 0.86 | 2.2 x 10-3 |

ADHD: attention deficit hyperactivity disorder; ANX: anxiety disorders; MDD: major depressive disorder; SCZ: schizophrenia. Males are coded as 0, females are coded as 1.

# Supplementary Figures

Chart, scatter chart

Description automatically generated

## S1 Fig. Forest plot of meta-analysis results for 20 PGC studies for the association between polygenic risk scores for ADHD with sex in individuals diagnosed with major depressive disorder (females coded as 1 and males coded as 0).

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