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A cross-platform Mendelian randomization study of mitochondrial DNA copy number across psychiatric disorders

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Abstract

Mitochondrial DNA copy number (mtDNA-CN) is an accessible peripheral proxy of mitochondrial biology, but its relationship to psychiatric liability remains difficult to interpret because blood mtDNA-CN is influenced by assay platform, tissue context and blood-cell composition. We used two-sample Mendelian randomization to evaluate genetically proxied mtDNA-CN across psychiatric phenotypes using four partially overlapping European-ancestry GWAS exposure panels spanning qPCR, SNP-array intensity and whole-genome-sequencing-derived measures with and without blood-cell-composition adjustment. Because the exposure panels substantially overlapped through UK Biobank, panel-level estimates were treated as correlated and synthesized with a design-effect correction rather than as independent replications. Among the five primary disorders, genetically proxied higher mtDNA-CN was associated with lower liability to autism spectrum disorder (ASD; β = -0.182, design-effect-corrected p = 5.97 × 10^-3; BH-FDR q = 0.030). ADHD showed a weaker directionally concordant estimate that did not survive correction, and the ASD-ADHD neurodevelopmental factor showed a similar nominal pattern but should not be interpreted as independent replication. No robust associations were observed for schizophrenia, bipolar disorder, major depressive disorder, general psychopathology or other disorder-class factors. Reverse-direction MR provided no evidence that psychiatric liability influenced mtDNA-CN, although instrument power was limited for several outcomes. Gene-set enrichment supported the mitochondrial relevance of the mtDNA-CN instruments, whereas brain eQTL colocalization nominated hypothesis-generating candidate genes without establishing mediation. These findings support an ASD-led inverse association between genetically proxied mtDNA-CN and neurodevelopmental liability, while broader mechanistic interpretations require validation.

Introduction

Each nucleated cell carries hundreds to thousands of copies of the circular mitochondrial genome, which encodes core components of the respiratory chain [1]. Oxidative phosphorylation is driven by a chemiosmotic proton gradient across the inner mitochondrial membrane [2]. Mitochondria also regulate cellular metabolism, calcium handling and apoptotic signaling [3–6]. Mitochondrial DNA copy number (mtDNA-CN), the per-cell abundance of mitochondrial genomes, is therefore often used as an accessible blood-based proxy for mitochondrial biogenesis and function. However, peripheral-blood mtDNA-CN depends on tissue context, assay method and nucleated blood-cell composition [7]. This limitation is particularly relevant in psychiatric genetics, where blood-derived molecular traits may capture systemic and hematological variation as well as mitochondrial biology. During neurodevelopment, when neuronal differentiation, synaptogenesis and circuit maturation impose high energetic demands, genetically regulated variation in mitochondrial biogenesis may be especially relevant but difficult to interpret observationally.

Observational studies have reported altered mtDNA-CN or mitochondrial abnormalities in several psychiatric phenotypes. In ADHD, peripheral mtDNA-CN has been reported to be increased and associated with symptom severity or treatment course, although available studies remain

heterogeneous [8–10]. In ASD, mitochondrial dysfunction and altered peripheral mtDNA-CN have been repeatedly described, including systematic-review, mechanistic-review and case-control evidence [11–13]. Evidence in bipolar disorder, major depressive disorder and schizophrenia is more variable, with inconsistent direction across leukocyte mtDNA-CN, circulating cell-free mitochondrial DNA, peripheral blood and postmortem brain studies [14–20]. A central question is therefore whether peripheral mtDNA-CN associations reflect upstream liability or downstream correlates of illness course, treatment exposure, inflammation, oxidative stress and blood-cell-composition differences.

mtDNA-CN is heritable and genetically tractable. A twin and family study estimated its heritability at approximately 0.65 (95% CI 0.50–0.72; roughly 65% of the population variance in blood mtDNA-CN attributable to genetic factors) [21], and large GWASs have identified nuclear loci involved in mitochondrial biogenesis, genome maintenance, nucleotide metabolism, platelet biology and related cellular processes [22–24]. This architecture enables Mendelian randomization (MR), which uses genetic instruments to estimate the effect of a lifelong genetically proxied difference in an exposure on disease liability while reducing reverse causation and residual confounding. Large recent GWASs are available for ADHD, ASD, bipolar disorder, major depressive disorder and schizophrenia [25–29]. Prior two-sample MR studies of mtDNA-CN and psychiatric outcomes have produced inconsistent results, with inverse associations reported for ASD or broader neuropsychiatric phenotypes in some analyses and null findings in others [30–34]; these prior studies are compared systematically in Table 1. These discrepancies may reflect differences in exposure panels, instrument selection, phenotype definitions and, critically, the treatment of overlapping UK Biobank samples.

Here, we evaluated genetically proxied mtDNA-CN across five primary psychiatric disorders, nine secondary psychiatric outcomes (anxiety, OCD, PTSD, anorexia nervosa, borderline personality disorder, Tourette syndrome, postpartum depression, and two antidepressant-response phenotypes) [35–42], six cross-disorder factors from a 14-disorder genomic structural-equation model [43], and standing height and BMI as negative-control and biological-contrast outcomes; substance use disorder [44] was additionally examined but excluded from forward Mendelian randomization for missing per-variant standard errors. We used four partially overlapping mtDNA-CN GWAS exposure panels spanning three assay/data-processing approaches: qPCR quantification, SNP-array intensity estimation and WGS-derived mtDNA-CN with and without blood-cell-composition adjustment. The analytic pipeline comprised instrumental-variable (IV) selection, forward MR, design-effect-corrected cross-panel synthesis, reverse-direction MR, negative controls, gene-set enrichment and exploratory brain eQTL colocalization (Fig. 1).

Materials and Methods

Data sources

GWAS summary statistics for mtDNA-CN were obtained from four large European-ancestry exposure panels derived from three source studies: Longchamps et al. [22], a qPCR meta-analysis of CHARGE and UK Biobank (N = 465 809); Chong et al. [23], an SNP-array AutoMitoC analysis in UK Biobank (N = 383

476); and two WGS-derived panels from Gupta et al. [24], comprising unadjusted and blood-cell-composition-adjusted mtDNA-CN phenotypes (N = 155 998 each). The two Gupta panels share the same individuals and WGS assay and differ only by blood-cell-composition adjustment; the adjusted panel was treated as a residualized mtDNA-CN measure. Detailed WGS variant mapping and exposure-level preprocessing are described in Supplementary Methods 1.

Primary psychiatric outcomes comprised ADHD (38 691 cases and 186 843 controls) [25], ASD (18 381 cases and 27 969 controls) [26], bipolar disorder (BD; European summary statistics from O'Connell et al.; maximum per-variant 59 287 cases and 781 022 controls) [27], major depressive disorder (MDD; European summary statistics from Adams et al.; 412 305 cases and 1 588 397 controls) [28], and schizophrenia (SCZ; PGC3 European, UK Biobank-deduplicated; 67 323 cases and 93 456 controls) [29]. Secondary outcomes included anxiety disorders, OCD, PTSD, anorexia nervosa, borderline personality disorder, Tourette syndrome, postpartum depression, substance use disorders and two antidepressant-response phenotypes [35–42, 44]. Substance use disorders were excluded from forward MR because per-variant standard errors were unavailable. Cross-disorder factor GWASs from Grotzinger et al. [43] provided a general psychopathology pFactor (a latent dimension indexing the variance shared across all 14 disorders in the model, i.e. transdiagnostic liability) and five domain factors (F1 compulsive, F2 schizo-bipolar, F3 neurodevelopmental, F4 internalizing and F5 substance/externalizing). Standing height and BMI from Yengo et al. [45] were used as the primary negative control and biological contrast, respectively. Yengo et al. combined GIANT and UK Biobank (total N ≈ 700 000), so these outcome GWASs share the same UK Biobank component as the mtDNA-CN exposure panels; they are therefore subject to the same overlap structure and are not fully independent specificity benchmarks. The null, homogeneous height result should be interpreted as a conservative specificity check. Residual overlap inflation would be expected to inflate, not suppress, any spurious association. Full sample sizes and metadata are provided in Supplementary Table 1.

Instrument selection and harmonization

Gupta WGS variants were harmonized to dbSNP rsIDs using a 1000 Genomes Phase 3 European chromosome-position-allele lookup and allele-frequency-informed strand resolution [46]. The Longchamps meta-analysis was filtered to imputation quality INFO ≥ 0.8. Across all mtDNA-CN exposures, analyses were restricted to autosomes; the X chromosome, mitochondrial contigs and the major histocompatibility complex (MHC) region were excluded to minimize complex LD and mapping artefacts. Duplicate-position handling, tri-allelic-site exclusion, palindromic-variant rules, MHC exclusion coordinates and WGS mapping details are provided in Supplementary Methods 1.

Genome-wide significant variants ($p < 5 \times 10^{-8}$) were selected as candidate instruments for each mtDNA-CN exposure. LD clumping used PLINK v1.90b6.21 [47] and the 1000 Genomes Phase 3 European reference panel [46] with a 10 000-kb window and $r^2 < 0.001$. Per-SNP F-statistics were calculated as $F = (\beta/SE)^2$, and variants with $F < 10$ were excluded [48]. Missing outcome variants were replaced by European LD proxies at $r^2 \geq 0.8$ where possible using LDlinkR [49], with proxy alleles aligned

to the target effect allele. The complete per-pair instrument list, Steiger-filter status and F-statistic distributions are provided in Supplementary Tables 3 and 4. Instrument allele frequencies were predominantly common, with a small minority of low-frequency instruments (MAF < 5%; two with MAF < 1%); to confirm the findings were not driven by low-frequency instruments, forward MR was repeated excluding instruments with MAF < 5% and, separately, MAF < 1%, and the ASD association remained significant and, if anything, strengthened (excluding MAF < 5%: design-effect-corrected β = −0.225, p = 1.08 × 10^−3; excluding MAF < 1%: essentially unchanged; Supplementary Table 17). Genetic instruments were additionally annotated with the Ensembl BioMart Human Short Variants dataset (GRCh38.p14) for PolyPhen and SIFT predictions, motif-feature (transcription-factor binding site) consequences and global minor-allele frequency.

Mendelian-randomization analyses

Forward MR analyses were conducted in TwoSampleMR v0.7.6 [50]. Exposure and outcome alleles were harmonized using allele-frequency-informed strand inference, and Steiger filtering [51] retained SNPs explaining more variance in mtDNA-CN than in the outcome. Full harmonization settings, including TwoSampleMR::harmonise_data(action = 2), palindromic-variant handling and PTSD Z-score reconstruction, are provided in Supplementary Methods 3. The inverse-variance-weighted (IVW) estimator [52] was the primary method. MR-Egger [53], weighted median [54] and MR-RAPS [55] were used as complementary estimators under different assumptions about invalid instruments. A Radial-MR outlier screen (modified second-order-weight IVW; outlier threshold α = 0.05/n) preceded IVW estimation; no ASD instruments were flagged, and recomputing IVW on the full Steiger-filtered instrument set (Supplementary Table 3) reproduces the reported per-panel and pooled ASD estimates exactly. MR-Egger intercepts, MR-PRESSO [56], Cochran's Q and leave-one-out analyses evaluated directional pleiotropy, outliers, heterogeneity and influential variants. After excluding substance use disorder, forward MR comprised 100 exposure-outcome tests across the four exposure panels and 25 outcomes (25 × 4, all evaluable; Supplementary Tables 2 and 10).

Because all four mtDNA-CN exposure panels include UK Biobank participants, panel-level IVW estimates are positively correlated rather than independent. We therefore quantified empirical inter-panel concordance before synthesis: averaged across evaluable outcomes, the pairwise Pearson correlation of panel-specific IVW β estimates was r = 0.70, with values ranging from 0.55 to 0.90 (Supplementary Table 10). Because panel-level IVW β estimates approach normality at these sample sizes and with negligible weak-instrument bias (per-instrument F > 10), the product-moment (Pearson) correlation is appropriate here; a rank-based Spearman correlation gave concordant values (mean 0.60, range 0.36–0.82). The primary cross-panel synthesis used this observed mean correlation as the working equicorrelation parameter (ρ = 0.70), rather than selecting ρ to optimize statistical significance [57]. Under k = 4 exposure panels, this corresponds to a design-effect variance inflation of 1 + (k − 1)ρ = 3.10 and an effective number of approximately 1.3 independent exposure panels. This correction preserves the inverse-variance pooled point estimate but inflates the standard error and p value to reflect shared-

sample dependence. The covariance structure, design-effect calculation, effective number of independent exposure panels and rho-sensitivity analyses are detailed in Supplementary Methods 5 and Supplementary Tables 10, 12 and 13. Sensitivity analyses repeated the synthesis across the observed correlation range (ρ = 0.55–0.90) and at the bivariate LDSC exposure-side estimate ρ_LDSC = 0.49 using GenomicSEM [58]. A naïve REML random-effects meta-analysis in metafor [59] was retained only as a sensitivity comparison; Q and I^2 were interpreted descriptively because shared samples reduce their evidential value. Implementation details for the naïve REML meta-analysis and the interpretation of Q and I^2 under shared samples are provided in Supplementary Methods 6.

The same MR pipeline was applied to six cross-disorder factor GWASs from Grotzinger et al. [43]. The factor-level implementation and correction framework are described in Supplementary Methods 7, with estimates in Supplementary Table 6. Hierarchical BH-FDR correction was applied across three families: the five primary disorders; the six cross-disorder constructs; and nine secondary outcomes comprising anxiety, OCD, PTSD, anorexia nervosa, BPD, Tourette syndrome, postpartum depression and two antidepressant-response phenotypes. Standing height and BMI were evaluated outside the FDR framework as specificity benchmarks.

Gene-set enrichment, colocalization and sensitivity analyses

To assess mitochondrial biological relevance, mtDNA-CN GWAS signals were tested for enrichment in the canonical 1 076-gene MitoCarta3.0 set and 143 sub-pathways using MAGMA [60, 61]. Gene-level statistics used a SNP-wise mean model with 1000 Genomes European LD reference and build-37 gene coordinates. MAGMA gene-window definitions, competitive enrichment settings and sub-pathway testing are detailed in Supplementary Methods 8 and Supplementary Table 11. The same pipeline was applied to ASD, ADHD and F3 outcome GWASs to test whether neurodevelopmental outcomes converged on the same mitochondrial gene sets.

Exploratory brain eQTL colocalization used coloc.abf [62] across 13 GTEx v10 brain tissues [63] for ASD, ADHD and F3, restricted to ± 500-kb windows around disorder-associated mtDNA-CN instruments. MitoCarta3.0 genes with posterior probability of a shared causal variant (PP4) ≥ 0.50 were considered hypothesis-generating candidates. Because windows were selected on disorder-associated mtDNA-CN instruments, a genome-wide false-positive rate could not be estimated. Liftover, harmonization and GTEx brain eQTL colocalization details are provided in Supplementary Methods, with results in Supplementary Table 14.

Three sensitivity analyses addressed sample overlap and directionality. First, UK Biobank-deduplicated GWASs were used where available for BD, MDD and BPD [39, 64, 65]. Second, MRlap [66], which couples bivariate LDSC [67] to IVW, was applied to the four exposures and the five primary disorders plus F3. Third, reverse-direction MR tested psychiatric liability as the exposure against each mtDNA-CN panel for the five primary disorders, F3, anxiety and OCD, using $p < 5 \times 10^{-8}$ instruments or $p < 1 \times 10^{-5}$ where fewer than five genome-wide loci were available. Reverse MR used a Bonferroni threshold of 0.05/32.

MRlap implementation, reverse-direction instrument thresholds, Bonferroni correction and negative-control pipelines are detailed in Supplementary Methods 9–11 and Supplementary Tables 7–9. These analyses were exploratory; no analysis plan was preregistered.

Analytic decisions and deviations, including SUD exclusion and the non-preregistered status of sensitivity analyses, are summarized in Supplementary Methods 12.

Results

Primary disorder analyses

Four mtDNA-CN exposure panels were assembled with strong instruments (Supplementary Tables 2–4). Instrument counts differed across panels, with Longchamps carrying the largest number of instruments and the blood-cell-composition-adjusted Gupta panel carrying the fewest, consistent with its more residualized exposure definition. Detailed per-pair instrument counts, Steiger-filter status, F-statistic distributions and harmonized instrument metadata are provided in Supplementary Tables 3 and 4, with full per-pair MR estimates across IVW, MR-Egger, weighted median and MR-RAPS [55], which is robust to weak instruments [68], in Supplementary Table 2.

The strongest association was observed for ASD. All four panels yielded negative IVW estimates (Fig. 2a,b). This remained stable across the assumed inter-panel correlation range ($p = 0.003$ at $rho = 0.55$ to $p = 0.012$ at $rho = 0.90$; Supplementary Table 12). MR-RAPS reproduced the negative ASD direction across panels, including nominal effects for Chong, Gupta raw and Gupta adjusted, and sensitivity-estimator scatter plots are shown in Supplementary Fig. 1.

ADHD estimates were directionally concordant but did not survive correction ($\beta = -0.079$, corrected $p = 0.173$). SCZ ($\beta = -0.047$, corrected $p = 0.40$), BD ($\beta = +0.016$, corrected $p = 0.71$) and MDD ($\beta = -0.005$, corrected $p = 0.80$) showed no significant effect. After design-effect correction and BH-FDR control across the five primary disorders, only ASD remained significant. The naïve random-effects synthesis produced smaller p values, including ADHD $p = 0.019$, illustrating the anti-conservative effect of treating overlapping panels as independent. Naïve REML estimates and cross-panel heterogeneity statistics are provided in Supplementary Results and Supplementary Table 10.

Secondary outcomes and cross-disorder factors

No secondary psychiatric outcome or antidepressant-response phenotype reached significance after design-effect and BH-FDR (false-discovery-rate) correction (Fig. 3a; Supplementary Table 5). Effect estimates were near zero for anxiety, OCD, PTSD, anorexia nervosa, BPD, postpartum depression and antidepressant non-remission. Antidepressant percentage improvement showed only a nominal trend, and Tourette syndrome showed a nominal risk-direction estimate under the naïve synthesis that was non-significant after design-effect correction. These results did not support a broad effect across

compulsive, internalizing, personality-disorder or antidepressant-response phenotypes. Full secondary-outcome estimates, including nominal effects that did not survive design-effect correction or BH-FDR correction, are provided in Supplementary Results and Supplementary Table 5.

Among the six cross-disorder constructs (Fig. 3b,c; Supplementary Table 6), the general psychopathology pFactor showed no effect (β = +0.002, corrected p = 0.85). F3 neurodevelopmental liability was the only factor with a negative and directionally concordant estimate across all four panels (β = −0.087, design-effect-corrected 95% CI − 0.154 to − 0.020; corrected p = 0.010), but this did not survive BH-FDR correction within the factor family (q = 0.062). F1 compulsive, F2 schizo-bipolar and F4 internalizing factors were null, and F5 substance/externalizing showed a non-significant risk-direction trend. Because F3 is a loading-weighted composite of ASD and ADHD, it should be interpreted as a factor-level summary of shared neurodevelopmental liability rather than independent replication. Full factor-level estimates and sensitivity results are provided in Supplementary Table 6.

Cross-panel concordance and biological annotation

Across outcomes included in the cross-panel concordance analysis, pairwise inter-panel correlations of panel-specific IVW β estimates ranged from r = 0.55 to r = 0.90 (mean r = 0.70; Fig. 2c; Supplementary Table 10), supporting the use of design-effect correction. Longchamps qPCR carried the highest mean instrument count and the lowest MR-PRESSO global-test pleiotropy rate, whereas the Gupta adjusted WGS panel carried fewer instruments and a higher pleiotropy-screen rate. Within-dataset Gupta raw versus Gupta adjusted concordance was incomplete, consistent with blood-cell-composition adjustment capturing a partly distinct component of the mtDNA-CN phenotype. Cross-panel instrument-quality metrics, directional-concordance summaries and pairwise inter-panel effect-size correlations are provided in Supplementary Results and Supplementary Table 10.

Gene annotations for the genetic instruments revealed significant SNPs with consensus predicted-damaging missense variants (both PolyPhen “probably damaging” and SIFT “deleterious”) in four genes: *DGUOK*, *HABP2*, *PLAUR* and *POLG2* (Supplementary Tables 3 and 18). *DGUOK* encodes deoxyguanosine kinase. The enzyme phosphorylates deoxyguanosine and deoxyadenosine in the mitochondrial matrix [69]. Deoxyguanosine kinase is an irreplaceable member of mitochondrial purine salvage pathway; damaging mutations hinder both mtDNA maintenance and bioenergetic output via limiting these purine precursors [70]. *DGUOK* rs74874677 [23] was the most common significant genetic instrument on the panels. rs17850455 is a *POLG2* mutation. *POLG2* transcribes for DNA polymerase gamma 2 accessory subunit, a mtDNA polymerase component, that supports mtDNA replication [71]. The *POLG2* mutations were commonly identified by mtDNA deletions [72]. *HABP2* (also known as *FSAP*) encodes a circulating hyaluronan-binding protein. Its expression is well documented in tissues with high mitochondrial activity (e.g. liver, pancreas, kidney and skeletal muscles); nonetheless, not in brain and heart. It does not only bind to hyaluronan but also to factor VII, urokinase-type plasminogen activator and fibrinogen. Thus, it can possibly act on blood coagulation and inflammation caused by dying cells [73]. The *HABP2* G534E variant (rs7080536) had been implicated in thrombosis and pulmonary embolism [74].

PLAUR encodes the urokinase-type plasminogen-activator surface receptor. These annotations are hypothesis-generating and do not by themselves establish a brain-mitochondrial mechanism: the POLG2 instrument (rs17850455) did not pass Steiger filtering in the primary-disorder analyses, and HABP2 encodes a secreted plasma protease that the authors note is not expressed in brain, so predicted-damaging status need not reflect a mitochondrial or brain-relevant effect.

MitoCarta3.0 enrichment supported the mitochondrial relevance of the mtDNA-CN exposure instruments (Fig. 4b; Supplementary Table 11). The canonical set showed positive enrichment in three exposure GWASs: Gupta adjusted (β = +0.099, p = 2.71 × 10^−4), Longchamps (β = +0.076, p = 9.35 × 10^−3) and Gupta raw (β = +0.067, p = 1.44 × 10^−2); Chong did not reach nominal significance. In contrast, ASD, ADHD and F3 outcome GWASs showed no positive mitochondrial enrichment, arguing against broad outcome-side mitochondrial convergence. Several sub-pathways related to mtDNA replication, maintenance and fusion were enriched in exposure panels, but these annotations do not by themselves establish mediation. Full canonical MitoCarta3.0 and sub-pathway enrichment results, including outcome-side enrichment tests for ASD, ADHD and F3, are provided in Supplementary Results and Supplementary Table 11.

Brain eQTL colocalization nominated seven MitoCarta3.0 genes with PP4 ≥ 0.50 (Supplementary Table 14). RECQL4 colocalized with ADHD across three brain regions and with F3 (PP4 = 0.96–0.97); SUOX colocalized with ADHD across all 13 regions (PP4 = 0.79–0.88); and MSRA (hippocampus PP4 = 0.77; cerebellum 0.54) and NT5DC2 (anterior cingulate 0.54) were the ASD-colocalizing candidates. Additional candidates included NDUFAF3, AMT and RMDN1. These results are hypothesis-generating because windows were selected around disorder-associated mtDNA-CN instruments and eQTL-disorder PP4 does not establish mediation. To probe potential mediation directly, three-way colocalization across mtDNA-CN, eQTL and disorder signals was flagged (colocalization with the disorder and with mtDNA-CN in at least one exposure panel) for 7 of 26 gene–tissue–disorder combinations, and for only 3 when concordant support across both mtDNA-CN panels was required (Supplementary Table 15), and SuSiE conditional fine-mapping did not place the lead eQTL within both the mtDNA-CN and disorder credible sets for any gene (Supplementary Table 16), reinforcing a hypothesis-generating rather than mediating interpretation. The complete GTEx v10 brain-tissue colocalization results are provided in Supplementary Table 14.

Sensitivity and negative-control analyses

Sensitivity analyses supported the primary interpretation (Fig. 4a,c; Supplementary Tables 7–9). UK Biobank-deduplicated BD, MDD and BPD outcomes remained non-significant and directionally consistent with the primary analyses. MRlap-corrected estimates were directionally concordant with the primary estimates across all 24 tested pairs, although correction magnitudes varied with cross-trait genetic-covariance intercepts and should not be interpreted as independent confirmation. UK Biobank-deduplicated outcome analyses and MRlap-corrected estimates are provided in Supplementary Tables 7 and 8.

Reverse-direction MR found no outcome-to-mtDNA-CN association surviving Bonferroni correction across 32 pairs, including all ASD, ADHD and F3 reverse tests. Full reverse-direction MR estimates, including nominal but correction-negative associations, are provided in Supplementary Results and Supplementary Table 9. Standing height showed no association and no cross-panel heterogeneity, whereas BMI showed expected cross-panel heterogeneity consistent with its known relationship to mitochondrial biology and cell-composition adjustment. The absence of a height association is consistent with a specificity check, noting that Yengo et al. includes UK Biobank participants and this result is therefore slightly conservative rather than a fully orthogonal null. Detailed standing-height and BMI negative-control estimates, including panel-level heterogeneity statistics, are provided in Supplementary Results and Supplementary Table 10.

Discussion

This study integrated four partially overlapping mtDNA-CN GWAS exposure panels and evaluated psychiatric outcomes at both disorder and cross-disorder factor levels. Because the exposure panels share substantial UK Biobank composition, they were treated as correlated sources of evidence rather than independent replications. Under this framework, the most robust finding was an ASD-led inverse association: genetically proxied higher mtDNA-CN was associated with lower ASD liability after design-effect correction and BH-FDR correction within the primary-disorder family. ADHD and the F3 neurodevelopmental factor showed directionally concordant but non-definitive evidence, whereas SCZ, BD, MDD, general psychopathology and other disorder-class factors showed no robust associations.

The ASD result aligns with prior MR evidence reporting protective mtDNA-CN associations with ASD [30–33]. A recent study reported shared genetic architecture between mtDNA-CN and these disorders but, instrumenting a blood-cell-composition-adjusted exposure, found no significant causal effect in bidirectional MR [34]. The present analysis extends this literature by explicitly modelling the correlation among overlapping mtDNA-CN exposure panels. The naïve meta-analysis produced much smaller p-values for ASD, ADHD and F3; the design-effect correction yielded a more conservative inference in which only ASD survived the primary-disorder correction and F3 remained nominal. This distinction is important because F3 is constructed from ASD and ADHD loadings and cannot be interpreted as an independent replication of the ASD result.

A neurodevelopmental interpretation is biologically plausible but should remain cautious. Mitochondrial bioenergetics influence neuronal excitability, dendritic arborization, synaptic function and neurogenesis during periods of high developmental energy demand [75, 76]. Reduced genetically proxied mtDNA-CN could therefore index lower mitochondrial reserve during developmental windows relevant to ASD liability. However, the present data cannot distinguish a direct mtDNA-CN pathway from correlated pleiotropy through nuclear pathways involved in nucleotide metabolism, platelet biology, immune signaling or cell proliferation. The F3 and ADHD findings support directional consistency, not definitive evidence of a shared ASD-ADHD causal pathway.

The absence of significant effects for SCZ, BD, MDD and PTSD contrasts with observational reports of altered mtDNA-CN or mitochondrial dysfunction in these disorders [14–20, 77]. This discrepancy is compatible with observational associations reflecting downstream illness processes, pharmacological exposure, oxidative stress, inflammation or blood-cell-composition differences rather than an upstream effect of genetically proxied mtDNA-CN. The null pFactor and non-neurodevelopmental factor results further argue against a broad pan-psychiatric mtDNA-CN mechanism. The nominal Tourette syndrome estimate should be interpreted cautiously because it did not survive design-effect correction, and the case count was limited.

The biological annotation analyses provide plausibility but not mediation. MitoCarta3.0 enrichment in three of four exposure GWASs indicates that the instruments capture mitochondrial biology, especially in the blood-cell-composition-adjusted Gupta panel. However, ASD, ADHD and F3 outcome GWASs did not show corresponding positive mitochondrial enrichment. Similarly, brain eQTL colocalization nominated RECQL4, SUOX and MSRA as candidate genes, but the strongest molecular signals were not ASD-specific and eQTL-disorder colocalization alone cannot establish that gene expression mediates the mtDNA-CN effect. Three-way triangulation across mtDNA-CN, eQTL and psychiatric outcome signals, ideally with conditional colocalization and multivariable MR, will be required before naming a mediator.

The sensitivity analyses address two major threats to interpretation. First, the ASD, ADHD and F3 outcome GWASs do not include UK Biobank participants, so their forward MR estimates are not driven by exposure-outcome sample overlap. Second, reverse-direction MR provided no evidence that psychiatric liability influences mtDNA-CN, although power was limited for outcomes requiring relaxed instrument thresholds. The standing-height negative control was null and homogeneous across panels, while BMI behaved as a biological contrast with cross-panel heterogeneity, consistent with the sensitivity of adiposity-mtDNA-CN associations to cell-composition adjustment.

Several limitations should be noted. Analyses were restricted to European ancestry and blood-derived mtDNA-CN, limiting ancestry and tissue generalizability. The effective number of independent exposure panels was small because of shared UK Biobank composition, and power varied substantially across outcomes, especially secondary disorders. Horizontal pleiotropy cannot be excluded, particularly through nuclear pathways involved in nucleotide metabolism, platelet biology or cell proliferation. The analyses also did not model environmental or gene-environment interactions, including perinatal hypoxia, prenatal infection and early-life stress [78, 79]. Brain-specific mtDNA-CN GWASs, multivariable MR, conditional colocalization and functional validation in neurodevelopmental models will be required to establish tissue specificity and mechanism.

A structured comparison with prior genetic-causal studies of mtDNA-CN and psychiatric or neurodevelopmental disorders is provided in Table 1 [30–34, 80, 81]. A protective association between genetically proxied mtDNA-CN and ASD has been reported by five two-sample MR analyses [30–33, 80], all of which instrumented mtDNA-CN from qPCR- or SNP-array-based GWASs that share UK Biobank participants with the outcome cohorts; their agreement therefore does not by itself constitute

independent replication. A sixth study instrumented mtDNA-CN from a whole-genome-sequencing GWAS with blood-cell-composition adjustment and found no significant MR effect on any disorder, including ASD, and no reverse effect [34]. This is consistent with our own data rather than in conflict with them: the ASD association is carried by the qPCR, SNP-array and unadjusted WGS panels (per-panel p = 0.003–0.015), whereas the blood-cell-composition-adjusted WGS panel — the same exposure used in that study — is not significant (β = −0.130, p = 0.087). Because blood mtDNA-CN partly reflects blood-cell composition, we cannot fully resolve whether the pooled estimate (OR 0.83, 95% CI 0.73–0.95; q = 0.030) reflects a mitochondrial effect that blood-cell-composition adjustment over-corrects, or a partly cell-composition-driven association in the less-adjusted panels; we therefore report the ASD result as robust to assay platform and shared-sample correlation but sensitive to blood-cell-composition adjustment, and interpret it as hypothesis-strengthening rather than definitive. Prior studies addressed sample overlap either not at all or by excluding UK Biobank outcomes [34], and none corrected for the correlation among multiple overlapping exposure panels; a genetic-overlap analysis likewise found no significant genetic correlation between mtDNA-CN and the psychotic-affective disorders [81], consistent with the neurodevelopmental specificity observed here. Relative to this literature, the present study is distinguished by a cross-platform exposure design, explicit design-effect and MRlap correction for shared-sample correlation, extension to cross-disorder genomic-SEM factors, and mechanistic MitoCarta3.0 enrichment and brain-eQTL colocalization.

In summary, across four partially overlapping mtDNA-CN exposure panels and a design-effect-corrected synthesis, genetically proxied higher mtDNA-CN was associated most robustly with lower ASD liability. ADHD and the ASD-ADHD neurodevelopmental factor showed directionally concordant but non-definitive evidence, and there was no robust effect on general psychopathology or other disorder classes. These findings nominate mitochondrial pathways for mechanistic investigation in neurodevelopmental disorders while emphasizing that broader causal and molecular interpretations remain exploratory.

Declarations

Data availability

The GWAS summary statistics for mtDNA-CN can be downloaded from Longchamps et al. 2022 (http://arkinglab.org/upload/mtDNA_CN_GWAS/mtDNA_CN.ALLm2.bgen.stats.gz), Chong et al. 2022 (GWAS Catalog accession GCST90026372), Gupta et al. 2023 blood-cell-composition-adjusted (GWAS Catalog accession GCST90268497), and Gupta et al. 2023 raw unadjusted (GWAS Catalog accession GCST90268498). Psychiatric outcome GWASs are available through the Psychiatric Genomics Consortium download portal and the figshare repository associated with the cross-disorder genomic structural-equation model. GIANT summary statistics were used for standing height and BMI. Harmonized exposure-outcome instruments, LDSC inter-panel correlation matrices and brain eQTL colocalization results generated for this study are available in the public repository (https://github.com/dryusufcicek/mtDNA-CN-psychiatric-mr).

Code availability

Analyses used TwoSampleMR, MR-PRESSO, MRlap, MendelianRandomization, metafor, GenomicSEM, coloc, pyliftover, MAGMA, PLINK v1.9 and R v4.4.2. The custom analysis pipeline for instrument selection, forward and reverse MR, design-effect-corrected synthesis, factor analyses, enrichment, colocalization and negative-control validation is publicly available at https://github.com/dryusufcicek/mtDNA-CN-psychiatric-mr, together with the derived per-analysis result tables.

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Author contributions

Y.Ç.: Investigation, Conceptualization, Methodology, Software, Formal analysis, Data curation, Visualization, Writing – original draft. M.C.Ç.: Investigation, Conceptualization, Writing – original draft. A.B.: Supervision, Formal analysis, Validation, Writing – review and editing. H.A.V.: Supervision, Validation, Writing – review and editing. All authors read and approved the final manuscript.

Declaration of generative AI

In accordance with the Nature Portfolio editorial policy on the use of generative AI in scientific publishing, the authors disclose the use of Anthropic Claude (model versions Opus 4.7, and 4.8) to refine the language and facilitate analytical discussions during manuscript drafting. The authors take full responsibility for the manuscript's accuracy, originality, and integrity, including all citations.

Competing interests

The authors declare no competing interests.

Ethics statement

This study used exclusively publicly available GWAS summary-statistics datasets. Ethical approval and informed consent were obtained in the original studies that generated these data. All analyses adhered to relevant institutional and international guidelines.

Additional information

Supplementary information is available at Translational Psychiatry's website. Correspondence and requests for materials should be addressed to Halil Aziz Velioğlu.

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Table

Table 1. Comparison with prior genetic-causal studies of mitochondrial DNA copy number and psychiatric or neurodevelopmental disorders. Prior two-sample Mendelian randomization (MR) and genetic-overlap studies are summarized alongside the present study. Effect estimates are given as reported in each source and were verified against the primary publications; the present-study effect is also shown as an odds ratio for comparability with the prior MR literature. NR, not reported; N.A., not applicable; bcc, blood-cell composition; UKB, UK Biobank; PGC, Psychiatric Genomics Consortium; NDD, neurodevelopmental disorder; ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder; SCZ, schizophrenia; BD, bipolar disorder; MDD, major depressive disorder; genomic-SEM, genomic structural equation modelling; MVMR, multivariable MR; GPA, genetic analysis incorporating pleiotropy and annotation; TS, Tourette syndrome; AD, Alzheimer's disease; AutoMitoC, automated mitochondrial copy-number estimation; GWAS-PW, pairwise GWAS; conjFDR, conjunctional false-discovery rate; FUMA, Functional Mapping and Annotation; eQTL, expression quantitative trait locus; NHANES, National Health and Nutrition Examination Survey; S-MultiXcan, multi-tissue transcriptome-

wide association; MRlap, sample-overlap-corrected MR; LDSC, linkage-disequilibrium score regression; rg, genetic correlation; sx, symptoms.

Study (design)	mtDNA-CN exposure (platform, N)	Outcomes	Instrument QC and overlap handling	Mechanism analysis	Key finding (ASD-anchored)
Lu 2024 [30] Bidirectional 2-sample MR	2 panels (UKB); platform NR; N 465 809 & 383 476	7 disorders	Steiger NR; overlap: none reported	None	ASD OR 0.73 (0.58–0.92); others null; reverse null
Niu 2024 [31] Bidirectional 2-sample MR (preprint)	1 panel (Chong, AutoMitoC, UKB); N 395 718	10 disorders (PGC + FinnGen)	F>10, Steiger no; overlap: none	None	ASD OR 0.735 (0.60–0.91); reverse: anxiety→ mtDNA-CN +
Fu 2025 [32] Bidirectional 2-sample MR	1 panel (Chong, AutoMitoC, UKB); N 395 718	13 disorders (PGC/FinnGen/ Within-Family)	F>10, Steiger no; overlap: none	NHANES methylmalonic-acid epidemiology	5 sig: ASD 0.80, BD 0.65, AD 0.15, dementia 0.52, depressive-sx 0.84
Qiu 2025 [33] Bidirectional 2-sample MR	2 panels (Chong + Longchamps); both UKB-based	3 NDD (ASD, ADHD, TS)	F>10, Steiger no; overlap: none	None	ASD OR 0.78 & 0.80; ADHD, TS null; reverse null
Hu 2024 [80] 2-sample MR + MVMR (mediation)	1 panel (Chong, UKB); N 383 476; mtDNA-CN 1 of 3 exposures	3 NDD (ASD, ADHD, TS)	Steiger yes; overlap: none	None	ASD OR 0.83 (0.69–0.99); ADHD, TS null
Pisanu 2024 [81] Genetic-overlap / pleiotropy (not MR)	1 panel (AutoMitoC, UKB); N 383 476; + telomere length	3 (BD, SCZ, MDD)	N.A. (not MR); overlap: LDSC rg only	eQTL annotation, druggability (no colocalization)	mtDNA-CN: no sig genetic correlation with any disorder; shared loci BD 4 / SCZ 12 / MDD 1
Xue 2026 [34] Genetic-overlap +	1 panel (Gupta WGS, bcc-adjusted, UKB); N 155 998	5 (ADHD, ASD, BD, MDD, SCZ); UKB excluded	F>10, Steiger, Radial-MR; overlap:	GPA/GWAS-PW/ conjFDR; 30 loci → 305 FUMA-	Sig genetic overlap with all 5; 30 loci; but MR null

Study (design)	mtDNA-CN exposure (platform, N)	Outcomes	Instrument QC and overlap handling	Mechanism analysis	Key finding (ASD-anchored)
bidirectional MR			UKB excluded	mapped genes; S-MultiXcan 87; enrichment	for all 5 (incl. ASD); reverse null
This study 2-sample MR + correlation-aware synthesis	4 panels / 3 platforms (qPCR, SNP-array, WGS×2: raw + bcc-adjusted); N 155 998–465 809	25 per panel (5 primary, 9 secondary, 6 genomic-SEM factors, 2 controls); 100 tests	Steiger; overlap: design-effect synthesis + MRlap	MAGMA × MitoCarta3.0; brain-eQTL coloc + SuSiE	ASD OR 0.83 (0.73–0.95), q=0.030 [β −0.182]; carried by less-adjusted panels, bcc-adjusted panel null (p=0.087); reverse null

Figures

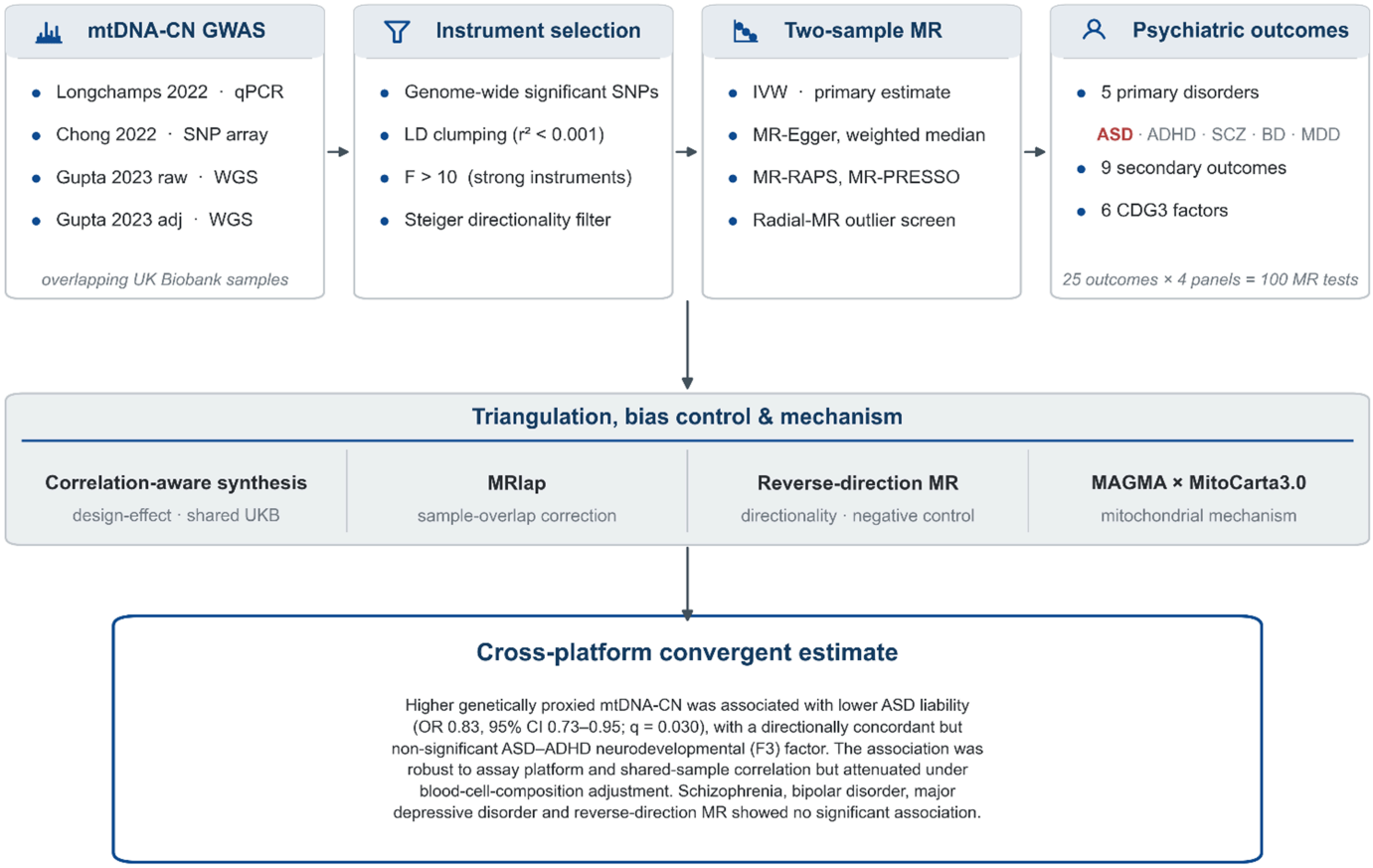


Figure 1

Study design and analytic workflow. Four mtDNA-CN GWAS exposure panels spanning three assay/data-processing approaches were assembled: qPCR quantification (Longchamps 2022), SNP-array intensity estimation (Chong 2022), and WGS-derived mtDNA-CN with and without blood-cell-composition adjustment (Gupta 2023 raw and adjusted), which share UK Biobank participants. Genetic instruments were defined by genome-wide significance, LD clumping, F-statistic strength filtering and Steiger directionality filtering, and entered two-sample MR with IVW as the primary estimator and MR-Egger, weighted median, MR-RAPS, MR-PRESSO and a Radial-MR outlier screen as sensitivity analyses. Outcomes comprised five primary psychiatric disorders (ASD, ADHD, SCZ, BD, MDD), nine secondary psychiatric and antidepressant-response outcomes, six cross-disorder genomic-SEM factors, and standing-height and BMI controls (25 outcomes per panel; 100 forward-MR tests). Cross-panel estimates were then triangulated through a correlation-aware, design-effect synthesis that accounts for the shared samples, alongside MRlap sample-overlap correction, reverse-direction MR and MAGMA × MitoCarta3.0 enrichment and brain-eQTL colocalization. The design-effect panel contrasts the naive and correlation-corrected evidence, in which only ASD survives correction among the five primary disorders; the ASD association is carried by the qPCR, SNP-array and unadjusted-WGS panels and attenuates under blood-cell-composition adjustment.

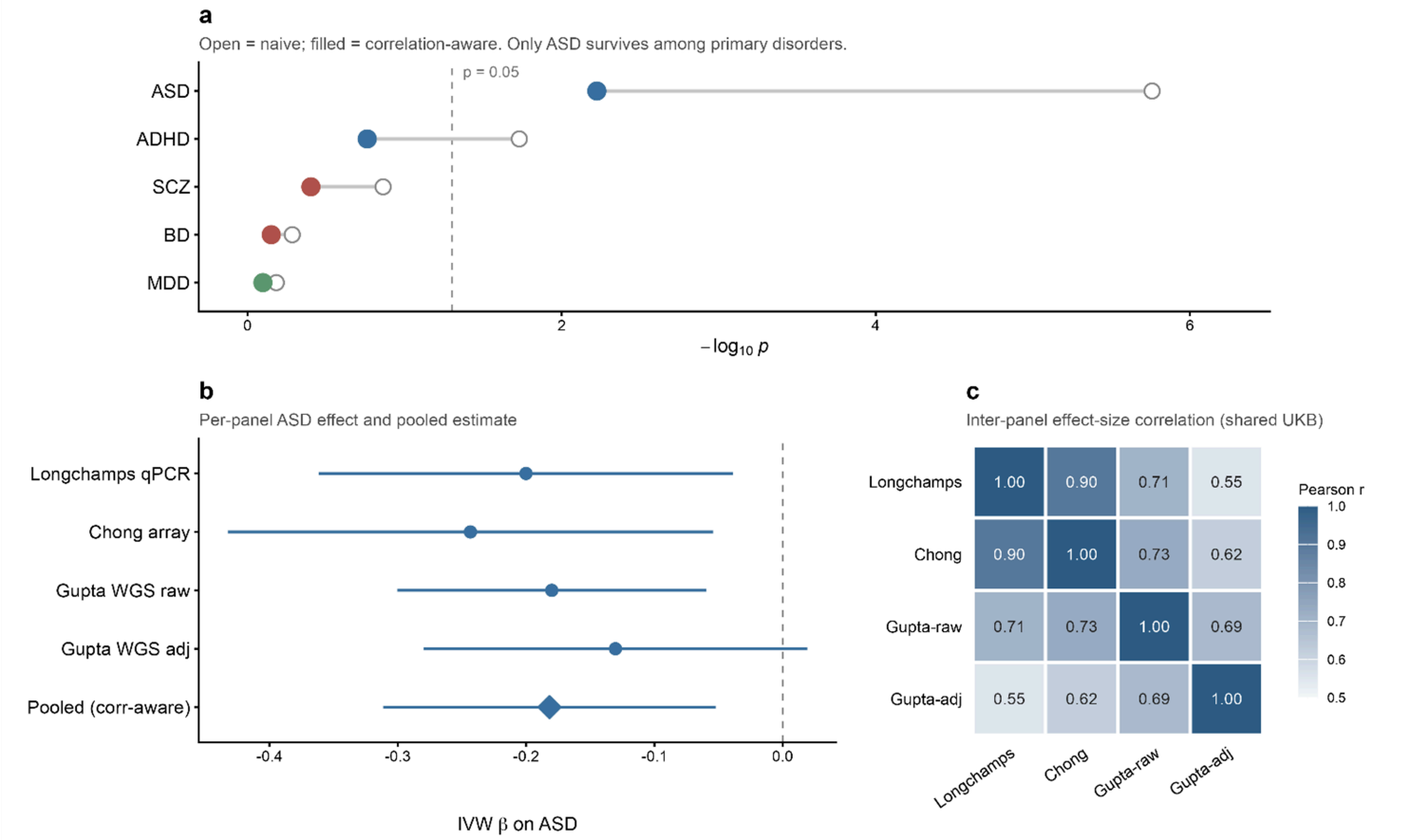


Figure 2

Genetically proxied mtDNA-CN effects on the five primary psychiatric disorders. (a) Statistical evidence under the naïve random-effects meta-analysis versus the design-effect-corrected synthesis. Only ASD remains significant after modelling shared-sample correlation among exposure panels. (b) Per-panel IVW estimates for mtDNA-CN on ASD (Longchamps β = −0.200, p = 0.015; Chong β = −0.243, p = 0.012; Gupta raw β = −0.180, p = 0.003; Gupta adjusted β = −0.130, p = 0.087) and the design-effect-corrected pooled estimate (β = −0.182; 95% CI −0.312 to −0.052; corrected p = 5.97 × 10^−3; BH-FDR q = 0.030). (c) Pairwise Pearson correlations of panel-specific IVW estimates across the outcomes included in the cross-panel concordance analysis between the four exposure panels, reflecting shared UK Biobank composition and motivating the design-effect correction.

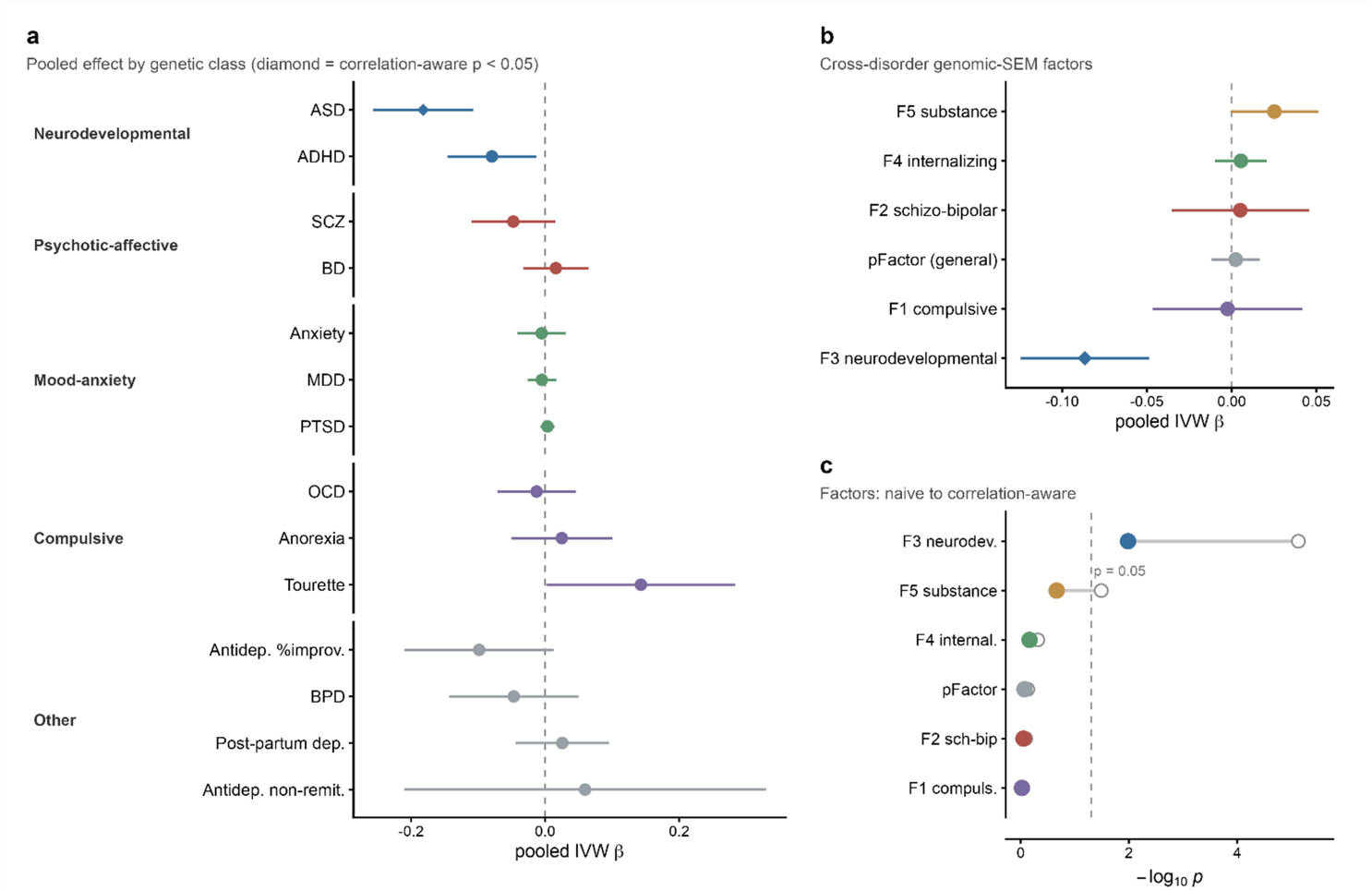


Figure 3

Cross-disorder architecture and neurodevelopmental specificity. (a) Pooled IVW effects on all psychiatric disorders and antidepressant-response outcomes, grouped by genetic class (neurodevelopmental, psychotic-affective, mood-anxiety, compulsive and other); diamonds mark correlation-aware significance. (b) Pooled IVW effects on the six cross-disorder genomic-SEM factors. F3 neurodevelopmental liability is directionally concordant but does not survive BH-FDR correction within the factor family. (c) Statistical evidence for factor-level effects under naïve and design-effect-corrected synthesis.

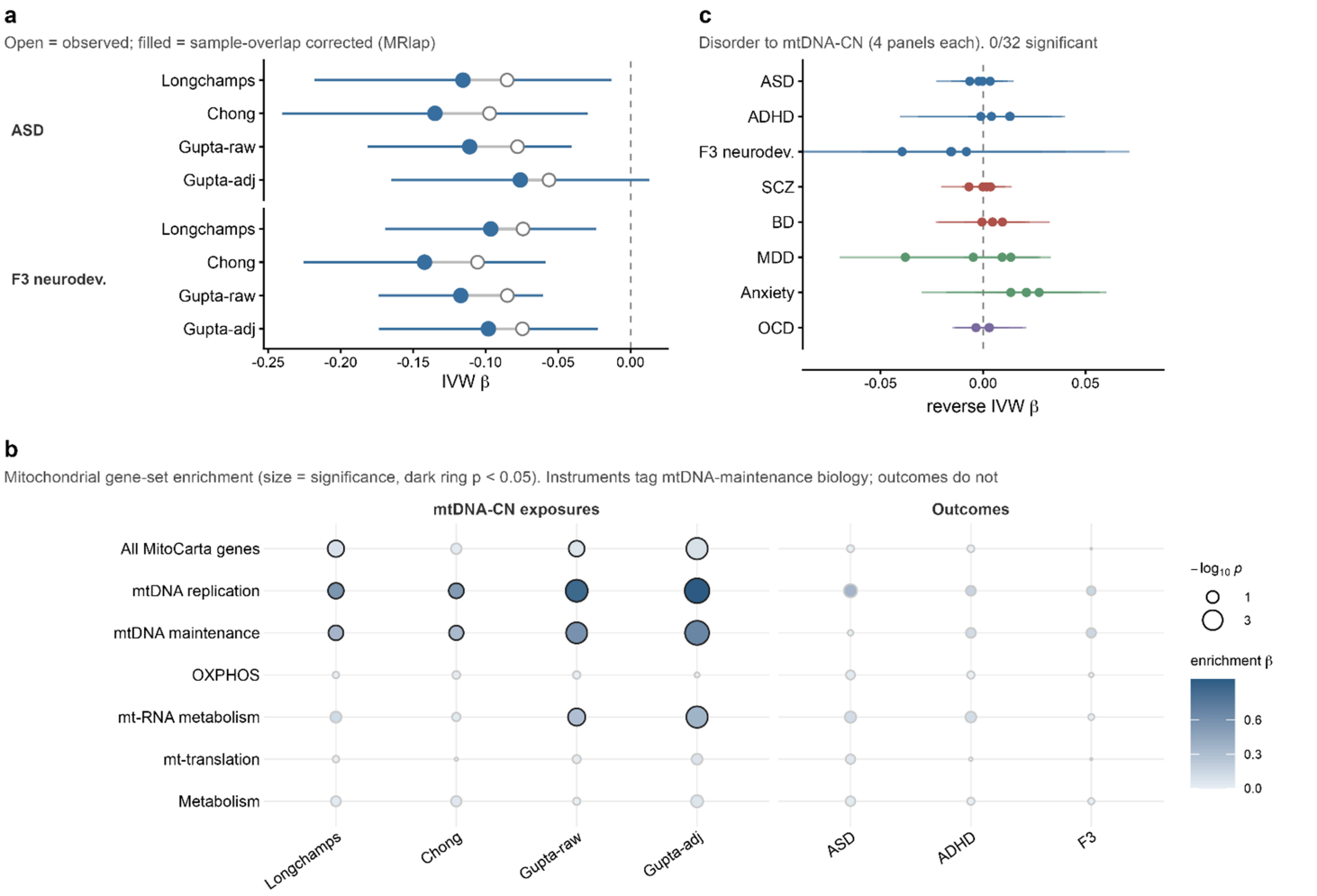


Figure 4

Robustness and mechanistic convergence. (a) MRlap sample-overlap correction for ASD and F3 across the four exposure panels. (b) MAGMA MitoCarta3.0 enrichment across mtDNA-CN exposures and neurodevelopmental outcome GWASs (canonical MitoCarta3.0 set: Gupta adjusted β = +0.099, p = 2.71 × 10^−4; Longchamps β = +0.076, p = 9.35 × 10^−3; Gupta raw β = +0.067, p = 1.44 × 10^−2; Chong not nominally significant). Exposure instruments show mitochondrial enrichment, whereas ASD, ADHD and F3 outcome GWASs do not show positive mitochondrial enrichment. (c) Reverse-direction MR (disorder to mtDNA-CN) across the four exposure panels; none of the 32 disorder-panel tests reached significance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1MRscatterASD.tif
- SupplementaryTablesS1S18.xlsx

- SupplementaryMethodsandResults.docx