Supplementary data - Genetic overlap between mood instability and alcohol-related traits suggests shared biological underpinnings

Validation with independent GWASs

Both the extent of polygenic overlap and genetic correlation were replicated for MOOD & GSCAN-AC (**Supplementary Figure 8 A&B**) with distinct log-likelihood optimum (**Supplementary Figure 8C**) and positive AIC value when compared to the model with minimum overlap. However, as with MVP-AUD, MiXeR was not deemed reliable for MOOD & PGC-AUD, as indicated by oscillating log-likelihood profile (**Supplementary Figure 8D**) producing large standard deviation of the number of 'causal' variants (SD =1,600 for 900 variants), and negative AIC compared to both minimum and maximum overlap (noticing that there was also a lack of enrichment in the corresponding Q-Q plot, data not shown). Consequently, conjFDR for MOOD and PGC-AUD yielded only one joint significant locus (**Supplementary Figure 9**). Effect directions of lead SNPs identified in discovery conjFDR analyses demonstrated a good agreement between discovery and validation GWASs on alcohol-related phenotypes (**Supplementary Table 1**). For AC, 15 of 18 (83%) loci were concordant (p =0.0037), while for AUD 14 out of 20 (70%) were concordant (p =0.0576). Finally, in order to investigate whether MiXeR MOOD & AUD results were due to power issues or to the genetic architecture of AUD itself, we ran MiXeR between MOOD and a meta-analyzed summary statistics of AUD from PGC + MVP samples (**Supplementary Figure 10**). We showed that, while the statistical power was overall increased (**Supplementary Figure 10A**), MiXeR estimates of phenotype-specific and shared fraction of "causal" variants remained unstable, as indicated by large standard deviation, erratic log-likelihood plot (**Supplementary Figure 10B**). AIC values for both minimum and maximum possible overlap were marginally positive. Finally, joint analyses for alcohol-related phenotypes with GSCAN-AC and either MVP

or PGC AUD samples yielded similar patterns of polygenic overlap, genetic correlation (**Supplementary Figure 11**) and jointly-associated loci (**Supplementary Figure 12**) as our discovery analyses.

Phenotypic associations between MOOD, AC and AUD

After adjustment on sex, age and ancestry, each linear relationship between MOOD and both alcohol-related phenotypes remained strong and significant (all p <0.001): AC ~ MOOD (β =0.025), AUD ~ MOOD (β =0.31), MOOD ~ AC (β =0. 059) and MOOD ~ AUD (β =0.063). In the model where MOOD and AC were entered together as independent variables, AUD was significantly associated with MOOD (β =0.32), with AC (β =0.03), and with their interaction term (β =-0.039); all p <0.001. Of note, comparing standardized coefficients obtained from regression on binary (MOOD, AUD) *vs.* continuous variables (AC) should be done with caution.

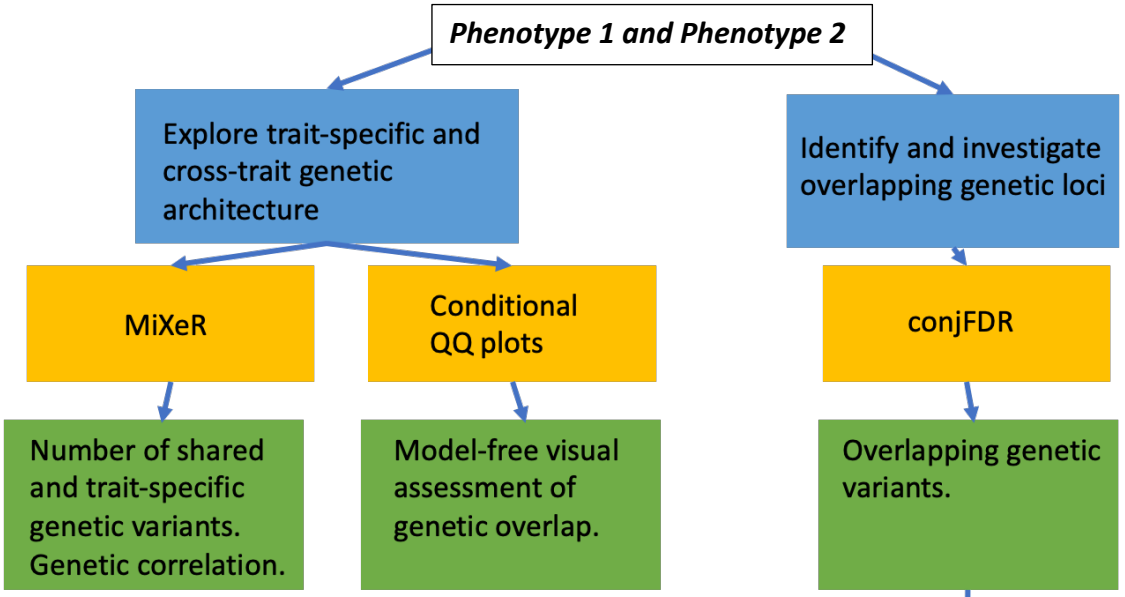
Exploratory analyses

BINGE GWAS in the UK Biobank was not powerful enough to get reliable Bivariate Mixer estimates (data not shown). However, conjFDR yielded 12 (BINGE & MOOD) and 10 (BINGE & AUD) significant jointly associated loci. Interestingly, only one lead SNP, rs4245150, was common to both analyses, and no SNP was shared between these analyses and conjFDR with MOOD and AC. **Supplementary Figure 13** shows MiXeR findings for all exploratory analyses. As regards the joint polygenicity of MOOD and AC quantity (ALCINTAKErint, **Supplementary Figure 13A**) vs. MOOD and AC frequency (DRINKALCw, **Supplementary Figure 13B**), we obtained similar results as for the total AUDIT-C, that is, complete polygenic overlap. However, conversely to the literature, both genetic correlations with MOOD were negative. The polygenic overlap of AC frequency and AUD was ~50% shared, while it was >90% shared for AC quantity; with both showing positive genetic correlation (again, stronger for quantity *vs.* frequency, 0.65 *vs.* 0.37; **Supplementary Figure 13C & D**). There was no difference in the

shared vs. unique polygenicity of MOOD and AC as a function of lifetime smoking status (**Supplementary Figure 14A & B**). Of note, MOOD and AUD MiXeR analyses showed different patterns: similar to that of the whole UK Biobank sample for ever-smokers, but showing complete overlap for never-smokers (**Supplementary Figure 14C & D**). However, AIC for the latter analysis was negative, indicating a lack of statistical power and, thus, barely interpretable results. Finally, it is noteworthy that the genetic correlation between MOOD GWASs conducted in never vs. ever smoked was =1."

Supplementary Tables and Figures

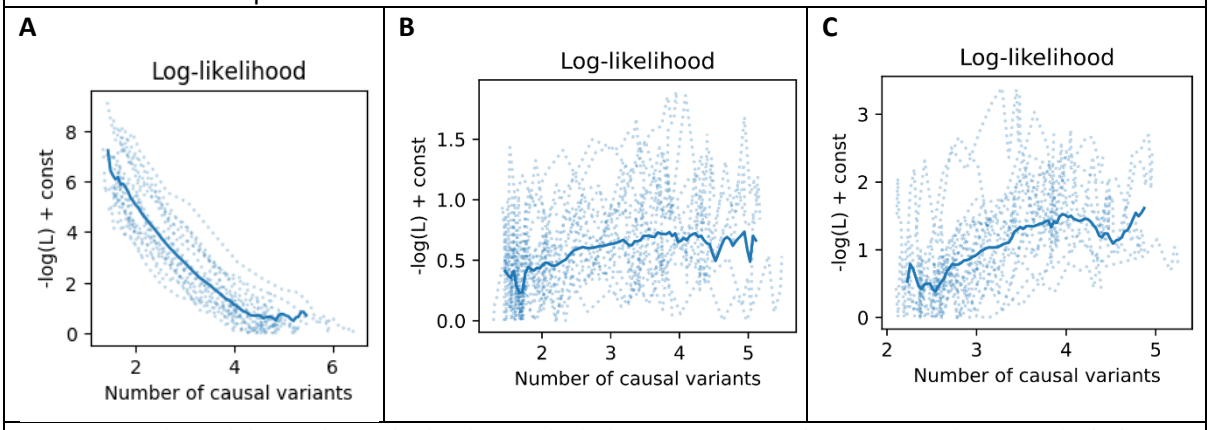
Supplementary Figure 1: overview of analyses and annotations



MiXeR	conjFDR
Causal (i.e. beyond LD issues) SNPs ?	
x	
Use of overlapping samples ?	
x	
Individual loci for further functional characterization?	
	x

FDR, false discovery rate; GWAS, genome-wide association study; FUMA, Functional Mapping and Annotation; SNP, single nucleotide polymorphism. Blue, scientific question; yellow, Analysis method/tool; green, output. The embedded table summarizes the comparative advantages of MiXeR *vs.* conjFDR.

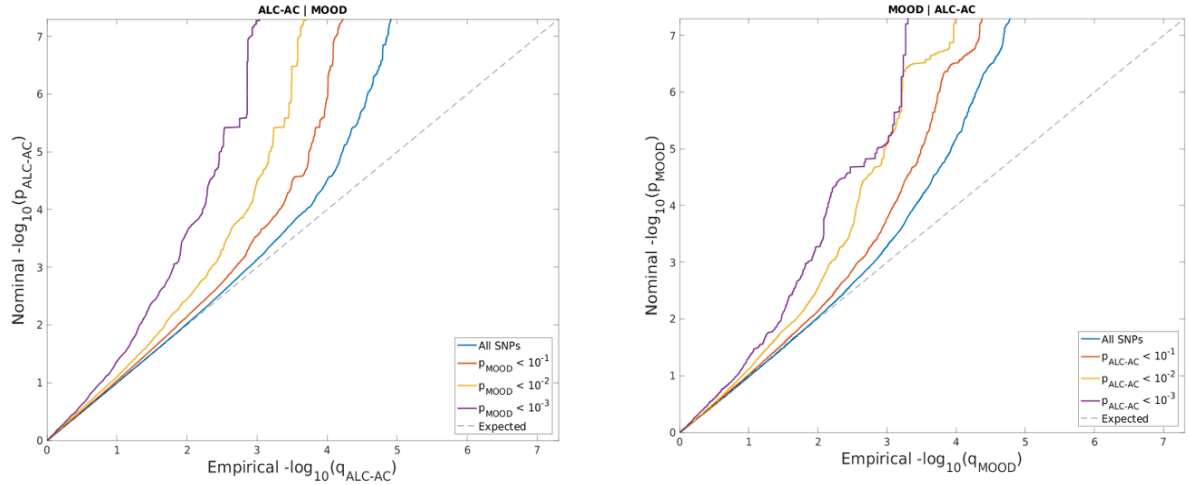
Supplementary Figure2: log-likelihood plots from the discovery MiXeR analysis. The number of causal variants is expressed in thousands.



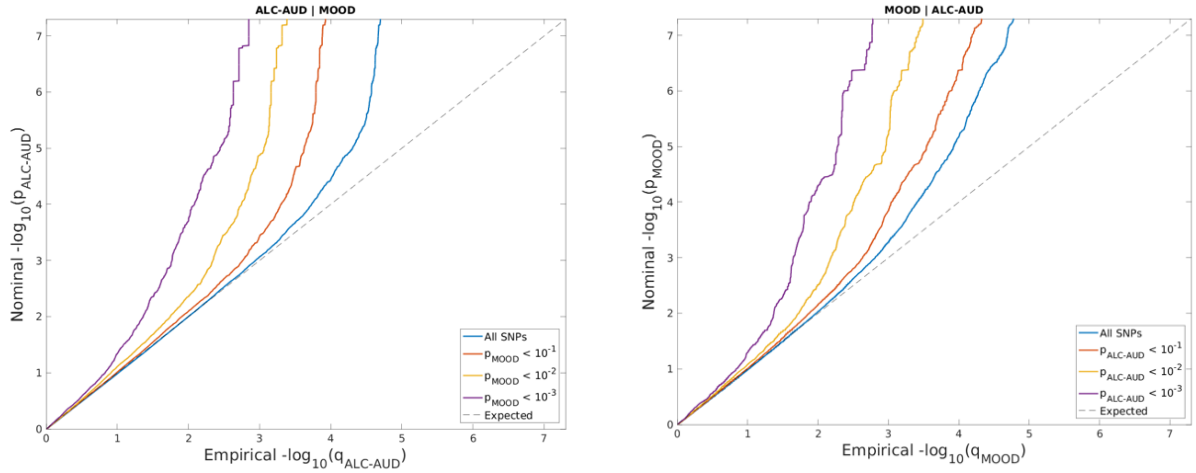
MOOD, mood instability in the UK biobank; AC, alcohol consumption in the MVP sample; AUD, alcohol use disorder in the MVP sample.

Supplementary Figure 3: Q-Q plots from MiXeR analyses showing enrichment for significant SNPs from trait one as the significance in the second trait increases.

A) AC|MOOD, MOOD|AC



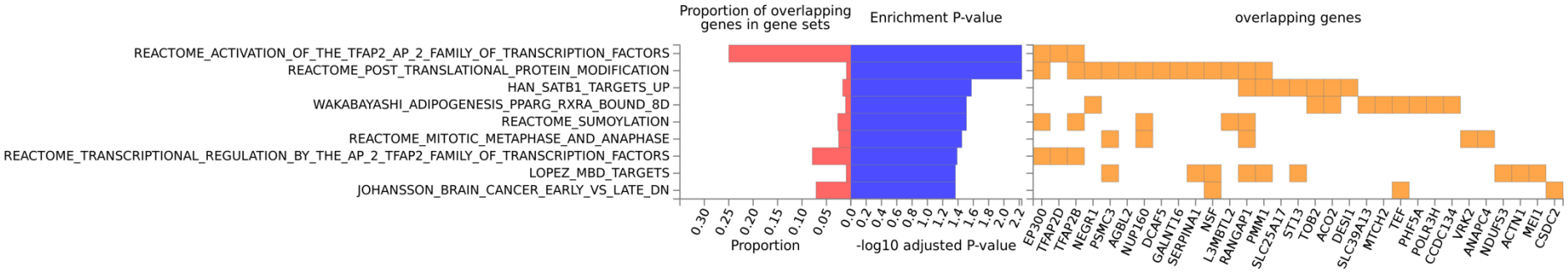
B) AUD|MOOD, MOOD|AUD



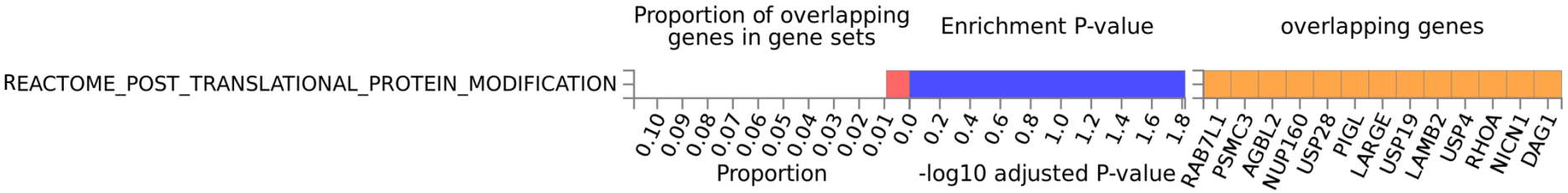
MOOD, mood instability in the UK biobank; AC, alcohol consumption in the MVP sample; AUD, alcohol use disorder in the MPV sample.

Supplementary Figure 4: signficantly enriched canonical pathways for (A) mood instability (MOOD) & alcohol consumption (AC) and (B) MOOD & alcohol use disorder (AUD). Gene sets were obtained by Functional Mapping and Annotation (FUMA) procedure based on the genes mapped from the discovery conjFDR analysis.

A)

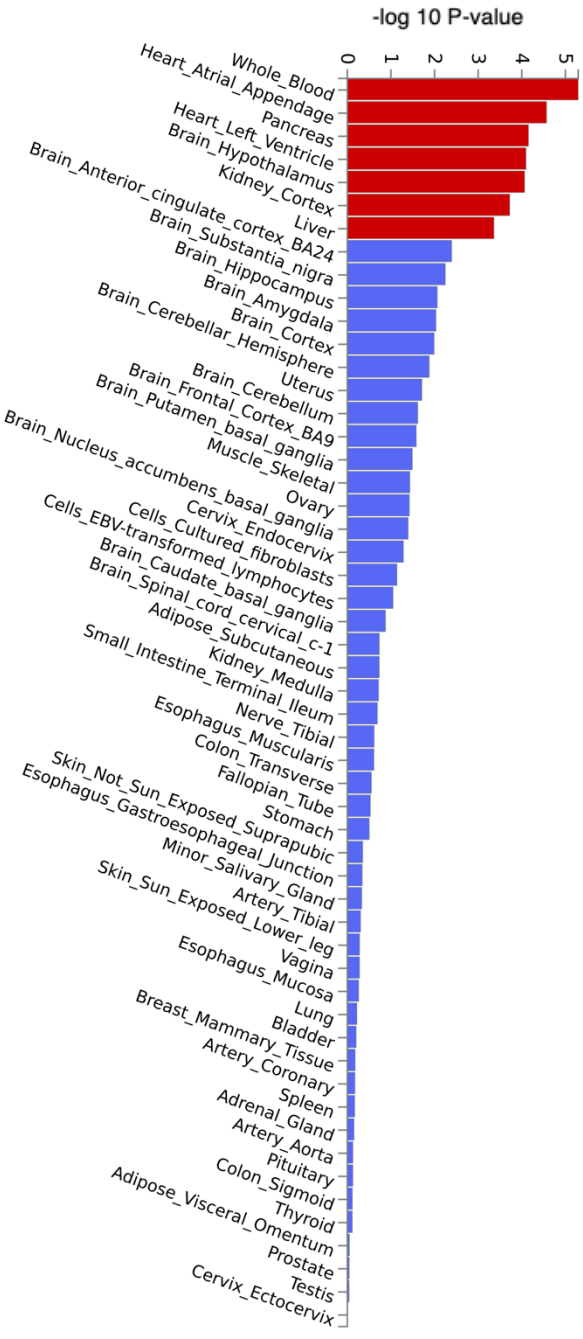


B)

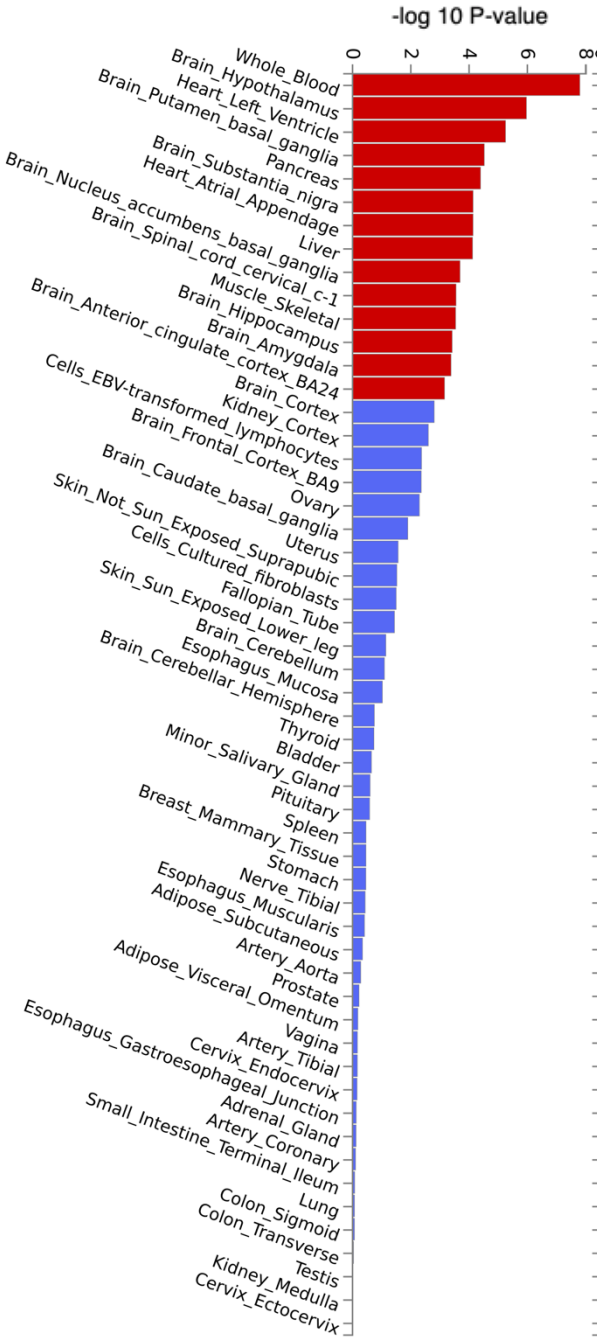


Supplementary Figure 5: increasing *p*-values for tissue-specific differential gene expression (both sides) for genes mapped after conjFDR for (A) mood instability and MVP-alcohol consumption and (B) mood instability and MVP-alcohol use disorder. Gene expression is obtained from GTEx V.8 (https://gtexportal.org/home/). Image was cropped, leaving some unwanted marks on panel B.

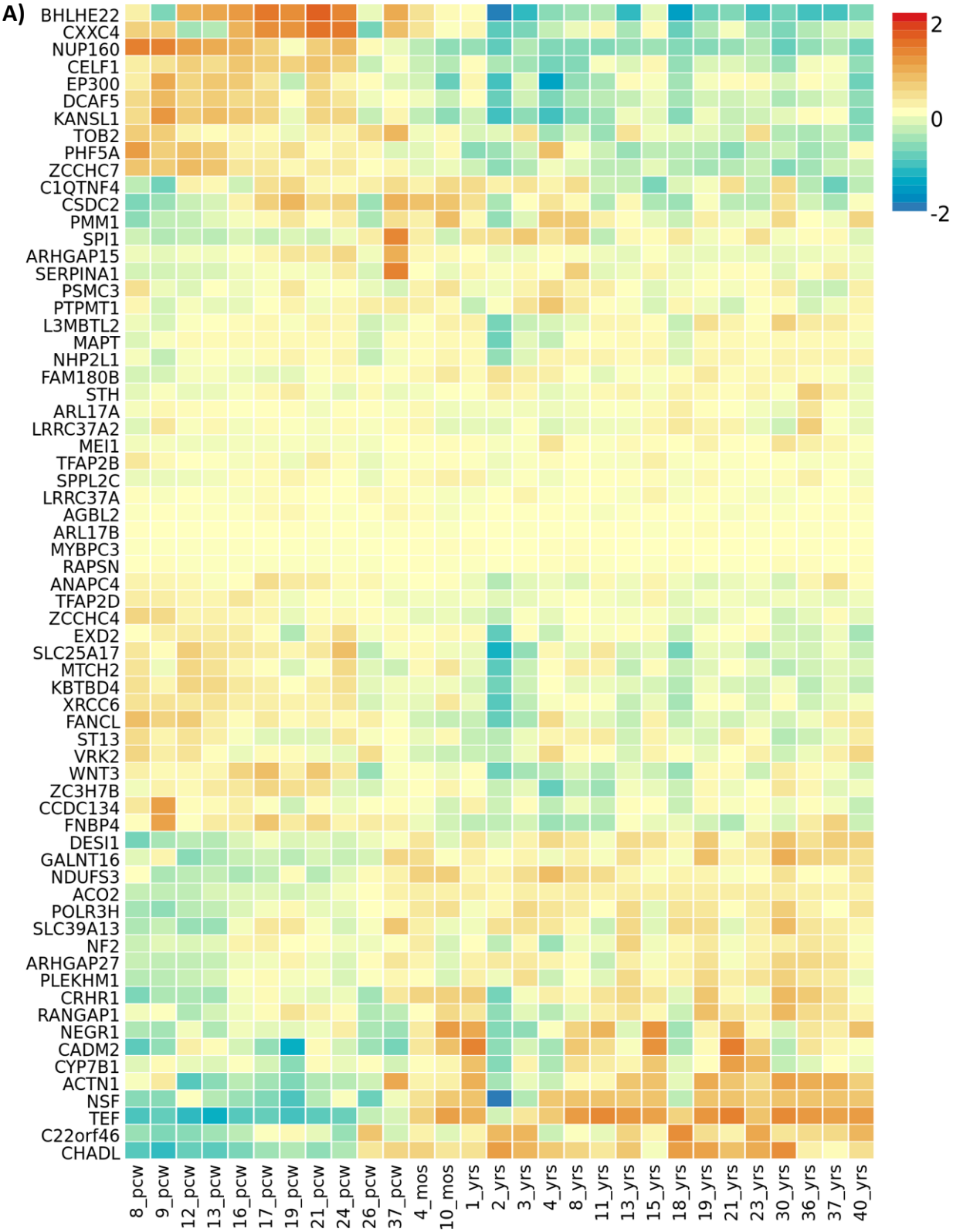
A)



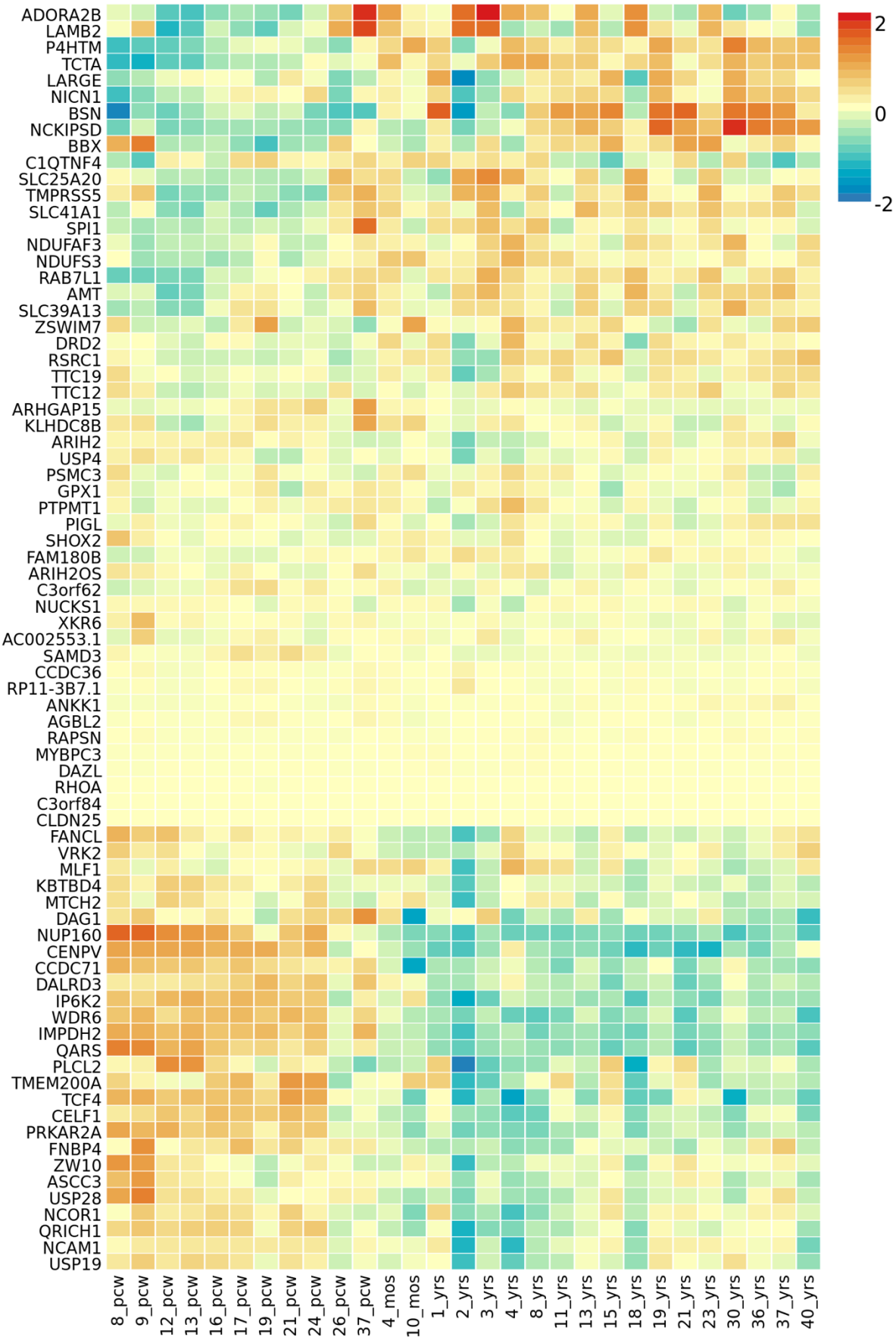
B)



Supplementary Figure 6: expression heatmaps for genes mapped after conjFDR for (A) mood instability and MVP-alcohol consumption and (B) mood instability and MVP-alcohol use disorder. Gene expression is obtained from BrainSpan data (https://www.brainspan.org/) at various developmental stages during fetal life, infancy, adolescence and adulthood.

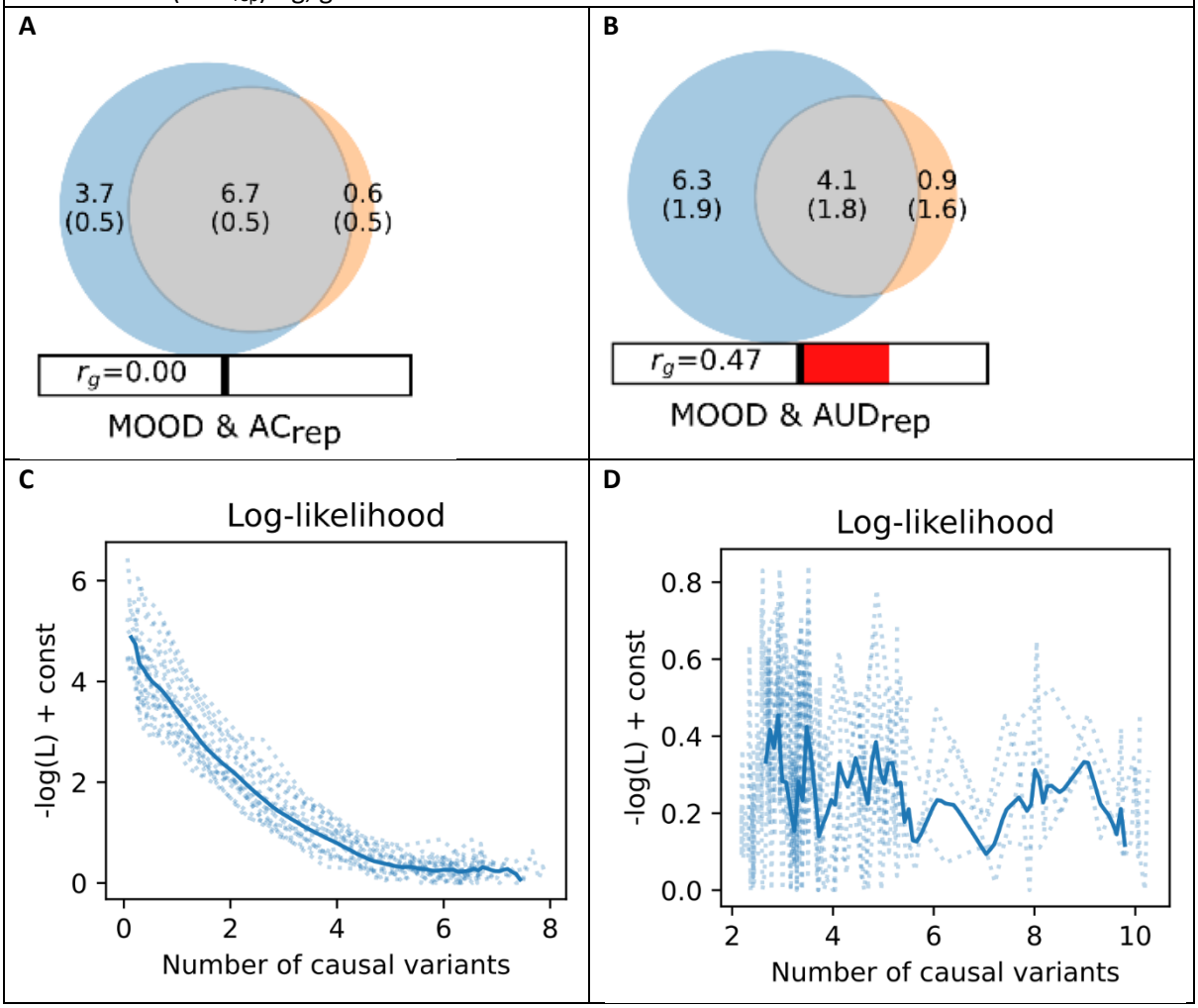


B)

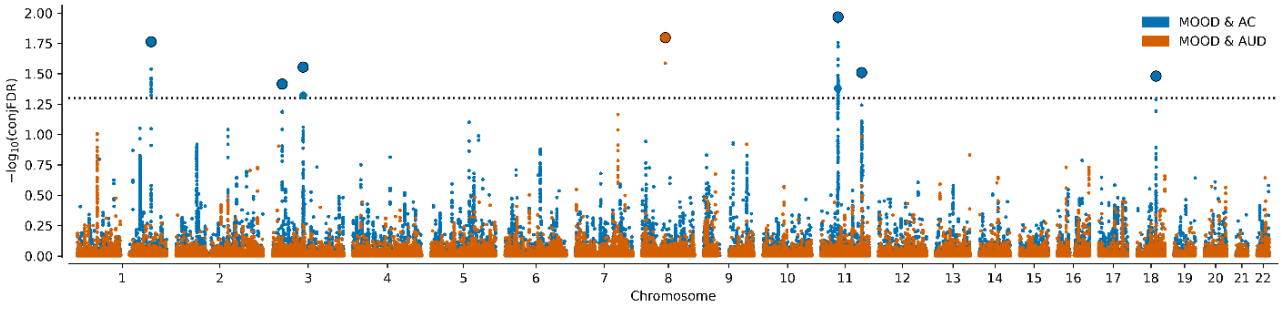

ADORA2B
LAMB2
P4HTM
TCTA
LARGE
NICN1
BSN
NCKIPSD
BBX
C1QTNF4
SLC25A20
TMPRSS5
SLC41A1
SPI1
NDUFAF3
NDUFS3
RAB7L1
AMT
SLC39A13
ZSWIM7
DRD2
RSRC1
TTC19
TTC12
ARHGAP15
KLHDC8B
ARIH2
USP4
PSMC3
GPX1
PTPMT1
PIGL
SHOX2
FAM180B
ARIH2OS
C3orf62
NUCKS1
XKR6
AC002553.1
SAMD3
CCDC36
RP11-3B7.1
ANKK1
AGBL2
RAPSN
MYBPC3
DAZL
RHOA
C3orf84
CLDN25
FANCL
VRK2
MLF1
KBTBD4
MTCH2
DAG1
NUP160
CENPV
CCDC71
DALRD3
IP6K2
WDR6
IMPDH2
QARS
PLCL2
TMEM200A
TCF4
CELF1
PRKAR2A
FNBP4
ZW10
ASCC3
USP28
NCOR1
QRICH1
NCAM1
USP19
8_pcw
9_pcw
12_pcw
13_pcw
16_pcw
17_pcw
19_pcw
21_pcw
24_pcw
26_pcw
37_pcw
4_mos
10_mos
1_yrs
2_yrs
3_yrs
4_yrs
8_yrs
11_yrs
13_yrs
15_yrs
18_yrs
19_yrs
21_yrs
23_yrs
30_yrs
36_yrs
37_yrs
40_yrs
2
0
-2

Supplementary Figure 7 : graphical representation of the PheWAS for15 novel SNPs for MOOD & AC (left) and MOOD & AUD (right). Plot obtained using the MRC IEU PheWAS tool. Red upwards arrows indicate positive effect size, blue downward arrows indicate negative effect size.

Supplementary Figure 8: validation for mood instability and alcohol-related phenotypes. Venn diagrams and Q-Q plots from MiXeR replication analyses showing polygenic overlap and enrichment for significant SNPs at decreasing thresholds. Analyses were performed using GWAS from UK biobank for mood instability (MOOD), GSCAN for alcohol consumption (AC_{rep}) and PGC for alcohol use disorder (AUD_{rep}). rg, genetic correlation.

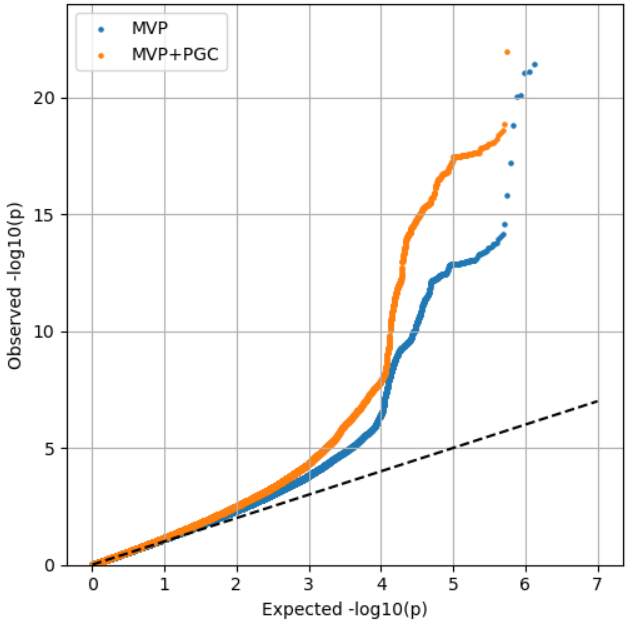


Supplementary Figure 9: conjFDR validation for mood instability and alcohol-related phenotypes. Manhattan plots for conjFDR between mood instability and GSCAN-alcohol consumption (MOOD & AC, in blue) and between mood instability and PGC-alcohol use disorder (MOOD & AUD, in brown).

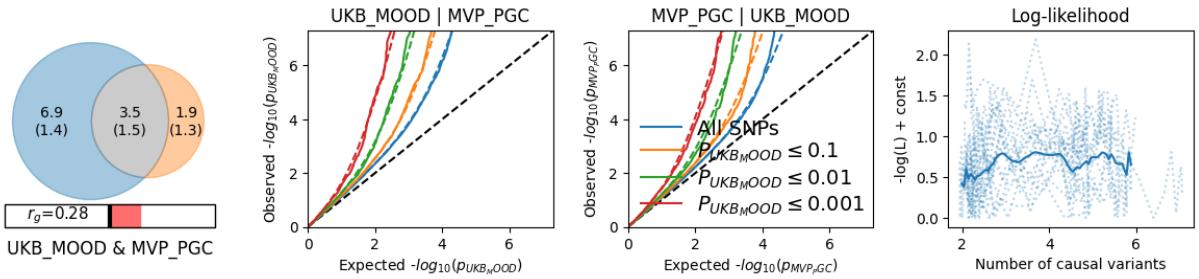


Supplementary Figure 10: Validation analyses for mood instability and alcohol use disorder using meta-analysis between the MVP and the PGC samples. (A) Q-Q plots from MVP alone *vs.* MVP+PGC AUD summary statistics. (B) Venn diagrams and Q-Q plots from MiXeR replication analyses showing polygenic overlap and enrichment for significant SNPs at decreasing thresholds. Analyses were performed using GWAS from UK biobank for mood instability (MOOD). rg, genetic correlation.

(A)

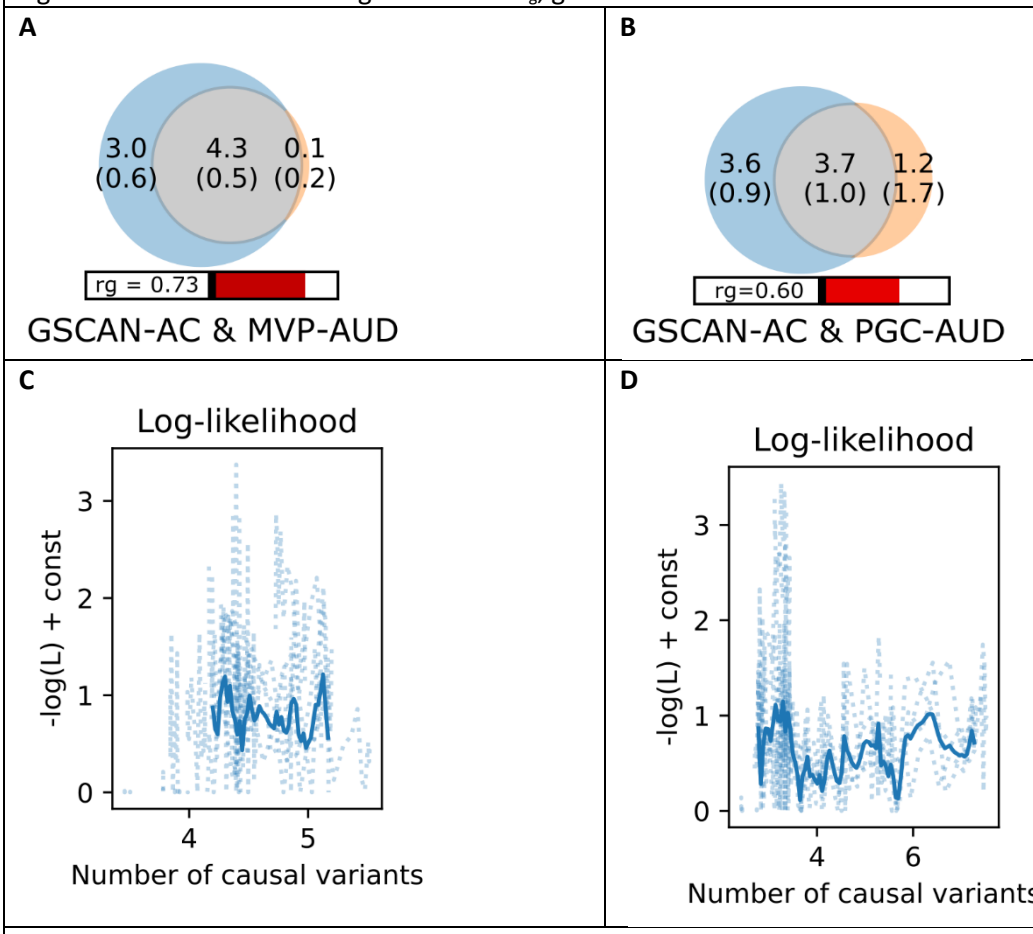


(B)



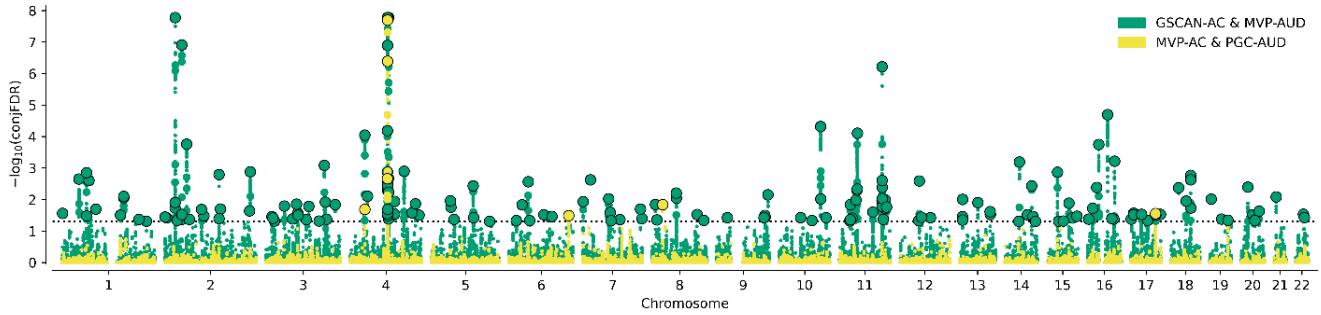
MOOD, mood instability in the UK biobank; MVP_PGC, meta-analysis of the alcohol use disorder in the Million Veteran Program + the Psychiatric Genomics Consortium samples.

Supplementary Figure 11: Validation for alcohol-related phenotypes. Venn diagrams and log-likelihood plots from MiXeR quasi-replication showing polygenic overlap and enrichment for significant SNPs at decreasing thresholds. r_g, genetic correlation.



MVP, Million Veteran Program; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine use; PGC, Psychiatric Genetics Consortium; AC, alcohol consumption; AUD, alcohol use disorder.

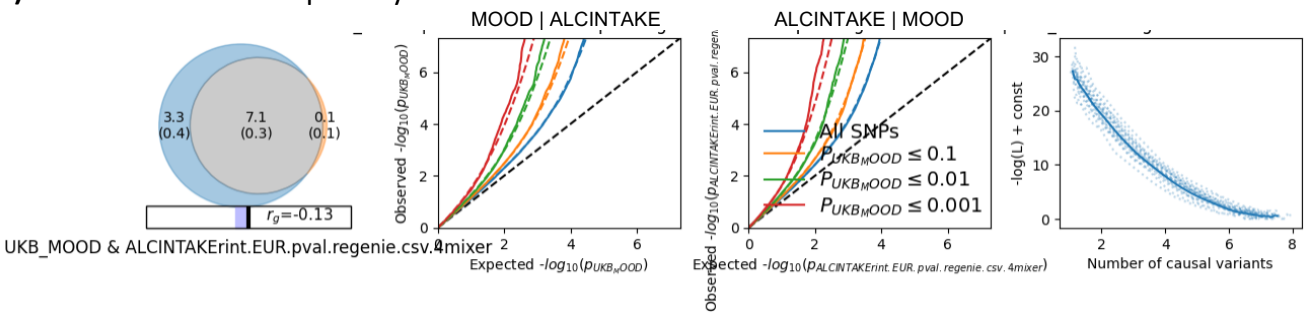
Supplementary Figure 12: Manhattan plots for conjFDR between GSCAN-alcohol consumption and MVP-alcohol use disorder (GSCAN-AC & MVP-AUD, in green) and between MVP-alcohol consumption and PGC-alcohol use disorder (MVP-AC & PGC-AUD, in yellow).



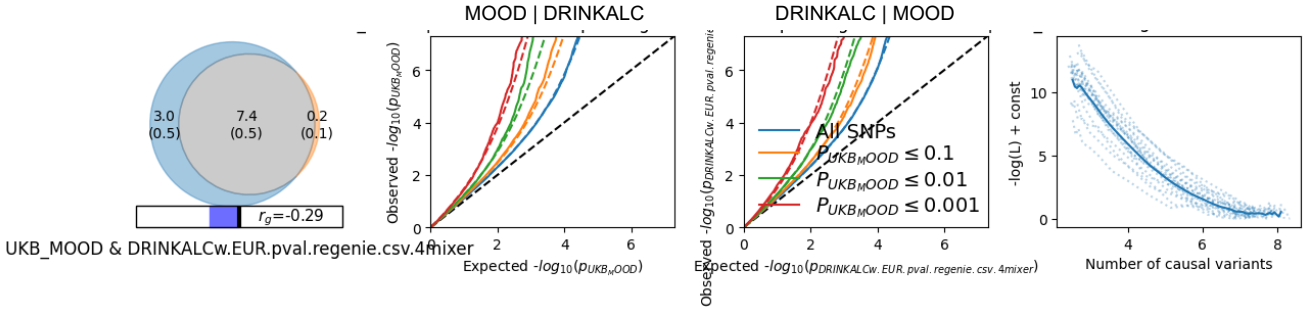
MVP, Million Veteran Program; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine use; PGC, Psychiatric Genetics Consortium. AC, alcohol consumption; AUD, alcohol use disorder.

Supplementary Figure 13: Exploratory analyses. Venn diagrams, Q-Q plots and log-likelihood plots from MiXeR showing polygenic overlap and enrichment for significant SNPs at decreasing thresholds between mood instability (MOOD) and AC phenotypes. r_g, genetic correlation.

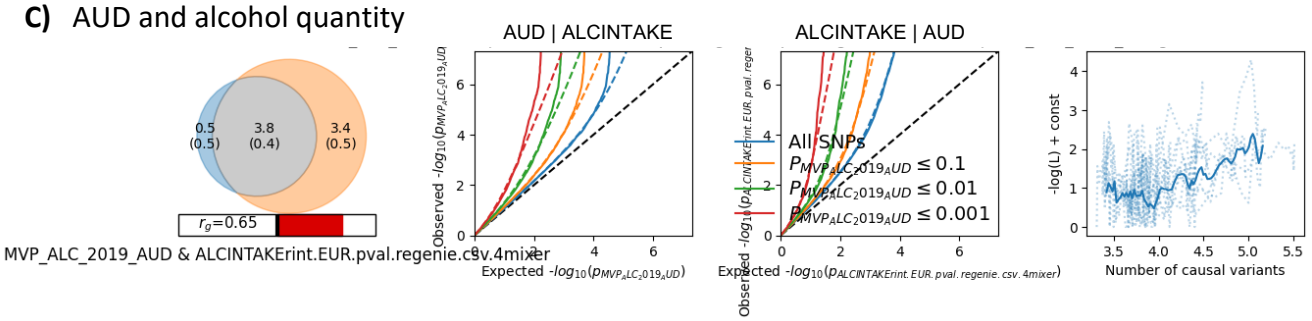
A) MOOD and alcohol quantity.



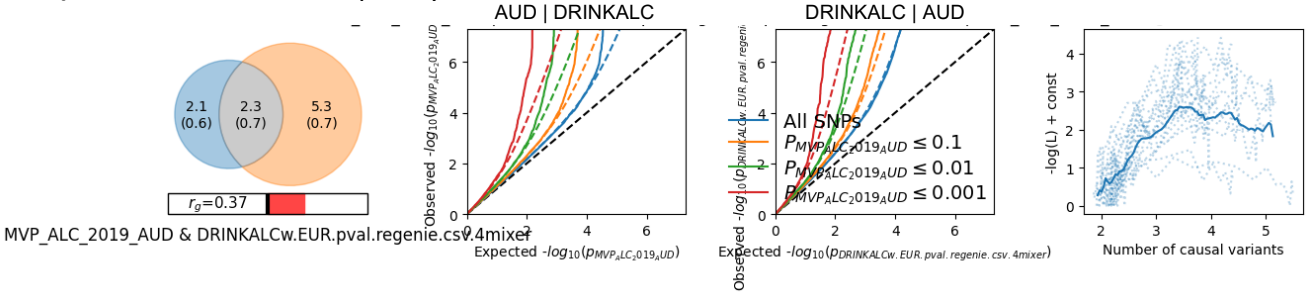
B) MOOD and alcohol frequency



C) AUD and alcohol quantity

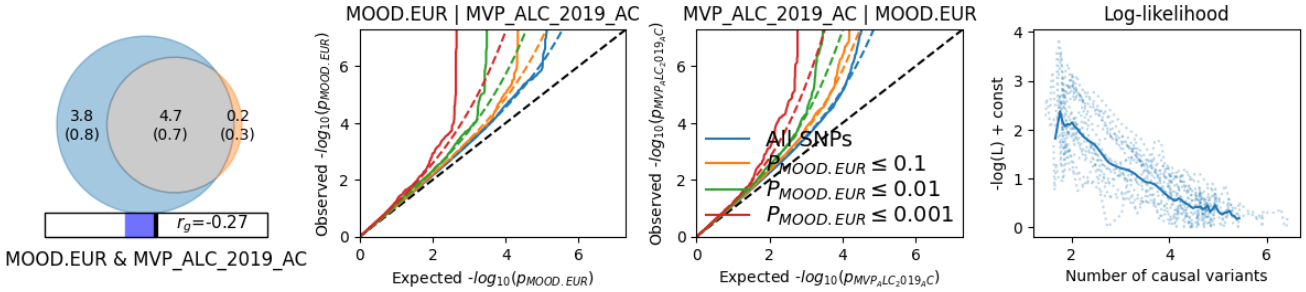


D) AUD and alcohol frequency

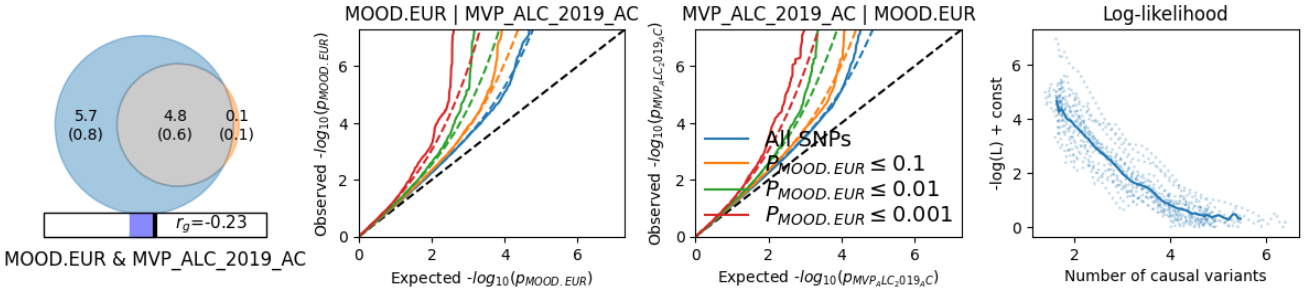


Supplementary Figure 14: Exploratory analyses. Venn diagrams, Q-Q plots and log-likelihood plots from MiXeR showing polygenic overlap and enrichment for significant SNPs at decreasing thresholds between mood instability (MOOD) and AC or AUD phenotypes as a function of lifetime tobacco smoking status. r_g, genetic correlation.

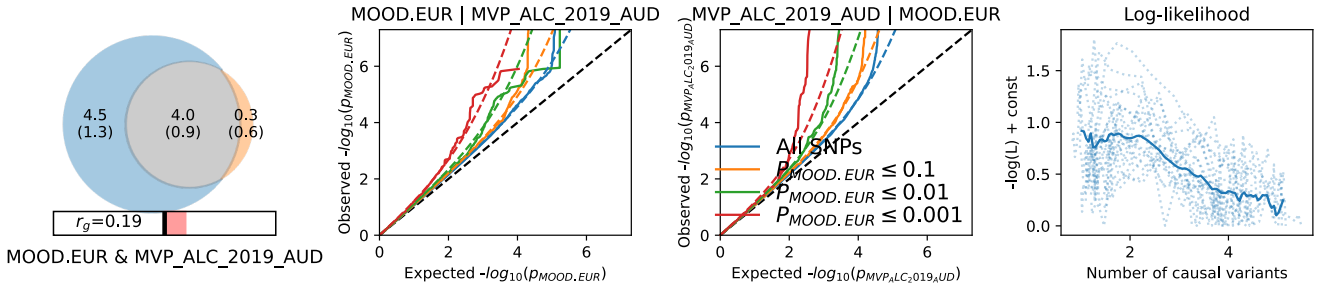
A) MOOD and AC in never-smokers



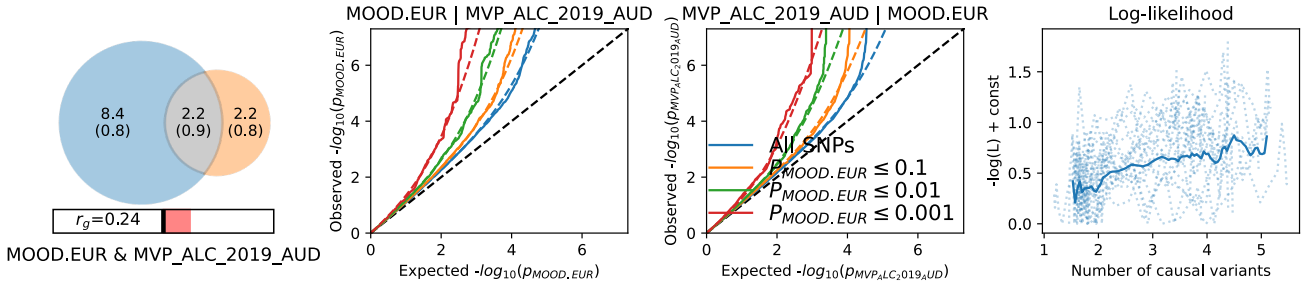
B) MOOD and AC in ever-smokers



C) MOOD and AUD in never-smokers



D) MOOD and AUD in ever-smokers



Supplementary Table 1: significant lead SNPs from conjunctional false discovery rate (conjFDR) analysis and their functional annotation for (A) mood instability and alcohol consumption and (B) mood instability and alcohol use disorder. *P*-values and Z scores are rounded for five digits. Novel SNPs for GWASs about MOOD, AC and AUD published as of June 1 2022 are written in bold. See supplementary File Supp_Table1.xlsx.

A) **MOOD and AC**

B) **MOOD and AUD**

Supplementary Table 2: overview of polygenic overlap and genetic correlation obtained by MiXeR in each discovery and validation analysis. Note that the degree of polygenic overlap is based on 90% of the joint genetic signal for the two phenotypes considered.

Phenotype 1	Phenotype 2	Sample 1	Sample 2	% shared polygenic overlap for phenotype 1	% shared polygenic overlap for phenotype 2	Genetic correlation (r_g)
Discovery analyses						
MOOD	AC	UKB	MVP	47	98	-0.22
	AUD			20	49	0.23
AC	AUD			51	58	0.52
Validation						
MOOD	AC	UKB	GSCAN	74	92	0
	AUD	UKB		39	82	0.47
AC	AUD	GSCAN	MVP	59	98	0.73
		MVP		51	58	0.52
		GSCAN	PGC	51	76	0.6
		GSCAN (without UKB)	PGC	45	61	0.52

MOOD, mood instability; AC, alcohol consumption; AUD, alcohol use disorder; UKB, UK Biobank; MVP, Million Veteran Program; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine use; PGC, Psychiatric Genetics Consortium; MDD, major depressive disorder; MVP_PGC, meta-analysis of the alcohol use disorder in the Million Veteran Program + the Psychiatric Genomics Consortium samples**.**

Supplementary Table 3: Lead SNPs with validation conjFDR *p* <0.05 for alcohol-related phenotypes.

SNP	CHR	BP	A1	A2	conjFDR discovery	Z or BETA discovery	conjFDR validation	Z or BETA validation
				MVP-AC & GSCAN-AC				
rs2312147	2	58222928	C	T	7.27E-09	0.03166	0.00414	0.00883
rs13411140*	2	144215811	C	T	0.0002872	0.02014	0.00435	0.00885
rs818219	3	85374589	C	T	0.0002052	0.01989	0.000324	0.0108
rs112635299	14	94838142	G	T	8.06E-07	0.1043	0.000492	0.0405
rs11039255*	11	47495746	G	T	5.18E-05	0.02297	0.00102	0.0103
				MVP-AUD & PGC-AUD				
rs4273169	2	144231309	A	G	2.63E-05	-4.204	0.00957	-2.591
rs1940701	11	112869404	C	T	0.0001677	3.763	0.03791	2.076
rs7933981	11	113438068	A	G	3.3E-10	-6.284	0.007097	-2.692
rs2958171	18	53072832	C	T	2.85E-05	4.185	0.03127	-2.154

SNP, single nucleotide polymorphism; CHR, chromosome; BP, position in base pairs; A1, alternate allele; A2, reference allele; MVP, Million Veteran Program; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine use; AC, alcohol consumption; AUD, alcohol use disorder; PGC, Psychiatric Genetics Consortium.

Supplementary Tables 4&5: PheWAS results for conjFDR with MOOD & AC and MOOD & AUD, respectively, using MRC IEU PheWAS tool. See Supplementary Files SupTab4_phewasAC.xlsx & SupTab5_phewasAUD.xlsx.