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Genome-wide meta-analysis of problematic alcohol use in 435,563 individuals yields insights into biology and relationships with other traits

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Problematic alcohol use (PAU) is a leading cause of death and disability worldwide. Although genome-wide association studies have identified PAU risk genes, the genetic architecture of this trait is not fully understood. We conducted a proxy-phenotype meta-analysis of PAU, combining alcohol use disorder and problematic drinking, in 435,563 European-ancestry individuals. We identified 29 independent risk variants, 19 of them novel. PAU was genetically correlated with 138 phenotypes, including substance use and psychiatric traits. Phenome-wide polygenic risk score analysis in an independent biobank sample (BioVU, *n* = 67,589) confirmed the genetic correlations between PAU and substance use and psychiatric disorders. Genetic heritability of PAU was enriched in brain and in conserved and regulatory genomic regions. Mendelian randomization suggested causal effects on liability to PAU of substance use, psychiatric status, risk-taking behavior and cognitive performance. In summary, this large PAU meta-analysis identified novel risk loci and revealed genetic relationships with numerous other traits.

Alcohol use and alcohol use disorder (AUD) are leading causes of death and disability worldwide[1]. Genome-wide association studies of AUD and problematic drinking as measured by different assessments have identified potential risk genes primarily in European populations[2–5]. Quantity-frequency measures of drinking—for example, the Alcohol Use Disorders Identification Test–Consumption (AUDIT-C), which sometimes reflect alcohol consumption in the normal range—differ genetically from AUD and measures of problematic drinking (for example, the Alcohol Use Disorders Identification Test–Problems (AUDIT-P)), and show a divergent set of genetic correlations[3,4]. The estimated single-nucleotide polymorphism (SNP)-based heritability (h^2) of AUD ranges from 5.6 to 10.0% (refs. [2–5]). To date, more than ten risk variants have been significantly associated with AUD and AUDIT-P ($P < 5 \times 10^{-8}$). Variants that have been mapped to several risk genes in multiple studies include *ADH1B* (Alcohol Dehydrogenase 1B (class I), Beta Polypeptide), *ADH1C* (Alcohol Dehydrogenase 1C (class I), Gamma Polypeptide), *ALDH2* (Aldehyde Dehydrogenase 2 Family Member, but only in some Asian samples), *SLC39A8* (Solute Carrier Family 39 Member 8), *GCKR* (Glucokinase Regulator) and *CRHR1* (Corticotropin Releasing Hormone Receptor 1). In the context of the known extensive polygenicity underlying AUD and AUDIT-P, we anticipate that additional significant risk loci can be identified by increasing sample size; this is also the pattern for genome-wide

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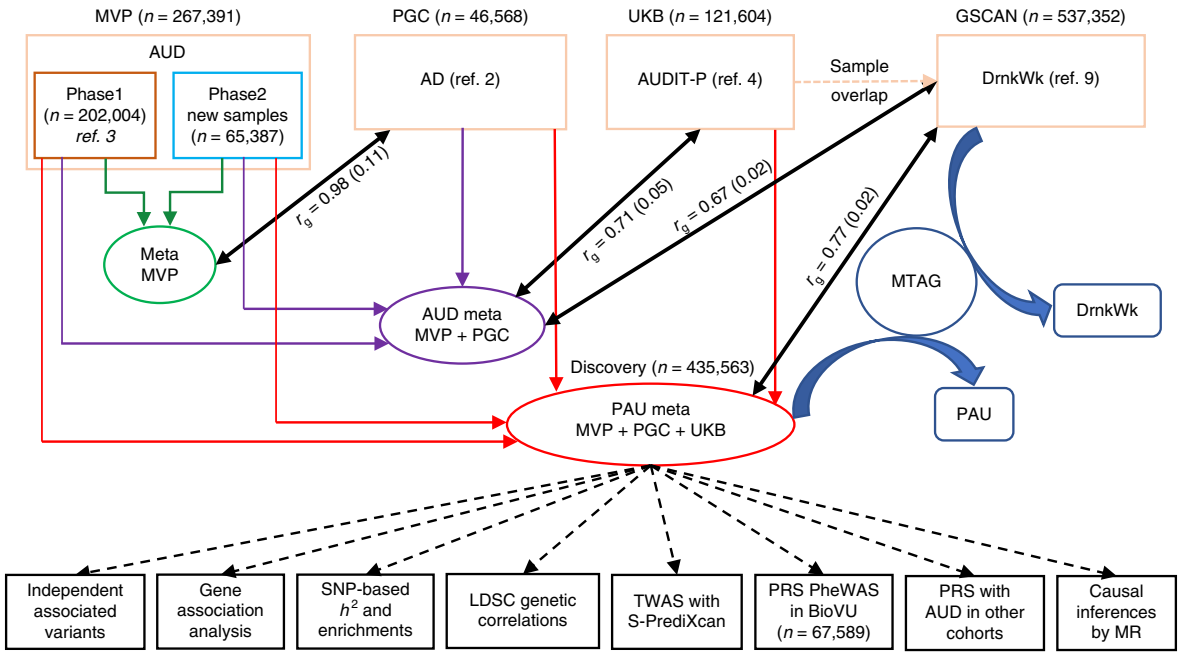

Fig. 1 | Overview of the analysis. The four datasets that were meta-analyzed as the discovery sample for PAU included MVP phase1, MVP phase2, PGC and UKB. MVP phase1 and phase2 were meta-analyzed, and the result was used to test the genetic correlation with PGC AD. An intermediary meta-analysis (AUD meta) combining MVP phase1, phase2 and PGC was then conducted to measure the genetic correlation with UKB AUDIT-P. Due to sample overlap between UKB and GSCAN, we used the AUD (intermediary) meta-analysis for MR analysis rather than the PAU (that is, from the final) meta-analysis. MTAG, which used the summary data from PAU and DrnkWk (DrnkWk) in GSCAN (without 23andMe samples, as those data were not available) as input to increase the power for each trait without introducing bias from sample overlap, returned summary results for PAU and DrnkWk separately. TWAS, transcriptome-wide association study.

association studies (GWAS) of heterogenous complex traits in general. We characterize both AUD per se and AUDIT-P as PAU. To identify additional risk variants and enhance our understanding of the genetic architecture of PAU, we conducted genome-wide meta-analysis of AUD and AUDIT-P in 435,563 individuals of European ancestry. Our understanding of the genetic architecture of PAU in African populations lags far behind that in Europeans; the largest sample of African-ancestry individuals published to date is 56,648, in the Million Veteran Program (MVP)[3], and results have not moved beyond a single genomic region that includes *ADH1B*. We limited the focus here to European samples because we could not achieve a substantial increment in African-ancestry subjects over previous studies.

Results

Figure 1 provides an overview of the meta-analysis of the four major datasets. The first is the GWAS of AUD in European Americans (EA) from MVP[6] (herein designated MVP phase1), comprising 202,004 individuals phenotyped for AUD (n_{case} = 34,658, $n_{control}$ = 167,346, $n_{effective}$ = 114,847) using International Classification of Diseases (ICD) codes[3]. The second, MVP phase2, included an additional 65,387 EA individuals from MVP (n_{case} = 11,337, $n_{control}$ = 54,050, $n_{effective}$ = 37,485) not previously analyzed. The third dataset is a GWAS of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) alcohol dependence (AD) from the Psychiatric Genomics Consortium (PGC), which included 46,568 European participants (n_{case} = 11,569, $n_{control}$ = 34,999, $n_{effective}$ = 26,853)[2]. The fourth dataset is a GWAS of AUDIT-P (a measure of problematic drinking) scores from a UK Biobank (UKB) sample[7] that included 121,604 European participants[4].

The genetic correlation (r_g) between MVP phase1 AUD and PGC AD was 0.965 (s.e.m. = 0.15, $P = 1.21 \times 10^{-10}$)[3]. The r_g between the entire MVP (meta-analysis of phase1 and phase2) and PGC was 0.98 (s.e.m. = 0.11, $P = 1.99 \times 10^{-19}$), justifying the meta-analysis of AUD across the three datasets ($n_{effective}$ = 179,185). We detected 24 risk variants in 23 loci in this intermediary meta-analysis (Fig. 2a and Supplementary Table 1). The r_g between UKB AUDIT-P and AUD (MVP + PGC) was 0.71 (s.e.m. = 0.05, $P = 8.15 \times 10^{-52}$) and the polygenic risk score (PRS) of AUD was associated with AUDIT-P in UKB (best *P* value threshold (PT_{best}) = 0.001, R^2 = 0.25%, $P = 3.28 \times 10^{-41}$; Supplementary Table 2 and Supplementary Fig. 1), justifying the proxy-phenotype meta-analysis of PAU across all four datasets (AUD and AUDIT-P, though highly correlated genetically, are not identical traits). The total sample size was 435,563 in the discovery analysis ($n_{effective}$ = 300,789).

Association results for PAU. Of 42 lead variants (mapping to 27 loci; Fig. 2b and Supplementary Table 3) that were genome-wide significant (GWS) for PAU, 29 were independently associated after conditioning on lead SNPs in the regions (see below and Table 1). Ten variants were previously identified through the same index SNPs or tagged SNPs located in or near the following genes: *GCKR*, *SIX3*, *KLB*, *ADH1B*, *ADH1C*, *SLC39A8*, *DRD2* and *FTO*[2–5]. Thus, 19 variants reported here are novel, of which 11 were located in gene regions, including *PDE4B* (phosphodiesterase 4B), *THSD7B* (thrombospondin type 1 domain containing 7B), *CADM2* (cell adhesion molecule 2), *ADH1B* (different from the locus identified previously), *DPP6* (dipeptidyl peptidase like 6), *SLC39A13* (solute carrier family 39 member 13), *TMX2* (thioredoxin related transmembrane protein 2), *ARID4A* (AT-rich interaction domain 4 A), *C14orf2* (chromosome 14 open reading frame 2), *TNRC6A* (trinucleotide repeat containing adaptor 6 A) and *FUT2* (fucosyltransferase 2). A novel rare *ADH1B* variant, rs75967634 ($P = 1.07 \times 10^{-9}$, with a minor allele frequency of 0.003), which causes a substitution of histidine for arginine, is in the same codon as rs2066702 (a well-known variant associated with AUD in African

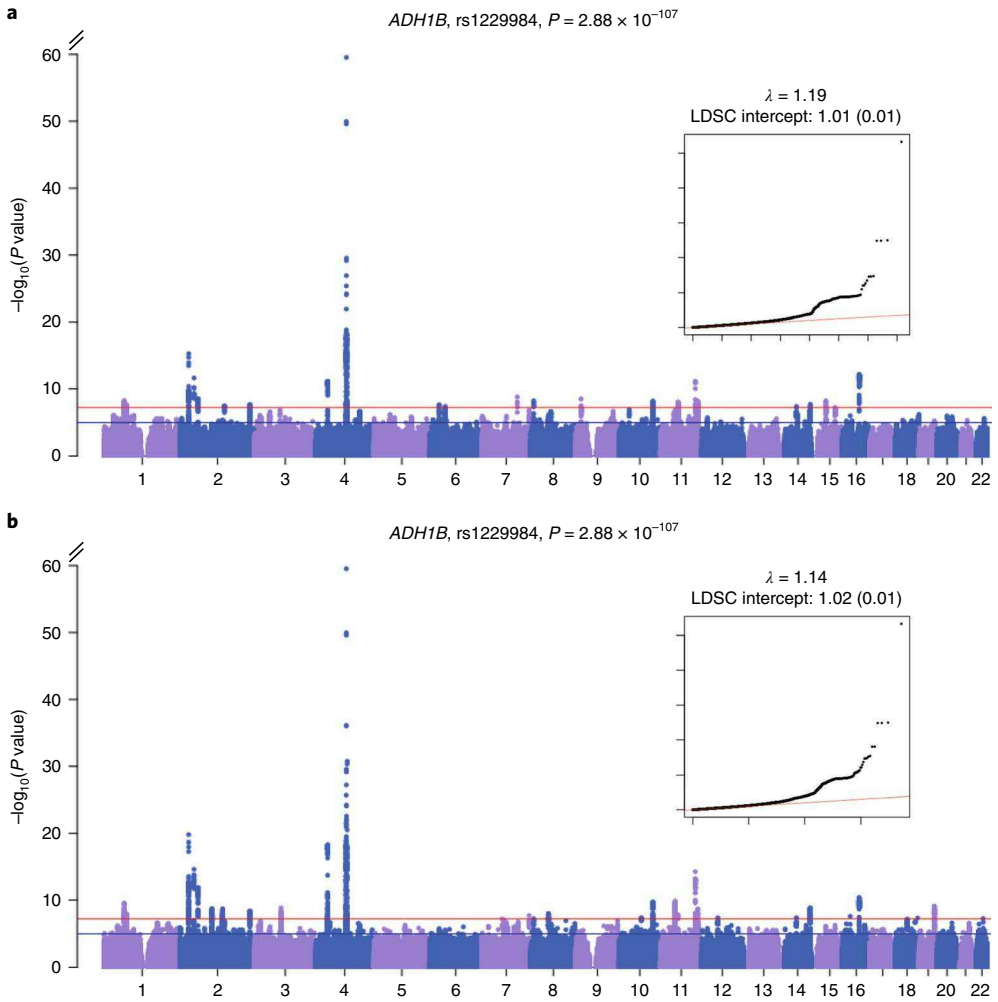

Fig. 2 | Association results for AUD and PAU meta-analyses. a, Manhattan and quantile–quantile plots for AUD (MVP + PGC), n_{case} = 57,564, $n_{control}$ = 256,395 and $n_{effective}$ = 179,185. **b**, Manhattan and quantile–quantile plots for PAU, n = 435,563 and $n_{effective}$ = 300,789. Effective sample size-weighted meta-analyses were performed using METAL. Red lines indicate GWS after correction for multiple testing ($P < 5 \times 10^{-8}$).

populations[3,8], but not polymorphic in European populations).This association is independent of rs1229984 in *ADH1B* and rs13125415 (a tag SNP of rs1612735 in MVP phase1 (ref. [3])) in *ADH1C*. The identification of rs75967634 demonstrates the greater power of the present study to detect risk variants in this region, beyond the frequently reported *ADH1B**rs1229984.

Moderate genetic correlation between AUD and alcohol consumption and pervasive pleiotropic effects of SNPs was demonstrated previously[2–4]. Some of the novel variants (10 of 19) identified in this study were also associated with other alcohol-related traits, including AUDIT-C score[3], total AUDIT score[4] and DrnkWk from the GWAS & Sequencing Consortium of Alcohol and Nicotine use (GSCAN) study[9] (described below and in Supplementary Table 3). Rs1402398, close to *VRK2*, was associated with AUDIT-C score (tagged by rs2683616)[3]; rs492602 in *FUT2* was associated with DrnkWk[9] and total AUDIT score[4]; and rs6421482, rs62250713, rs2533200, rs10717830, rs1783835, rs12296477, rs61974485 and rs72768626 were associated with DrnkWk, either directly or through tag SNPs in high-linkage disequilibrium (LD)[9]. Analysis conditioned on DrnkWk shows that 11 of the 29 independent variants were independently associated with PAU (that is, not mediated by DrnkWk; Supplementary Table 3).

Gene-based association analysis identified 66 genes that were associated with PAU at GWS ($P < 2.64 \times 10^{-6}$; Supplementary Table 4). *DRD2*, which has been extensively studied in many fields of neuroscience, was among these genes and was previously reported in both UKB[4] and MVP phase1 (ref. [3]). Among the 66 genes, 46 are novel, including *ADH4* (alcohol dehydrogenase 4 (class II), pi polypeptide), *ADH5* (alcohol dehydrogenase 5 (class III), chi polypeptide) and *ADH7* (alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide), extending alcohol-metabolizing gene associations beyond the well-known *ADH1B* and *ADH1C*; *SYNGAP1* (synaptic Ras GTPase activating protein 1), *BDNF* (brain-derived neurotrophic factor) and others. Certain genes show associations with multiple traits, including previous associations with AUDIT-C (four genes in MVP phase1, 12 in UKB), total AUDIT score (19 genes in UKB) and DrnkWk (46 genes in GSCAN, which includes results for DrnkWk after multi-trait analysis of GWAS (MTAG)[10] analysis).

Examination of the 66 associated genes for known drug–gene interactions through the Drug Gene Interaction Database v.3.0.2 (ref. [11]) showed 327 interactions between 16 genes and 325 drugs (Supplementary Table 5). Of these 16 genes with interactions, *DRD2* had the most drug interactions (n = 177) followed by *BDNF* (n = 68) and *PDE4B* (n = 36).

SNP-based h^2 and partitioning heritability enrichment. We used linkage disequilibrium (LD) score regression (LDSC)[12] to estimate SNP-based h^2 in the different datasets and meta-analyses (Fig. 3).

Table 1 | GWS associations for PAU

Chr	Pos (hg19)	rs ID	Gene	A1	A2	EAF	*Z*	*P*	Direction
1	66419905	**rs6421482**	*PDE4B*[a]	A	G	0.4363	−6.315	2.7×10^{-10}	----
1	73848610	**rs61767420**	[]	A	G	0.3999	5.714	1.11×10^{-8}	++++
2	27730940	rs1260326	*GCKR*[a]	T	C	0.4033	−9.296	1.4×10^{-20}	--+-
2	45141180	rs494904	*SIX3*[b]	T	C	0.5961	−7.926	2.26×10^{-15}	----
2	58042241	**rs1402398**	*VRK2*[b]	A	G	0.6274	7.098	1.27×10^{-12}	++++
2	104134432	**rs9679319**	[]	T	G	0.4797	−6.01	1.86×10^{-9}	----
2	138264231	**rs13382553**	*THSD7B*[a]	A	G	0.766	−6.001	1.97×10^{-9}	----
2	227164653	**rs2673136**	*IRS1*[b]	A	G	0.6387	−5.872	4.31×10^{-9}	----
3	85513793	**rs62250713**	*CADM2*[a]	A	G	0.368	6.049	1.46×10^{-9}	++++
4	39404872	rs13129401	*KLB*[b]	A	G	0.4532	−8.906	5.29×10^{-19}	----
4	100229016	**rs75967634**	*ADH1B*[a]	T	C	0.003	−6.098	1.07×10^{-9}	--?-
4	100239319	rs1229984	*ADH1B*[a]	T	C	0.0302	−22	2.9×10^{-107}	---?
4	100270452	rs13125415	*ADH1C*[a]	A	G	0.5849	−9.073	1.16×10^{-19}	----
4	103198082	rs13135092	*SLC39A8*[a]	A	G	0.9192	11.673	1.75×10^{-31}	++++
7	153489074	**rs2533200**	*DPP6*[a]	C	G	0.5163	−5.631	1.79×10^{-8}	----
8	57424874	**rs2582405**	*PENK*[b]	T	C	0.237	5.751	8.86×10^{-9}	++++
10	72907951	**rs7900002**	*UNC5B*[b]	T	G	0.6012	-5.503	3.7×10^{-8}	--+-
10	110537834	rs56722963	[]	T	C	0.2551	-6.374	1.85×1^{-10}	----
11	47423920	**rs10717830**	*SLC39A13*[a]	G	GT	0.674	6.422	1.34×10^{-10}	++++
11	57480623	**rs576859**	*TMX2*[a]	A	C	0.3272	5.67	1.43×10^{-8}	+++?
11	113357710	rs138084129	*DRD2*[b]	A	AATAT	0.6274	7.824	5.13×10^{-15}	++++
11	113443753	rs6589386	*DRD2*[b]	T	C	0.4323	−7.511	5.88×10^{-14}	----
11	121542923	**rs1783835**	*SORL1*[b]	A	G	0.4569	−5.979	2.24×10^{-9}	----
12	51903860	**rs12296477**	*SLC4A8*[b]	C	G	0.5469	5.484	4.15×10^{-8}	++++
14	58765903	**rs61974485**	*ARID4A*[a]	T	C	0.2646	5.506	3.67×10^{-8}	++++
14	104355883	**rs8008020**	*C14orf2*[a]	T	C	0.4175	6.062	1.35×10^{-9}	++++
16	24693048	**rs72768626**	*TNRC6A*[a]	A	G	0.9448	5.591	2.26×10^{-8}	++++
16	53820813	rs9937709	*FTO*[a]	A	G	0.585	6.602	4.06×10^{-11}	++++
19	49206417	**rs492602**	*FUT2*[a]	A	G	0.5076	−6.143	8.08×10^{-10}	----

The total sample size is 435,563; effective sample size from each cohort was used for sample size-weighted meta-analyses ($n_{effective}=300{,}789$) using METAL. Listed are the 29 independent variants that were significant genome-wide. Variants labeled in bold are novel associations with PAU. Chr, chromosome. A1, effect allele; A2, other allele; EAF, effective allele frequency. Directions are for the A1 allele in MVP phase1, MVP phase2, PGC and UKB datasets. Pos (hg19), the position of human genome assembly GRCh37/hg19; [], no protein-coding gene within 500 kb. [a]Protein-coding gene containing the lead SNP. [b]Protein-coding gene nearest to the lead SNP.

Because of the unbalanced case/control ratio, we used effective sample size rather than actual sample size in MVP (following the PGC AD GWAS[2]). The h^2 of PAU (the meta result) was 0.068 (s.e.m. = 0.004). The h^2 of AUD in the MVP meta-analysis (phases 1 and 2) was 0.095 (s.e.m. = 0.006) and 0.094 (s.e.m. = 0.005) in the meta-analysis that combined MVP and PGC.

Partitioning heritability enrichment analyses using LDSC[13,14] showed the most significantly enriched cell type group to be central nervous system (CNS; $P=3.53\times10^{-9}$), followed by adrenal and pancreas ($P=1.89\times10^{-3}$) and immune and hematopoietic ($P=3.82\times10^{-3}$; Supplementary Fig. 2). Significant enrichments were also observed in six baseline annotations, including conserved regions, conserved regions with 500 bp extended (ext), fetal DHS (DNase I hypersensitive sites) ext, weak enhancers ext, histone mark H3K4me1 ext and transcription start site (TSS) ext (Supplementary Fig. 3). We also investigated heritability enrichments using Roadmap data, which contain six annotations (DHS, H3K27ac, H3K4me3, H3K4me1, H3K9ac and H3K36me3) in a subset of 88 primary cell types and tissues[14,15]. Significant enrichments were observed for H3K4me1 and DHS in fetal brain, and H3K4me3 in both fetal brain and brain germinal matrix (Supplementary Table 6). Although no heritability enrichment was observed in tissues using gene expression data from the Genotype-Tissue Expression Project (GTEx)[16], the top nominally enriched tissues were all in brain (Supplementary Fig. 4).

Functional enrichments. MAGMA tissue expression analysis[17,18] using GTEx showed significant enrichments in several brain tissues, including cerebellum and cortex (Supplementary Fig. 5). Although no enrichment was observed via MAGMA gene set analysis using gene-based *P* values of all protein-coding genes, the 152 genes prioritized by positional, expression quantitative trait loci (eQTL) and chromatin interaction mapping were enriched in several gene sets, including ethanol metabolic processes (Supplementary Table 7).

Genetic correlations with other traits. We estimated the genetic correlations between PAU and 715 publicly available sets of GWAS summary statistics, which included 228 published sets and 487 unpublished sets from UKB. After Bonferroni correction ($P<6.99\times10^{-5}$), 138 traits were significantly correlated with

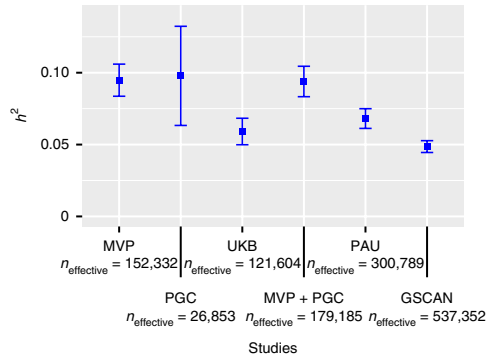

Fig. 3 | Estimated SNP-based h^2. Shown are h^2 results for single datasets or meta-analysis between datasets, from published studies or analyzed here. MVP is the phase1–phase2 MVP meta-analysis and PAU is the discovery meta-analysis. Effective sample sizes ($n_{effective}$) were used in LDSC. Center values are the estimated h^2, and error bars indicate 95% confidence intervals.

Fig. 4 | Genetic correlations with published traits. LDSC was applied to test genetic correlation between PAU and 715 traits. Of 228 published traits, 26 were genetically correlated with PAU after Bonferroni correction ($P < 6.99 \times 10^{-5}$). ADHD, attention deficit hyperactivity disorder. Center values are the estimated genetic correlation, and error bars indicate 95% confidence intervals.

PAU (Supplementary Table 8). Among the 26 published correlated traits, DrnkWk showed the highest correlation with PAU ($r_g = 0.77$, s.e.m. = 0.02, $P = 3.25 \times 10^{-265}$), consistent with the overall quantity of alcohol consumed being a key domain of PAU[5,19]. Several smoking traits and lifetime cannabis use were positively genetically correlated with PAU, consistent with the high comorbidity between alcohol and other substance use disorders in the general population[20]. Among psychiatric disorders, major depressive disorder (MDD; $r_g = 0.39$, s.e.m. = 0.03, $P = 1.43 \times 10^{-40}$) showed the highest genetic correlation with PAU, extending the evidence for a shared genetic contribution to MDD- and alcohol-related traits[21,22]. PAU was positively correlated with risk-taking behavior, insomnia, CYP2A6 activity and other traits, and negatively correlated with cognitive traits and parents' age at death. These findings are in line with the known adverse medical, psychiatric and social consequences of problem drinking (Fig. 4).

Transcriptomic analyses. We used S-PrediXcan[23] to predict gene expression and the mediating effects of variation on gene expression on PAU. Forty-eight tissues from GTEx[16] release v.7 and whole blood samples from the Depression Genes and Networks (DGN) study[24] were analyzed as reference transcriptomes (Supplementary Table 9). After Bonferroni correction, 103 gene–tissue associations were significant, representing 39 different genes, some of which were identified in multiple tissues (Supplementary Table 10). For example, *C1QTNF4* (C1q and TNF related 4) was detected in 18 tissues, including brain, gastrointestinal, adipose and liver. None of the four significant alcohol dehydrogenase genes (*ADH1A*, *ADH1B*, *ADH4* and *ADH5*) was associated with expression in brain tissue, but they were associated with expression in other tissues—adipose, thyroid, gastrointestinal and heart. These cross-tissue associations indicate that there are widespread functional consequences of PAU risk-associated genetic variation at the expression level.

Although the sample size for tissues used for eQTL analysis limits our ability to detect associations, there are substantial common eQTLs across tissues[16]. Integrating evidence from multiple tissues can increase the power to detect genes relative to the tissues tested individually, at least for shared eQTLs. We applied S-MultiXcan[25] to the summary data for PAU using all 48 GTEx tissues as reference transcriptomic data. The expression of 34 genes was significantly associated with PAU, including *ADH1B*, *ADH4*, *ADH5*, *C1QTNF4*, *GCKR* and *DRD2* (Supplementary Table 11). Among the 34 genes, 27 overlapped with genes detected by S-PrediXcan.

PAU PRS for phenome-wide associations. We calculated PRS for PAU in 67,589 individuals of European descent from the Vanderbilt University Medical Center's biobank, BioVU. We conducted a phenome-wide association study (PheWAS) of PRS for PAU, adjusting for sex, age (calculated as the median age across an individual's medical record) and the top ten principal components (PCs) of ancestry. We standardized the PRS so that the odds ratios (ORs) correspond to a s.d. increase in the PRS. After Bonferroni correction, 31 of the 1,372 phenotypes tested were significantly associated with PAU PRS, including alcohol-related disorders (OR = 1.46, s.e.m. = 0.03, $P = 3.34 \times 10^{-40}$), alcoholism (OR = 1.33, s.e.m. = 0.03, $P = 3.85 \times 10^{-28}$), tobacco use disorder (OR = 1.21, s.e.m. = 0.01, $P = 2.71 \times 10^{-38}$), 6 respiratory conditions and 17 additional psychiatric conditions (Fig. 5 and Supplementary Table 12).

PAU PRS with AD in independent samples. We tested the association between PAU PRS and AD in three independent samples: the iPSYCH group ($n_{case} = 944$, $n_{control} = 11{,}408$, $n_{effective} = 3{,}487$); University College London (UCL) Psych Array ($n_{case} = 1{,}698$, $n_{control} = 1{,}228$, $n_{effective} = 2{,}851$); and UCL Core Exome Array ($n_{case} = 637$, $n_{control} = 9{,}189$, $n_{effective} = 2{,}383$). The PAU PRSs were significantly associated with AD in all three samples, with the most variance explained in the UCL Psych Array sample, which includes the most AD cases ($PT_{best} = 0.001$, $R^2 = 2.12\%$, $P = 8.64 \times 10^{-14}$). In the iPSYCH group and UCL Core Exome Array samples, the maximal variance explained was 1.61% ($PT_{best} = 0.3$, $P = 1.87 \times 10^{-22}$) and 0.77% ($PT_{best} = 5 \times 10^{-8}$, $P = 1.65 \times 10^{-7}$), respectively (Supplementary Table 13).

Mendelian randomization. We tested the bidirectional causal effects between other traits and AUD (MVP + PGC), rather than PAU; the UKB AUDIT-P GWAS sample was excluded to minimize overlap with other GWAS for putative exposures. (When we refer to exposure having causal effect on outcome, this should be understood to mean susceptibility or liability to exposure having causal effect on susceptibility or liability to outcome.) We limited the exposures to those genetically correlated with PAU and which yielded more than ten available instruments providing a robust causal estimate.

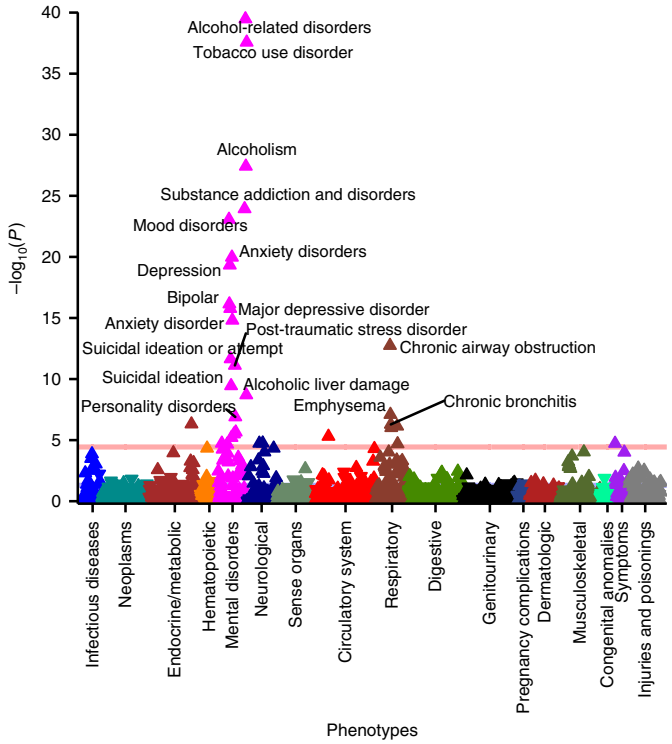

Fig. 5 | Phenome-wide associations with PAU PRS in BioVU. The polygenic score for PAU was calculated for 67,588 participants in BioVU using PRS-CS; 1,372 phenotypes were tested, and Bonferroni correction ($P < 3.64 \times 10^{-5}$) was applied.

Among the 15 tested exposures on AUD, seven showed evidence of a causal effect on liability to AUD (Table 2). DrnkWk and ever smoked regularly have a positive causal effect on AUD risk by all four methods, without violating Mendelian randomization (MR) assumptions through horizontal pleiotropy (MR–Egger intercept. $P > 0.05$). General risk tolerance was causally related to AUD risk, and the estimate was robust after correction for horizontal pleiotropy. The 'worry' subcluster of neuroticism and number of sexual partners shows evidence of positive causal effects on liability to AUD with at least one method, while cognitive performance and educational attainment show evidence of negative causal effects. As an exposure, AUD has a positive causal effect on DrnkWk and a negative causal effect on educational attainment, indicating bidirectional causality. There is no evidence of a causal effect of AUD on other traits (Table 3).

Joint analysis of PAU and DrnkWk using MTAG. We conducted a joint analysis of PAU and DrnkWk using MTAG, which can increase the power for each trait without introducing bias from sample overlap[10]. MTAG analysis increased the GWAS-equivalent sample size (n_{Eq}) for PAU to 514,790 (that is, a 71.1% increase from the original effective sample size ($n_{Eq} = 300{,}789$, $n = 435{,}563$). In this analysis, we observed an increase in the number of independent variants for PAU to 119, 76 of which were conditionally independent (Supplementary Fig. 6a and Supplementary Table 14). For DrnkWk, MTAG analysis increased n_{Eq} to 612,968 from 537,352, which yielded 141 independent variants, 86 of which were conditionally independent (Supplementary Fig. 6b and Supplementary Table 15).

The MTAG analysis also increased the power for the functional enrichment analysis. MAGMA gene set analysis for PAU after MTAG analysis detected ten enriched Gene Ontology terms, including 'regulation of nervous system development' ($P_{Bonferroni} = 8.80 \times 10^{-4}$), 'neurogenesis' ($P_{Bonferroni} = 0.010$) and 'synapse' ($P_{Bonferroni} = 0.046$) (Supplementary Table 16).

Discussion

We report here a genome-wide meta-analysis of PAU in 435,563 individuals of European ancestry from the MVP, PGC and UKB datasets. MVP is a mega-biobank that has enrolled >750,000 subjects (for whom genotype data on 313,977 subjects were used in this study), with rich phenotype data assessed by questionnaires and from electronic health records (EHRs). Currently, MVP is the largest single cohort available with diagnostic information on AUD[3,6]. PGC is a collaborative consortium that has led the effort to collect smaller cohorts with DSM-IV AD[2]. UKB is a population-level cohort with the largest available sample with AUDIT-P data[4].

Our discovery meta-analysis of PAU yielded 29 independent variants of which 19 were novel, with 0.059–0.113 of the phenotypic variance explained in different cohorts or meta-analyses. The value of h^2 in the phase1–phase2 MVP meta-analysis was 0.095 (s.e.m. = 0.006), which was higher than MVP phase1: 0.056 (s.e.m. = 0.004, in MVP phase1, where only the actual (as opposed to effective) sample size was used)[3]. The h^2 of AD in PGC was 0.098 (s.e.m = 0.018), comparable to the reported liability-scale h^2 (0.090, s.e.m. = 0.019)[2]. Functional and heritability analyses consistently showed enrichments in brain regions and gene expression regulatory regions, providing biological insights into the etiology of PAU. Variation associated with gene expression in the brain is central to PAU risk, a conclusion that is also consistent with our previous GWAS in MVP of both alcohol consumption and AUD diagnosis[3]. The enrichments in regulatory regions point to specific brain tissues relevant to the causative genes; the specific interactions between 16 genes and 325 drugs may provide targets for the development of medications to manage PAU. Potential targets identified include the D_2 dopamine receptor (encoded by *DRD2*) and phosphodiesterase 4B (encoded by *PDE4B*). The presence of risk variation at these loci also suggests that they may be 'precision medicine' targets.

We also found that PAU was significantly genetically correlated with 138 other traits. The top correlations were with substance use and substance-related disorders, MDD, schizophrenia and several other neuropsychiatric traits. In a conceptually similar analysis, we performed a PheWAS of PAU PRS in BioVU, which confirmed in an independent sample the genetic correlations between PAU and multiple substance use disorders, mood disorders and other psychiatric traits. We also used MR to infer causal effects of the above traits on liability to AUD (we tested AUD excluding UKB samples to avoid sample overlap) using selected genetic instruments. We found evidence of positive causal relationships from DrnkWk (bidirectional), ever smoked regularly, worry subcluster and number of sexual partners, while cognitive performance and educational attainment (bidirectional) showed protective effects on liability to AUD. In comparison, we detected few causal effects from AUD to other traits, possibly because of lack of power since there are fewer instrumental variants for AUD available in our study than for many comparison GWAS.

The study has other limitations. First, only European populations were included; therefore, the genetic architecture of PAU in other populations remains largely unknown. To date, the largest non-European sample to undergo GWAS for alcohol-related traits is African American, which was reported in the MVP phase1 sample (17,267 cases; 39,381 controls, an effective sample size of 48,015), with the only associations detected on chromosome 4 in the *ADH* gene locus (where several *ADH* genes map)[3]. The collection of substantial numbers of non-European subjects will require a concerted effort by investigators in our field. Second, despite the high genetic correlation between AUD and AUDIT-P, they are not identical traits. We conducted a meta-analysis of the two traits to

Table 2 | Causal effects on AUD (MVP + PGC) by MR

Exposure (no. of instruments)	Ref.	IVW[27]		Weighted median[28]		MR-Egger[29]		MR-Egger intercept *P*	MR-PRESSO[30]			GSMR[31]		
		β (s.e.m.)	*P*	*β* (s.e.m.)	*P*	*β* (s.e.m.)	*P*		Outliers (*n*)	*β* (s.e.m.)	*P*	HEIDI-outliers (*n*)	*β* (s.e.m.)	*P*
DrnkWk (58)	9	0.89 (0.06)	**1.80×10^{-46}**	0.89 (0.08)	**2.89×10^{-26}**	0.91 (0.20)	**3.80×10^{-6}**	0.898	0	0.89 (0.06)	**1.58×10^{-20}**	2	0.92 (0.05)	**6.37×10^{-79}**
Ever smoked regularly (199)	9	0.32 (0.02)	**8.72×10^{-51}**	0.33 (0.02)	**4.2×10^{-43}**	0.26 (0.08)	**1.21×10^{-3}**	0.471	3	0.33 (0.02)	**1.34×10^{-37}**	6	0.34 (0.01)	**1.84×10^{-115}**
Current versus former smoker (12)	9	0.04 (0.09)	0.678	0.00 (0.06)	0.978	−0.33 (0.22)	0.140	0.078	5	0.02 (0.04)	0.692	0	0.04 (0.03)	0.292
Cigarettes per day (33)	9	0.04 (0.06)	0.475	−0.10 (0.04)	0.010	−0.18 (0.09)	0.034	1.27×10^{-3}	5	0.09 (0.06)	0.151	4	0.01 (0.03)	0.643
MDD (78)	32	0.14 (0.03)	**8.42×10^{-6}**	0.14 (0.03)	**2.79×10^{-6}**	−0.17 (0.20)	0.390	0.113	5	0.14 (0.03)	**3.73×10^{-6}**	1	0.15 (0.02)	**1.65×10^{-18}**
Schizophrenia (110)	33	0.04 (0.01)	**2.47×10^{-6}**	0.04 (0.01)	**4.96×10^{-6}**	−0.05 (0.04)	0.202	0.016	4	0.04 (0.01)	**6.03×10^{-8}**	5	0.06 (0.01)	**4.65×10^{-26}**
Bipolar disorder (23)	34	0.03 (0.01)	0.012	0.03 (0.02)	0.049	−0.05 (0.07)	0.423	0.120	0	0.03 (0.01)	0.020	0	0.03 (0.01)	6.56×10^{-3}
Depressed affect subcluster (56)	35	0.19 (0.06)	1.75×10^{-3}	0.24 (0.05)	**5.44×10^{-6}**	−0.20 (0.28)	0.462	0.147	7	0.23 (0.04)	**1.12×10^{-6}**	5	0.26 (0.04)	**6.80×10^{-13}**
Neuroticism (131)	35	0.20 (0.04)	**1.10×10^{-7}**	0.20 (0.04)	**1.10×10^{-7}**	−0.26 (0.16)	0.097	2.64×10^{-3}	6	0.19 (0.03)	**5.83×10^{-8}**	4	0.17 (0.02)	**3.44×10^{-12}**
Worry subcluster (61)	35	0.13 (0.06)	0.020	0.17 (0.05)	**8.06×10^{-4}**	0.04 (0.26)	0.890	0.702	7	0.19 (0.04)	**8.64×10^{-5}**	5	0.21 (0.03)	**7.40×10^{-11}**
Number of sexual partners (64)	36	0.31 (0.04)	**3.27×10^{-12}**	0.36 (0.05)	**9.00×10^{-16}**	0.51 (0.20)	0.011	0.309	4	0.33 (0.04)	**1.14×10^{-12}**	3	0.34 (0.03)	**6.13×10^{-28}**
General risk tolerance (64)	36	0.26 (0.06)	**7.37×10^{-6}**	0.28 (0.07)	**5.93×10^{-5}**	0.88 (0.25)	**3.69×10^{-4}**	9.62×10^{-3}	0	0.26 (0.06)	**3.18×10^{-5}**	0	0.28 (0.05)	**1.91×10^{-9}**
Insomnia (159)	37	0.05 (0.01)	**1.90×10^{-5}**	0.03 (0.01)	5.31×10^{-3}	−0.00 (0.05)	0.993	0.288	7	0.04 (0.01)	**3.89×10^{-4}**	8	0.04 (0.01)	**3.51×10^{-6}**
Cognitive performance (134)	38	−0.08 (0.02)	**1.03×10^{-3}**	−0.05 (0.03)	0.044	−0.21 (0.12)	0.086	0.282	4	−0.08 (0.02)	4.21×10^{-3}	3	−0.09 (0.02)	**6.20×10^{-8}**
Educational attainment (570)	38	−0.22 (0.02)	**1.32×10^{-25}**	−0.21 (0.02)	**1.45×10^{-17}**	−0.24 (0.08)	2.21×10^{-3}	0.781	4	−0.21 (0.02)	**1.37×10^{-23}**	16	−0.23 (0.02)	**1.6×10^{-51}**

P values labeled in bold are significant after multiple testing correction ($P < 1.32\times10^{-3}$). Traits labeled in bold are those having a causal effect on AUD by at least one method and consistent for the direction of effect by all five methods. IVW, inverse variance-weighted linear regression. Outliers (*n*), number of pleiotropic variants removed from the MR estimate. HEIDI-outliers (*n*), number of pleiotropic variants removed from the MR estimate. Depressed affect subcluster, depressed affect subcluster of neuroticism. Worry subcluster, worry subcluster of neuroticism. Outliers are variants showing evidence of horizontal pleiotropy and that were removed before the causal estimate was made.

Table 3 | Causal effects of AUD (MVP + PGC) on other traits by MR

Outcome (no. of instruments)	Ref.	IVW[27]		Weighted median[28]		MR-Egger[29]		MR-Egger intercept *P*	MR-PRESSO[30]			GSMR[31]		
		β (s.e.m.)	*P*	*β* (s.e.m.)	*P*	*β* (s.e.m.)	*P*		Outliers (*n*)	*β* (s.e.m.)	*P*	HEIDI-outliers (*n*)	*β* (s.e.m.)	*P*
DrnkWk (17)	9	0.34 (0.05)	$\mathbf{3.16\times10^{-10}}$	0.31 (0.04)	$\mathbf{1.62\times10^{-12}}$	0.61 (0.39)	0.117	0.479	2	0.30 (0.04)	$\mathbf{1.31\times10^{-6}}$	1	0.28 (0.03)	$\mathbf{1.72\times10^{-25}}$
Ever smoked regularly (20)	9	0.08 (0.04)	0.021	0.04 (0.03)	0.186	−0.04 (0.06)	0.544	0.032	4	0.07 (0.03)	0.028	2	0.08 (0.02)	$\mathbf{6.94\times10^{-6}}$
Lifetime cannabis use (21)	39	0.05 (0.17)	0.763	−0.32 (0.13)	0.013	−0.44 (0.27)	0.100	0.027	3	0.17 (0.17)	0.320	2	−0.07 (0.08)	0.345
Current versus former smoker (24)	9	0.05 (0.03)	0.113	0.03 (0.03)	0.374	0.01 (0.07)	0.917	0.482	1	0.04 (0.03)	0.197	1	0.04 (0.02)	0.061
Cigarettes per day (23)	9	0.06 (0.04)	0.125	0.05 (0.04)	0.185	−0.06 (0.08)	0.431	0.073	0	0.06 (0.04)	0.139	0	0.06 (0.02)	0.011
Age of initiation of smoking (24)	9	−0.05 (0.03)	0.065	−0.06 (0.04)	0.109	0.07 (0.05)	0.147	0.004	1	−0.11 (0.03)	0.001	0	−0.05 (0.02)	0.027
MDD (23)	32	0.11 (0.11)	0.320	0.04 (0.09)	0.646	−0.81 (0.51)	0.112	0.064	10	0.14 (0.08)	0.118	5	0.00 (0.05)	0.914
Depressive symptom (23)	40	0.01 (0.05)	0.794	−0.04 (0.05)	0.402	−0.26 (0.21)	0.207	0.177	1	−0.02 (0.04)	0.673	0	0.01 (0.04)	0.736
PGC cross-disorder (22)	41	0.31 (0.18)	0.086	0.16 (0.19)	0.382	−2.28 (1.10)	0.038	0.017	0	0.31 0.18	0.100	0	0.31 (0.12)	0.010
ADHD (24)	42	0.25 (0.17)	0.132	−0.14 (0.16)	0.405	−0.44 (0.29)	0.122	0.005	1	0.18 (0.14)	0.220	1	0.18 (0.11)	0.101
Schizophrenia (21)	33	0.45 (0.20)	0.026	0.21 (0.10)	0.045	0.00 (0.29)	0.999	0.047	6	0.24 (0.08)	0.009	6	0.24 (0.08)	0.004
Bipolar disorder (22)	34	−0.06 (0.18)	0.732	−0.03 (0.14)	0.812	−0.20 (0.31)	0.511	0.569	2	−0.02 (0.14)	0.893	2	−0.01 (0.11)	0.931
Depressed affect subcluster (22)	35	0.02 (0.04)	0.650	−0.02 (0.03)	0.594	−0.08 (0.08)	0.313	0.131	4	0.02 (0.03)	0.508	1	0.00 (0.02)	0.845
Neuroticism (22)	35	0.01 (0.04)	0.840	−0.01 (0.03)	0.641	−0.06 (0.07)	0.388	0.234	4	−0.02 (0.03)	0.591	3	−0.03 (0.02)	0.112
Worry subcluster (24)	35	0.03 (0.04)	0.393	0.01 (0.03)	0.754	−0.04 (0.07)	0.591	0.239	4	0.01 (0.03)	0.820	3	−0.01 (0.02)	0.777
Subjective wellbeing (22)	40	−0.02 (0.05)	0.70	−0.05 (0.05)	0.264	0.03 (0.27)	0.921	0.860	3	−0.06 (0.04)	0.132	1	−0.05 (0.03)	0.092
Number of sexual partners (23)	36	0.09 (0.05)	0.058	−0.00 (0.03)	0.941	−0.00 (0.09)	0.966	0.219	7	0.05 (0.04)	0.225	4	0.02 (0.02)	0.266
General risk tolerance (24)	36	0.05 (0.03)	0.096	−0.03 (0.03)	0.323	00.06 (0.06)	0.251	0.015	3	0.07 (0.03)	0.053	0	0.05 (0.02)	0.002
Insomnia (24)	37	0.08 (0.06)	0.157	0.06 (0.06)	0.367	−0.04 (0.11)	0.744	0.196	1	0.12 (0.06)	0.050	2	0.10 (0.04)	0.020
Cognitive performance (22)	38	−0.03 (0.0)	0.460	−0.08 (0.03)	0.021	−0.09 (0.09)	0.295	0.440	3	−0.08 (0.04)	0.054	1	−0.05 (0.02)	0.030
Educational attainment (20)	38	−0.06 (0.03)	0.055	−0.10 (0.02)	$\mathbf{7.38\times10^{-6}}$	−0.12 (0.06)	0.024	0.152	3	−0.07 (0.02)	6.04×10^{-3}	5	−0.08 (0.02)	$\mathbf{3.12\times10^{-7}}$
Mother's age at death (24)	43	−0.03 (0.04)	0.424	−0.02 (0.06)	0.692	−0.01 (0.08)	0.886	0.764	0	−0.03 0.03	0.342	0	−0.03 (0.04)	0.424
Father's age at death (24)	43	−0.05 (0.05)	0.352	−0.09 (0.06)	0.113	−0.08 (0.10)	0.408	0.671	1	−0.03 (0.05)	0.523	0	−0.05 (0.04)	0.206

P values labeled in bold are significant after multiple testing correction ($P < 1.32\times10^{-3}$). Traits labeled in bold are those having a causal effect from AUD by at least one method and consistent for the direction of effect by all five methods.

increase the power for the association study of PAU and, consequently, associations specific to AUD or AUDIT-P could have been attenuated. Third, there was no opportunity for replication of the individual novel variants. Because the variants were detected in more than 430,000 subjects and have small effect sizes, a replication sample with adequate power would also have to be very large and no such sample is currently available. To validate the findings, we conducted PRS analyses in three independent cohorts which showed strong association with AUD. Although this indicates that our study had adequate power for variant detection, it does not address the validity of the individual variants discovered.

The present GWAS study of PAU is very large. Previous work has shown that the genetic architecture of AUD (and PAU) differs substantially from that of alcohol consumption[2–4]. There have been larger studies of alcohol quantity/frequency measures[9,26]; alcohol consumption data are available in many EHRs, and thus they were included in many studies of other primary traits including cardiac disease. AUD diagnoses are collected much less commonly. The three-item AUDIT-C is a widely used measure of alcohol consumption that is often available in EHRs, but the full ten-item AUDIT, which allows the assessment of AUDIT-P, is not as widely available. Despite the high genetic correlation between, for example, PAU and DrnkWk ($r_g = 0.77$), very different patterns of genetic correlation and pleiotropy have been observed via LDSC and other methods for these different kinds of index of alcohol use[2–5]. PAU captures pathological alcohol use: physiological dependence and/or significant psychological, social or medical consequences. Quantity/frequency measures may capture alcohol use that is in the normal, or any way nonpathological, range. As such, we argue that although quantity/frequency measures are important for understanding the biology of habitual alcohol use, PAU is the more clinically important trait. Thus, we did not meta-analyze PAU with DrnkWk directly, but used MTAG analysis instead, recognizing that they are different traits. These circumstances underscore the need to assemble a large GWAS sample of PAU to inform its biology, and our study moves towards this goal via the identification of numerous and previously unidentified risk loci—we increased known PAU loci from 10 to 29, nearly tripling our knowledge of specific risk regions. Similarly, we identified 66 gene-based associations of which 46 were novel—again roughly tripling current knowledge. MTAG analysis increased locus discovery to 119, representing 76 independent loci, by leveraging information from DrnkWk[9]. By the same token, we provide a major increment in information about the biology of PAU, offering considerable fodder for future studies that will be required to delineate the biology and function associated with each risk variant. We anticipate that knowledge of the functional effects of the variants will contribute eventually to personalized treatment of PAU, facilitating identification of individuals with PAU who may be most treatment responsive or for whom a specific medication may be most efficacious.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-020-0643-5.

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Methods

MVP datasets. The MVP is a mega-biobank supported by the US Department of Veterans Affairs (VA), enrollment for which began in 2011 and is ongoing. Phenotypic data were collected using questionnaires and the VA electronic health records, and a blood sample was obtained from each participant for genetic studies. Two phases of genotypic data have been released and were included in this study. MVP phase1 contains 353,948 subjects, of whom 202,004 EA with AUD diagnoses were included in a previous GWAS and the summary statistics were used in this study[3]. MVP phase2 released data on another 108,416 subjects, of whom 65,387 EAs with AUD diagnosis information were included in this study. Following the same procedures as for MVP phase1, participants with at least one inpatient or two outpatient alcohol-related ICD-9/10 codes from 2000 to 2018 were assigned a diagnosis of AUD.

Ethics statement: The Central VA Institutional Review Board (IRB) and site-specific IRBs approved the MVP study. All relevant ethical regulations for work with human subjects were followed in the conduct of the study, and informed consent was obtained from all participants.

Genotyping for both phases of MVP was performed using a customized Affymetrix Biobank Array. Imputation and quality control methods for MVP phase1 were described in detail in Kranzler et al.[3]. Similar methods were used for MVP phase2. Before imputation, phase2 subjects or SNPs with genotype call rate <0.9 or high heterozygosity were removed, leaving 108,416 subjects and 668,324 SNPs. Imputation for MVP phase2 was done separately from phase1; both were performed with EAGLE2 (ref. [44]) and Minimac3 (ref. [45]) using 1000 Genomes Project phase3 data[46] as the reference panel. Imputed genotypes with posterior probability ≥0.9 were transferred to best-guess genotypes (the remainder were treated as missing genotype calls). A total of 6,635,093 SNPs with INFO scores >0.7, genotype call rates or best-guess rates >0.95, Hardy–Weinberg equilibrium (HWE) $P > 1\times10^{-6}$ and minor allele frequency (MAF) > 0.001 remained for GWAS.

We removed subjects with mismatched genotypic and phenotypic sex and one subject randomly from each pair of related individuals (kinship coefficient[47] threshold = 0.0884), leaving 107,438 phase2 subjects for subsequent analyses. We used the same processes as in MVP phase1 to define EAs. First, we ran PC analysis (PCA) on 74,827 common SNPs (MAF > 0.05) shared by MVP and the 1000 Genomes phase3 reference panels using FastPCA[48]. We then clustered each participant into the nearest reference population according to the Euclidean distances between the participant and the centers of the five reference populations using the first ten PCs. A second PCA was performed for participants who were clustered to the reference European population, and outliers were removed if any of the first ten PCs were >3 s.d. from the mean, leaving 67,268 EA subjects.

Individuals <22 or >90 years of age and those with a missing AUD diagnosis were removed from the analyses, leaving 65,387 phase2 EAs (11,337 cases and 54,050 controls). GWAS was then performed on the MVP phase2 dataset. We used logistic regression implemented in PLINK v.1.90b4.4 (ref. [49]) for the AUD GWAS, correcting for age, sex and the first ten PCs. The mean age wass 63.2 years (s.d. = 13.4) in the entire MVP sample, with 92.5% male. Data collection and analysis were not performed blind to the conditions of the experiments.

PGC summary statistics. We used the 46,568 European-ancestry subjects (11,569 cases and 34,999 controls) from 27 cohorts that were analyzed by the PGC. The phenotype was lifetime DSM-IV diagnosis of AD. The summary data were downloaded from the PGC website (https://www.med.unc.edu/pgc/) with full agreement to the PGC conditions. Allele frequencies were not reported in the summary data. We used allele frequencies from the 1000 Genome European sample as proxy measures in PGC for certain downstream analyses.

UKB summary statistics. The UKB included 121,604 White-British unrelated subjects with available AUDIT-P scores. Past-year AUDIT-P was assessed by seven questions: (1) frequency of inability to cease drinking; (2) frequency of failure to fulfill normal expectations due to drinking alcohol; (3) frequency of needing a morning drink of alcohol after a heavy drinking session; (4) frequency of feeling guilt or remorse after drinking alcohol; (5) frequency of memory loss due to drinking alcohol; (6) been injured or injured someone else through drinking alcohol; and (7) had a relative, friend or health worker who was concerned about, or suggested, a reduction in alcohol consumption. The AUDIT-P was $\log_{10}$-transformed for GWAS (see ref. [4] for details). We removed SNPs with INFO <0.7 or call rate <0.95.

Meta-analyses. Meta-analyses were performed using METAL[50]. The meta-analysis within MVP (for the purpose of genetic correlation analysis with PGC AD) was conducted using an inverse variance-weighted method, because the two subsets were from the same cohort. The meta-analyses for AUD (MVP + PGC) and PAU (MVP + PGC + UKB) were performed using the sample size-weighted method. Given the unbalanced ratios of cases to controls in MVP samples, we calculated effective sample sizes for meta-analysis following the approach used by the PGC:

$$n_{\text{effective}} = \frac{4}{\frac{1}{n_{\text{case}}} + \frac{1}{n_{\text{control}}}}$$

The calculated effective sample sizes in MVP and reported effective sample sizes in PGC were used in meta-analyses and all downstream analyses. Because AUDIT-P in UKB is a continuous trait, we used actual sample sizes for that trait. For the AUD meta-analysis, variants present in only one sample (except MVP phase1, which is much larger than the others), or with heterogeneity test $P < 5\times10^{-8}$, were removed, leaving 7,003,540 variants. For the PAU meta-analysis, variants present in only one sample (except MVP phase1 or UKB), or with heterogeneity test $P < 5\times10^{-8}$, and variants with effective sample size <45,118 (15% of the total effective sample size) were removed, leaving 14,069,427 variants.

AUD polygenic risk score in UKB. We calculated AUD PRS for each of the 82,930 unrelated subjects in UKB (application no. 41910) who had nonmissing AUDIT-P information[7]. A PRS was calculated as the sum of the number of effective alleles with *P* values less than a given threshold, weighted by the effect sizes from AUD meta-analysis (MVP + PGC). We analyzed ten *P* value thresholds: 5×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4}, 0.001, 0.05, 0.3, 0.5 and 1.0, and clumped the AUD summary data by LD with $r^2 < 0.3$ in a 500-kb window. We then tested the association between AUD PRS and AUDIT-P, corrected for age, sex and ten PCs. The analysis was performed using PRSice-2 (ref. [51]).

Independent variants and conditional analyses. We identified the independent variant ($P < 5\times10^{-8}$) in each locus (1-Mb genomic window) based on the smallest *P* value and $r^2 < 0.1$ with other independent variants, and assigned these variants to the independent variant clump. Any two independent variants <1 Mb apart and whose clumped regions overlapped were merged into one locus. Given the known long-range LD for the ADH gene cluster on chromosome 4, we defined chr4q23–q24 (~97.2–102.6 Mb) as one locus. When multiple independent variants were present in a locus, we ran conditional analyses using GCTA-COJO[52] to define conditionally independent variants. For each variant other than the most significant (index), we tested the marginal associations conditioning on the index variant using Europeans ($n = 503$) from the 1000 Genomes as the LD reference sample. Variants with significant marginal associations ($P < 5\times10^{-8}$) were defined as conditionally independent variants (that is, independent when conditioned on other variants in the region) and subject to another round of conditional analyses for each significant association.

For the conditionally independent variants for AUD or PAU, we also conducted a multi-trait analysis conditioning on GSCAN DrnkWk[9] using GCTA-mtCOJO[31] to identify variants associated with AUD or PAU, but not DrnkWk (that is, not alcohol consumption per se). Europeans from the 1000 Genomes were used as the LD reference. For variants missing in GSCAN, we used proxy variants ($P < 5\times10^{-8}$) in high LD with the locus for analyses. Whereas conditional analyses require the beta (effect size) and s.e.m., we calculated these using *Z*-scores (*z*), allele frequency (*p*) and sample size (*n*) from the meta-analyses[53]:

$$\text{beta} = \frac{z}{\sqrt{2p(1-p)(n+z^2)}}$$
$$\text{s.e.m.} = \frac{1}{\sqrt{2p(1-p)(n+z^2)}}$$

Gene-based association analysis. Gene-based association analysis for PAU was performed using MAGMA implemented in FUMA[17,18], which uses a multiple regression approach to detect multimarker effects that account for SNP *P* values and LD between markers. We used default settings to analyze 18,952 autosomal genes, with $P < 2.64\times10^{-6}$ (0.05/18,952) considered GWS.

Drug–gene interaction. For the genes identified as significant by MAGMA, we examined drug–gene interaction through the Drug Gene Interaction Database v.3.0.2 (ref. [11]) (http://www.dgidb.org/), a database of integrated drug–gene interaction information based on 30 sources.

SNP-based h^2 and partitioning heritability enrichment. We used LDSC[12] to estimate the SNP-based h^2 for common SNPs mapped to HapMap3 (ref. [54]), with Europeans from the 1000 Genomes Project[46] as the LD reference panel. We excluded the major histocompatibility complex region (chr6: 26–34 Mb).

We conducted portioning h^2 enrichment analyses for PAU using LDSC in different models[13,14]. First, we analyzed a baseline model consisting of 52 functional categories that included genomic features (coding, intron, untranslated region and so on), regulatory annotations (promoter, enhancer and so on), epigenomic annotations (H3K27ac, H3K4me1, H3K3me3 and so on) and others (see ref. [13] for details; Supplementary Fig. 3). We then analyzed cell type group h^2 enrichments with ten cell types: CNS, adrenal and pancreas, immune and hematopoietic, skeletal muscle, gastrointestinal, liver, cardiovascular, connective tissue and bone, kidney and other (see ref. [13] for details; Supplementary Fig. 2). Third, we used LDSC to test for enriched heritability in regions surrounding genes with the highest tissue-specific expression, using 53 human tissue or cell type RNA-seq data from GTEx[16], or enriched heritability in epigenetic markers from 396 human epigenetic annotations (six features in a subset of 88 primary cell types or tissues) from the Roadmap Epigenomics Consortium[15] (see ref. [14] for details; Supplementary Fig. 4 and Supplementary Table 6). For each model, the number

of tested annotations was used to calculate a Bonferroni corrected $P < 0.05$ as a significance threshold.

Gene set and functional enrichment. We performed gene set analysis for PAU for curated gene sets and Gene Ontology terms using MAGMA[17,18]. We then used MAGMA for gene property analyses to test the relationships between tissue-specific gene expression profiles and PAU–gene associations. We analyzed gene expression data from 53 GTEx (v.7) tissues. We also performed gene set analysis on the 152 prioritized genes using MAGMA. Gene sets with adjusted $P < 0.05$ were considered as significant.

Genetic correlation. We estimated the genetic correlation (r_g) between traits using LDSC[55]. For PAU, we estimated r_g with 218 published traits in LD Hub[56], 487 unpublished traits from UKB (integrated in the LD Hub) and recently published psychiatric and behavioral traits[9,32,34–39,42,57,58], bringing the total number of tested traits to 715 (Supplementary Table 8). For traits reported in either multiple studies or UKB, we selected the published version of the phenotype or used the largest sample size. Bonferroni correction was applied, and correlation was considered significant at a P value threshold of 6.99×10^{-5}.

S-PrediXcan and S-MultiXcan. To perform transcriptome-wide association analysis, we used S-PrediXcan[23] (a version of PrediXcan that uses GWAS summary statistics[59]) to integrate transcriptomic data from GTEx[16], and DGN[24] to analyze the summary data from the PAU meta-analysis. Forty-eight tissues with sample size >70 from GTEx release v.7 were analyzed, totaling 10,294 samples. DGN contains RNA sequencing data from the whole blood of 992 genotyped individuals. The transcriptome prediction model database and the covariance matrices of the SNPs within each gene model were downloaded from the PredictDB repository (http://predictdb.org/, released 8 January 2018). Only individuals of European ancestry in GTEx were analyzed. S-PrediXcan was performed for each of the 49 tissues (48 from GTEx and 1 from DGN), for a total of 254,345 gene–tissue pairs. Significant association was determined by Bonferroni correction ($P < 1.97 \times 10^{-7}$).

Considering the limited eQTL sample size for any single tissue and the substantial sharing of eQTLs across tissues, we applied S-MultiXcan[25], which integrates evidence across multiple tissues using multivariate regression to improve association detection. Forty-eight tissues from GTEx were analyzed jointly. The threshold for condition number of eigenvalues was set to 30 when truncating singular-value decomposition components. In total, 25,626 genes were tested in S-MultiXcan, leading to a significant P value threshold of 1.95×10^{-6} (0.05/25,626).

PAU PRS for phenome-wide associations. Polygenic scores were generated using polygenic risk scores–continuous shrinkage (PRS-CS)[60] on all genotyped individuals of European descent ($n = 67{,}588$) in BioVU. PRS-CS uses a Bayesian framework to model linkage disequilibrium from an external reference set and a continuous shrinkage prior on SNP effect sizes. We used the 1000 Genomes Project Phase 3 European sample[46] as the LD reference. Additionally, we used the PRS-CS auto option, which allows the software to learn the continuous shrinkage prior from the data. Polygenic scores were constructed from PRS-CS auto-adjusted summary statistics containing 811,292 SNPs. All individuals used for polygenic scoring were genotyped on the Illumina Multi-Ethnic Global Array (MEGA). Genotypes were filtered for SNP (95%) and individual (98%) call rates, sex discrepancies and excessive heterozygosity. For related individuals, one of each pair was randomly removed (pi_hat > 0.2). SNPs showing significant associations with genotyping batch were removed. Genetic ancestry was determined by PCA performed using EIGENSTRAT[61]. Imputation was completed using the Michigan Imputation Server[45] and the Haplotype Reference Consortium[62] as the reference panel. Genotypes were then converted to hard calls, and filtered for SNP imputation quality ($R^2 < 0.3$), individual missingness (>2%), SNP missingness (>2%), MAF (<1%) and HWE ($P < 1 \times 10^{-10}$). The resulting dataset contained 9,330,483 SNPs on 67,588 individuals of European ancestry.

We conducted PheWAS[63] of the PAU PRS by fitting a logistic regression model to 1,372 case/control phenotypes to estimate the odds of each diagnosis given the PAU polygenic score, controlling for sex, median age across the medical record, top ten PCs of ancestry and genotyping batch. We required the presence of at least two ICD codes that mapped to a PheWAS disease category (Phecode Map 1.2) to assign 'case' status. A phenotype was required to have at least 100 cases to be included in the analysis. PheWAS analyses were run using the PheWAS R package[64]. Bonferroni correction was applied to test for significance ($P < 3.64 \times 10^{-5}$, 0.05/1,372).

PAU PRS in independent samples. We calculated PAU PRS in three independent samples, where we tested the association between PAU PRS and AD corrected for age, sex and ten PCs. Ten P value thresholds were applied in all samples.

iPSYCH group. DNA samples for cases and controls were obtained from newborn bloodspots linked to population registry data[65]. Cases were identified with the ICD-10 code F10.2 (AD; $n = 944$); controls were from the iPSYCH group ($n = 11{,}408$; $n_{effective} = 3{,}487$). The iPSYCH sample was genotyped on the Psych Array (Illumina). GWAS quality control (QC), imputation against the 1,000 Genomes Project panel[46] and association analysis using the Ricopili pipeline[66] were performed. The current study is part of a general study in iPSYCH investigating the comorbidity of alcohol misuse and psychiatric disorders.

UCL Psych Array. Cases were identified with ICD-10 code F10.2 ($n = 1{,}698$) and comprised 492 individuals with a diagnosis of alcoholic hepatitis and who had participated in the Steroids or Pentoxifylline for Alcoholic Hepatitis (STOPAH) trial (ISRCTN88782125; EudraCT no. 2009-013897-42), and 1,206 subjects recruited from the AD arm of the DNA Polymorphisms in Mental Health (DPIM) study; controls were UK subjects who had either been screened for an absence of mental illness and harmful substance use ($n = 776$), or were random blood donors ($n = 452$; total $n = 1{,}228$; $n_{effective} = 2{,}851$). The sample was genotyped on the Psych Array (Illumina). GWAS QC was performed using standard methods, and imputation was done using the Haplotype Reference Consortium (HRC) panel[67] on the Sanger Imputation server (https://imputation.sanger.ac.uk/). Association testing was performed using Plink1.9 (ref. [49]).

UCL Core Exome Array. Cases had an ICD-10 diagnosis of F10.2 ($n = 637$), including 324 individuals with a diagnosis of alcoholic hepatitis who had participated in the STOPAH trial and 313 subjects recruited from the AD arm of the DPIM study; controls were unrelated UK subjects from the UK Household Longitudinal Study ($n = 9{,}189$; $n_{effective} = 2{,}383$). The sample was genotyped on the Illumina Human Core Exome Array (Illumina). GWAS QC was performed using standard methods, and imputation was done using the HRC panel[67] on the Sanger Imputation server (https://imputation.sanger.ac.uk/). Association testing was performed with Plink1.9 (ref. [49]).

MR. We used MR to investigate the bidirectional causal relationships between PAU liability and traits that were significantly genetically correlated ($P < 6.99 \times 10^{-5}$). However, all or most of the published traits in recent large GWAS include UKB data. To avoid biases caused by overlapping samples in MR analysis, we tested only the relationship between published traits and AUD (MVP + PGC). For robust causal effect inference, we limited the traits studied to those with more than ten available instruments (association $P < 5 \times 10^{-8}$). For causality on AUD, 15 exposures were analyzed (Table 2) and, for causality from AUD on others, 23 traits were tested. We applied Bonferroni correction for the 38 hypotheses, interpreting $P < 1.32 \times 10^{-3}$ (0.05/38) as significant.

Four methods—weighted median[28], IVW, random-effects model[27] and MR–Egger[29]—implemented in the R package MendelianRandomization v.0.3.0 (ref. [68]), MR–pleiotropy residual sum and outlier (MR–PRESSO)[30], and generalized sparse matrix reduction (GSMR)[31] were used for MR inference. Evidence of average pleiotropic effects was examined by the MR–Egger intercept test, where a non-zero intercept indicates horizontal pleiotropy[29]. Individual variants with horizontal pleiotropy were detected by MR–PRESSO, and an outlier test was applied to correct horizontal pleiotropy via outlier removal. Pleiotropic variants were also detected by the HEIDI test in GSMR, and removed from causal inference. Instrumental variants that are associated with outcome ($P < 5 \times 10^{-8}$) were removed. For instrumental variants missing in the outcome summary data, we used the results of the best-proxy variant with the highest LD ($r^2 > 0.8$) with the missing variant. If the MAF of the missing variant was <0.01, or none of the variants within 200 kb had LD $r^2 > 0.8$, we removed the instrumental variant from the analysis. Palindromic SNPs (A/T or G/C alleles) with MAF [0.4, 0.5], which can introduce ambiguity into the identity of the effect allele, were also removed.

MTAG between PAU and DrnkWk. Multiple trait analysis between PAU and DrnkWk from GSCAN was performed on summary statistics with multi-trait analysis of GWAS (MTAG) v.1.0.7 (ref. [10]). The summary data of DrnkWk were generated from 537,352 subjects, excluding the 23andMe samples that were not available to us for inclusion. We analyzed variants with a minimum effective sample size of 80,603 (15%) in DrnkWk, and a minimum effective sample size of 45,118 (15%) in PAU, which left 10,613,246 overlapping variants.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The full summary-level association data from the meta-analysis are available through dbGaP at accession no. phs001672.v3.p1.

Code availability

Kinship analysis was performed using KING (http://people.virginia.edu/~wc9c/KING/). PCAs were performed using EIGENSOFT (https://data.broadinstitute.org/alkesgroup/EIGENSOFT/). Imputation was performed using EAGLE2 (https://data.broadinstitute.org/alkesgroup/Eagle/), Minimac3 (https://genome.sph.umich.edu/wiki/Minimac3), Sanger imputation server (https://imputation.sanger.ac.uk/) or RICOPILI (https://data.broadinstitute.org/mpg/ricopili/), the choice depending on the sample. GWAS was performed using PLINK (https://www.cog-genomics.org/plink2). Meta-analyses were performed using METAL (https://genome.sph.umich.edu/wiki/METAL_Documentation). Polygenic risk score

analyses were performed using PRSice-2 (https://www.prsice.info/) or PRS-CS (https://github.com/getian107/PRScs). GCTA (https://cnsgenomics.com/software/gcta/#Overview) was used for identification of independent loci (GCTA-COJO), multi-trait conditional analysis (GCTA-mtCOJO) and MR (GCTA-GSMR). LDSC (https://github.com/bulik/ldsc) was used for heritability estimation, genetic correlation analysis (also using LD Hub (http://ldsc.broadinstitute.org/)) and heritability enrichment analyses. FUMA (https://fuma.ctglab.nl/) was used for gene association, functional enrichment and gene set enrichment analyses. Transcriptomic analyses were performed using S-PrediXcan and S-MultiXcan (https://github.com/hakyimlab/MetaXcan). PheWAS analyses were run using the PheWAS R package (https://github.com/PheWAS/PheWAS). The Mendelian Randomization R Package (https://cran.r-project.org/web/packages/MendelianRandomization/index.html) and MR–PRESSO (https://github.com/rondolab/MR-PRESSO) were used for MR analyses. MTAG (https://github.com/omeed-maghzian/mtag) was used for multiple trait analysis.

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Author contributions

H.Z., J.G., H.R.K. and A.A.P. conceived the analyses. H.Z. and J.G. wrote the first draft and prepared all drafts for submission. J.G. supervised and H.Z. accomplished primary analyses. J.M.S., S.S.-R., T.-K.C., D.F.L., Z.C., B.L. and A.M. conducted additional analyses. J.G., H.R.K., A.A.P., L.K.D., H.J.E. and A.A. supervised additional analyses. J.M.S., S.S.-R., T.-K.C., A.A.P., A.M. and L.K.D. prepared individual datasets and provided summary statistics or results. R.P., R.L.K., R.V.S., J.H.T., M.Y.M., S.R.A., M.R.T., M.N., M.M., A.D.B., E.C.J., A.C.J., A.M., L.K.D. and H.R.K. provided critical support regarding phenotypes and data in individual datasets. J.G., A.C.J. and H.R.K. provided resource support. All authors reviewed the manuscript and approved it for submission.

Competing interests

H.R.K. is a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which was supported over the past 3 years by AbbVie, Alkermes, Ethypharm, Indivior, Lilly, Lundbeck, Otsuka, Pfizer, Arbor, and Amygdala Neurosciences. H.R.K. and J.G. are named as inventors on PCT patent application no. 15/878,640, entitled Genotype-guided dosing of opioid agonists, filed 24 January 2018.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41593-020-0643-5.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data collection was performed previously for each included cohort.
Data analysis	All software used in the analyses is described in the manuscript. All are publicly available and links or references are provided in the manuscript. EAGLE2, Minimac3, KING, FastPCA, PLINK v1.90b4.4, METAL, PRSice-2, GCTA-COJO (1.92.1 beta6), GCTA-mtCOJO (1.92.1 beta6), MAGMA, FUMA, Drug Gene Interaction Database v3.0.2, LDSC, LD Hub, S-PrediXcan, S-MultiXcan, PRS-CS, EIGENSTRAT, PheWAS R package, RicopiliMendelianRandomization v0.3.0, MR-PRESSO, GSMR, and MTAG were used.

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The full summary-level association data from the meta-analysis are available through dbGaP: [https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001672.v3.p1] (accession number phs001672.v3.p1). Summary statistics of AUD from PGC and AUDIT-P from UK Biobank are available on the Psychiatric Genomics Consortium's downloads page (https://www.med.unc.edu/pgc/download-results/). 1000 Genomes Project phase 3 reference data is available on The International Genome Sample Resource's page (https://www.internationalgenome.org/data). Summary statistics from GSCAN are available through https://genome.psych.umn.edu/index.php/GSCAN. Drug–gene interaction information is available http://www.dgidb.org/downloads. Dataset for partitioning heritability enrichment analyses are available through https://data.broadinstitute.org/alkesgroup/LDSCORE/. Summary statistics of the rest of the analyzed traits for genetic correlation analysis are available at LD Hub (http://ldsc.broadinstitute.org/), or PGC (https://www.med.unc.edu/pgc/download-results/), or SSGAC (https://

www.thessgac.org/data), or CNCR (https://ctg.cncr.nl/software/summary_statistics). Gene expression data used by S-PrediXcan/S-MultiXcan is available at PredictDB (http://predictdb.org/).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We used a series of standard quality control methods to yield a total N = 435,563 for analysis. We have used all available European American samples with both genotype and phenotype data in MVP phase 1 and phase2, and the summary statistics from the published samples. We did not do a specific power calculation.
Data exclusions	In MVP, subjects with genotype call rate < 0.9 or high heterozygosity were removed. Subjects with no demographic information or whose genotypic and phenotypic sex did not match were also removed. We also removed one subject randomly from each pair of related individuals. To differentiate population groups, we performed PCA analyses and participants with PC scores > 3 standard deviations from the mean of any of the 10 PCs were removed as outliers. Finally, individuals < 22 or > 90 years old and those with missing AUD diagnosis were removed. Those exclusion criteria were applied in previous MVP studies. The summary statistics from PGC and UK Biobank were publicly available, we did not do further data cleaning.
Replication	Polygenic risk scores for PAU were generated in 3 independent cohorts: iPSYCH (n=12,352), UCL Psych Array (n=2,926), UCL Core Exome Array (n=9,826). We did not attempt to replicate the individual SNP association due to the limited sample sizes in the replication samples.
Randomization	Not applicable since this is GWAS study.
Blinding	Genotyping was done blind to phenotype

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n/a	Involved in the study
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☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
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☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
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☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	All participants are of European ancestry. In the MVP samples, the mean age is 63.2 (SD=13.4), and 92.5% are males. Only summary statistics from PGC and UK Biobank were used, which have been described in previous studies.
Recruitment	MVP participants were recruited through the U.S. Veterans Administration (VA) Million Veteran Program, which advertised and solicited patients receiving medical care through the VA. They gave informed consent for use of their self-report information and access to their electronic medical record. They also provided a blood sample for DNA extraction and genotyping. The MVP samples are predominantly male (>92%), which might limit the power to detect female specific loci. PGC participants were recruited separately for each cohort according to their respective study designs. UK Biobank participants were recruited across the UK.
Ethics oversight	Research involving MVP in general is approved by the VA Central IRB; the current project was also under the supervision of IRBs in West Haven, New Haven, and Philadelphia.

Note that full information on the approval of the study protocol must also be provided in the manuscript.