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Novel alcohol-related genes suggest shared genetic mechanisms with neuropsychiatric disorders

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ABSTRACT

Excessive alcohol consumption is one of the main causes of death and disability worldwide. Alcohol consumption is a heritable complex trait. We conducted a meta-analysis of genome-wide association studies (GWAS) of gram/day (g/d) alcohol consumption in UK-Biobank, AlcGen and CHARGE+ consortia accumulating 480,842 people of European descent to decipher the genetic architecture of alcohol intake. We identified 46 novel, common loci, and investigated their potential functional significance using magnetic resonance imaging data and gene expression studies. Our results identify genetic pathways associated with alcohol consumption and suggest shared genetic mechanisms with neuropsychiatric disorders including schizophrenia.

Excessive alcohol consumption is a major public health problem that is responsible for 2.2% and 6.8% age-standardized deaths for women and men respectively[1]. Most genetic studies of alcohol use focus on alcohol dependency, although the population burden of alcohol-related disease mainly reflects a broader range of alcohol consumption behaviors[2]. Small reductions in alcohol consumption could have major public health benefits; even moderate amounts of alcohol/day may have significant impact on mortality[3].

Alcohol consumption is a heritable complex trait[4], but genetic studies to date have robustly identified only a small number of associated genetic variants [5-8]. These include variants in the aldehyde dehydrogenase (ADH) gene family, a group of enzymes that catalyze the oxidation of aldehydes[9], including a cluster of genes on chromosome 4q23 (*ADH1B, ADH1C, ADH5, ADH6, ADH7*)[6].

Here, we report a GWAS meta-analysis of alcohol intake (log transformed g/day) among people of European ancestry drawn from UK Biobank (UKB)[10], the Alcohol Genome-Wide Consortium (AlcGen) and the Cohorts for Heart and Aging Research in Genomic Epidemiology Plus (CHARGE+) consortia. Briefly, UKB is a prospective cohort study of ~500,000 individuals recruited between the ages of 40 and 69 years. Participants were asked to report their average weekly and monthly alcohol consumption through a self-completed touchscreen questionnaire[10]. Based on these reports, we calculated the g/d alcohol intake (**Methods**). Participants were genotyped using a customized array with imputation from the Haplotype Reference Consortium (HRC) panel[11], yielding ~7 million common single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) $\geq$ 1% and imputation quality score [INFO] $\geq$ 0.1. After quality control (QC) and exclusions (**Methods**) we performed GWAS of alcohol consumption using data from 404,731 UKB participants of European descent under an additive genetic model (**Methods and Supplementary Table 1**). We found that genomic inflation in the UKB analysis was λ_{GC}=1.45, but did not adjust for inflation as the LD score regression intercept was 1.05, indicating that this was due to polygenicity rather than to population stratification[12]. The estimated SNP-wide heritability of alcohol consumption in the UKB data was 0.09.

We also carried out GWAS in 25 independent studies from the AlcGen and CHARGE+ consortia including 76,111 participants of European descent for which alcohol g/d could be calculated (**Supplementary Table 2**). Various arrays were used for genotyping, with imputations performed using either the 1,000 Genomes Reference Panel or the HRC platforms (**Supplementary Table 3**). After QC, we applied genomic

control at the individual study level and obtained summary results for ~7 million SNPs with imputation quality score ≥ 0.3 (**Methods**).

We combined the UKB, AlcGen and CHARGE+ results using a fixed effects inverse variance weighted approach for a total of 480,842 individuals[13]. To maximize power, we performed a single-stage analysis to test common SNPs with MAF ≥ 1%. We set a stringent *P*-value threshold of $P < 5 \times 10^{-9}$ to denote significance in the combined meta-analysis[14], and required signals to be at $P < 5 \times 10^{-7}$ in UKB, with same direction of effect in UKB and AlcGen plus CHARGE+, to minimize false positive findings. We excluded SNPs within 500kb of variants reported as genome-wide significant in previous GWAS of alcohol consumption[5,6], identified novel loci by requiring SNPs to be independent of each other (LD $r^2 < 0.1$), and selected the sentinel SNP within each locus according to lowest *P*-value (**Methods**).

We then tested for correlations of alcohol-associated SNPs with Magnetic Resonance Imaging (MRI) phenotypes of brain, heart and liver, and gene expression. We tested the sentinel SNPs for association with other traits/diseases and *Drosophila* mutant models were used to investigate functional effects on ethanol-induced behavior.

RESULTS

Our meta-analysis identified 46 novel loci associated with alcohol consumption (log transformed g/day) **(Fig. 1 and Table 1**). All inferential statistics for the novel loci are reported in Table 1 whereas heterogeneity metrics are presented in **Supplementary Table 4**. In addition, we discovered a further eight variants in the combined analysis at nominal genome-wide significance ($P < 1 \times 10^{-8}$) that may also be associated with alcohol intake (**Supplementary Table 5**). The most significantly associated variant, rs1991556 ($P = 4.5 \times 10^{-23}$), is an intronic variant in *MAPT* gene that encodes the microtubule-associated protein tau, and was found through Phenoscanner not only to be associated with dementia[15] and Parkinson's disease[16,17], but also with neuroticism, schizophrenia[18]and other traits[19-21] (**Methods, Fig. 2 and Supplementary Table 6**). The second most significantly associated variant is rs1004787 ($P = 6.7 \times 10^{-17}$), near *SIX3* gene, which encodes a member of the sine oculis homeobox transcription factor family involved in eye development[22]. The third SNP is rs13107325 ($P = 1.3 \times 10^{-15}$), a missense SNP in *SLC39A8* (https://www.ncbi.nlm.nih.gov/gene/64116), a gene that encodes a member of the SLC39 family of metal ion transporters, which has been associated with

schizophrenia[23] as well as inflammatory bowel disease, cardiovascular and metabolic phenotypes [24 25-27] in previous GWAS (**Fig. 2 and Supplementary Table 6**).
Another of our most significant variants, an intronic SNP rs7121986 (P = 6.2 x 10^{-14}) in *DRD2* (https://www.ncbi.nlm.nih.gov/gene/1813), encodes the dopamine receptor D2 that has been associated with cocaine addiction, neuroticism and schizophrenia[18]. We also found significant associations with SNP rs988748 (P = 4.4 x 10^{-9}) in the *BDNF* gene (https://www.ncbi.nlm.nih.gov/gene/627, that encodes a member of the nerve growth factor family of proteins and rs7517344, which is near *ELAVL4* (https://www.ncbi.nlm.nih.gov/gene/1996) *(*P = 2.0 x 10^{-10}), the gene product of which is involved in BDNF regulation[28]. Previous studies have suggested that a variant in *BDNF* is associated with alcohol consumption and that alcohol consumption modulates BDNF expression[29].

Additionally, we found association of alcohol consumption with SNP rs838145 (P = 3.2 x 10^{-15}), which has been associated with macronutrient intake in a previous GWAS[30]. This variant is nearest *IZUMO* (https://www.ncbi.nlm.nih.gov/gene/284359*)* in a locus of around 50kb that spans a number of genes including *FGF21* (https://www.ncbi.nlm.nih.gov/gene/26291), whose gene product FGF21 is a liver hormone involved in the regulation of alcohol preference, glucose and lipid metabolism[31]. We previously reported significant association of alcohol intake with SNP rs11940694 in *KLB* (https://www.ncbi.nlm.nih.gov/gene/152831), an obligate receptor of FGF21 in the brain[5], and we strongly replicated that finding here (P = 3.3 x 10^{-68}).

As well as variants in *KLB* and in the alcohol dehydrogenase locus (smallest P = 1.2 x 10^{-125}), we found support (P = 1 x 10^{-5}) for association of common variants in the three other alcohol intake-related loci previously reported in GWAS (**Supplementary Table 7**), including SNP rs6943555 in *AUTS2* (https://www.ncbi.nlm.nih.gov/gene/26053) (P = 2.9 x 10^{-6}). In addition, we found a novel alcohol intake-related SNP rs1421085 in *FTO* (https://www.ncbi.nlm.nih.gov/gene/79068) in high LD (r^2 = 0.92) with a variant reported previously as genome-wide significant for association with alcohol dependence[32].

Conditional analysis using Genome-wide Complex Trait Analysis (GCTA) did not reveal any independent secondary signals related to alcohol consumption. Among ~14,000 individuals in the independent Airwave cohort[33] (**Methods**), 7% of the variance in alcohol consumption was explained by the novel and known common

variants. Using weights from our analysis, we constructed an unbiased weighted genetic risk score (GRS) in Airwave (**Methods**) and found a strong association of the novel and known variants on alcohol consumption levels (P = 2.75 x 10^{-14}), with mean difference in sex-adjusted alcohol intake of 2.6 g/d comparing the top vs the bottom quintile of the GRS (**Supplementary Table 8**).

Associations with MRI imaging phenotypes

We functionally characterized novel variants by carrying out single-SNP analyses of the imaging phenotypes in UKB (**Methods**), focusing on brain (N=9,702), heart (N=10,706) and liver (N=8,479).

With Bonferroni correction (corrected P-value 6.6 x 10^{-6}, corresponding to 0.05/46 SNPs*164 imaging phenotypes), we found significant positive associations between SNP rs13107325 in *SLC39A8* and the volumes of multiple brain regions; All inferential statistics for these associations are reported in **Supplementary Table 9**. The strongest associations were with putamen (left: P = 2.5 x 10^{-45}, right: P = 2.8 x 10^{-47}), ventral striatum (left: P = 9.5 x 10^{-53}, right: P = 9.6 x 10^{-51}) and cerebellum (strongest association for left I-IV volume; P = 1.2 x 10^{-9}) (**Supplementary Table 9**); similar findings were recently reported in a GWAS on brain imaging in UKB[34]. The other significant association was for rs1991556 with the parahippocampal gyrus (P = 1.2 x 10^{-6}).

We then tested these brain regions for association with alcohol consumption and found a significant effect for the left (t_{8601} = -3.7; beta $\pm$ SE = -0.0019 $\pm$ 0.0005; P = 2.0 x 10^{-4}) and right (t_{8601} = -3.65; beta $\pm$ SE = -0.0070 $\pm$ 0.0005; P = 2.6 x 10^{-4}) putamen. Finally, we used data from N= 8,610 individuals and performed a mediation analysis using a standard three-variable path model, bootstrapping 10,000 times to calculate the significance of the mediation effect of putamen volume for genetic influences on alcohol consumption (**Methods**). We found evidence that the effect of SNP rs13107325 in *SLC39A8* on alcohol intake is partially mediated via its association with left (t_{8601} = -3.03; beta $\pm$ SE = -0.27 $\pm$ 0.09; P = 1.9 x 10^{-3}) and right (t_{8601} = -2.82; beta $\pm$ SE = -0.27 $\pm$ 0.09; P = 1.7 x 10^{-3}) putamen volume (**Fig. 3 and Supplementary Table 10**). To exclude the possibility of an inverse causal pathway we performed additional analyses in UKB non-drinkers (N =589). With 10,000 random permutations, associations of rs13107325 with both left and right putamen remained significant (left putamen: t_{541}=1.06; P = 0.02; right putamen: t_{541}=0.38; P =

0.04) indicating that the association between rs13107325 and putamen regions is not mediated by alcohol intake.

We did not find any significant associations of novel SNPs with either cardiac (left ventricular mass or end diastolic volume or right ventricular end diastolic volume) (**Supplementary Table 11**) or liver fat measures on MRI (**Supplementary Table 12**), after adjustment for multiple testing.

Effects of SNPs on gene expression

We carried out expression quantitative trait loci eQTL analyses using the Genotype-Tissue Expression (GTEx) and the UK Brain Expression Consortium (UKBEC) datasets; 34 of the 53 novel and known SNPs associated with alcohol consumption have a significant effect on gene expression in at least one tissue, including 33 SNPs that affect gene expression in the brain (**Supplementary Tables 13 and 14, and Supplementary Figures 1-3**). We found that the most significant eQTLs often do not involve the nearest gene and that several of the SNPs affect expression of different genes in different tissues. For example, SNP rs1991556 in the *MAPT* gene (https://www.ncbi.nlm.nih.gov/gene/4137) affects expression of 33 genes overall, with most significant effects on the expression of the non-protein coding genes *CRHR1-IT1 (*also known as *C17orf69* or *LINC02210*) (https://www.ncbi.nlm.nih.gov/gene/147081) and *LRRC37A4P* (https://www.ncbi.nlm.nih.gov/gene/?term=LRRC37A4P), near *MAPT*, across a wide range of tissues including brain, adipose tissue and skin ($P = 7.2 \times 10^{-126}$ to $P = 2.5 \times 10^{-6}$) **(Supplementary Figure 2)**. Similarly, the A-allele at SNP rs2071305 within *MYBPC3* (https://www.ncbi.nlm.nih.gov/gene/4607) affects the expression of several genes and is most significantly associated with increased expression of *C1QTNF4* (https://www.ncbi.nlm.nih.gov/gene/114900) across several tissues ($P = 1.9 \times 10^{-25}$ to $P = 8.4 \times 10^{-5}$).

Several of these eQTLs were found to affect expression of genes known to be involved in reward and addiction. SNP rs1053651 in the *TCAP-PNMT-STARD3* gene cluster affects expression of the *PPP1R1B* gene (also known as *DARPP-32*) (https://www.ncbi.nlm.nih.gov/gene/84152) which encodes a protein that mediates the effects of dopamine in the mesolimbic reward pathway[35]. Other known addiction-related genes include *ANKK1* (https://www.ncbi.nlm.nih.gov/gene/255239) and *DRD2* (expression affected by SNP rs7121986) implicated in alcohol and nicotine dependence[36,37], *CRHR1* (https://www.ncbi.nlm.nih.gov/gene/1394) (affected by SNP rs1991556) involved in

stress-mediated alcohol dependence[38,39] and *PPM1G* (SNP rs1260326) (https://www.ncbi.nlm.nih.gov/gene/5496) whose epigenetic modification was reported to be associated with alcohol abuse[40].

Over-representation enrichment analyses based on functional annotations and disease-related terms indicated that genes whose expressions are affected by the identified eQTLs are most significantly enriched for terms related to abdominal (n=91) and other malignant cancers, motor function (n= 5) and cellular homeostasis (n= 22) (**Supplementary Figure 4**). We performed a gene-based analysis and repeated the over-representation enrichment analysis adding the new set of identified genes (**Supplementary Table 15**). The results were similar supporting an enrichment for abdominal (n=100) and other cancers, as well as motor function (n=5) and cellular homeostasis (n=24) (**Supplementary Figure 5**).

Other traits and diseases

Using LD score regression[12], we assessed genetic correlations between alcohol consumption and 235 complex traits and diseases from publicly available summary GWAS statistics (**Methods).** All results including their statistics (i.e. r_g, standard errors, z value and P value) are included in **Supplementary Table 16**. The strongest positive genetic correlations based on false discovery rate $P < 0.02$ were found for smoking (r_g= 0.42, $P = 1.0 \times 10^{-23}$) and HDL cholesterol levels (r_g= 0.26, $P = 5.1 \times 10^{-13}$). We also found negative correlations for sleep duration (r_g= -0.14, $P = 3.8 \times 10^{-7}$) and fasting insulin levels (r_g= -0.25, $P = 4.5 \times 10^{-6}$). A significant genetic correlation was also found with schizophrenia (r_g= 0.07, $P = 3.9 \times 10^{-3}$) and bipolar disorder (r_g= 0.15, $P = 5.0 \times 10^{-4}$) (**Supplementary Table 16**). Over-representation enrichment analysis using WebGestalt[41] (http://www.webgestalt.org) showed that our list of novel and known variants is significantly enriched for several diseases and traits including developmental disorder in children ($P = 7.3 \times 10^{-5}$), epilepsy ($P = 1.4 \times 10^{-4}$), heroin dependence ($P = 5.7 \times 10^{-4}$) and schizophrenia ($P = 8.4 \times 10^{-4}$) (**Supplementary Figure 6**). The result of the Mendelian randomization analysis (**Methods**) to assess a potential causal effect of alcohol on schizophrenia risk, using the inverse variance weighted approach, was not significant ($P = 0.089$), with large heterogeneity of the estimates of the tested variants.

Functional studies in *Drosophila*

Based on our GWAS and brain imaging findings we took forward SNP rs13107325 in *SLC39A8* (alias *Zip8* gene) for additional testing in *Drosophila*, which employ

conserved mechanisms to modulate ethanol-induced behaviors[42,43]. First, we overexpressed human *Zip8* using a Gal4-driver that included expression in neurons involved in multiple ethanol-induced behaviors[43]. Flies carrying *ics^{Gal4}/+ UAS-hZip8/+* showed a slight, but significant, resistance to ethanol-induced sedation compared to control flies (t_{30} = 2.3; Hedge's *g* = 0.80; 95% CI: 0.08 – 1.53; *P* = 0.026; N = 16 per genotype). Ethanol tolerance, induced with repeat exposures spaced by a 4-hour recovery, was unchanged in these flies (t = 1.0; *P* = 0.33; **Fig. 4a**). We next used the same Gal4-driver to knock down the endogenous *Drosophila* ortholog of *hZip8*, namely *dZip71B*. This caused the flies to display naïve sensitivity to ethanol-induced sedation (t_{14} = 3.98; Hedge's *g* = -1.84; 95% CI: -0.67 – -3.01; *P* = 0.0014; N = 8 per genotype), and in addition, these flies developed greater tolerance to ethanol upon repeat exposure (t_{14} = 4.80; Hedge's *g* = 2.29; 95% CI: 1.03 – 3.55; *P* = 0.0003; **Fig. 4b**). To corroborate this phenotype, we then tested flies transheterozygous for two independent transposon-insertions in the middle of the *dZip71B* gene (**Supplementary Figure 7**) and found that these *dZip71B$^{Mi\,/\,MB}$* flies also displayed naïve sensitivity (t_{14} = 3.23; Hedge's *g* = -1.54; 95% CI: -0.42 – -2.65; *P* = 0.006) and increased ethanol-induced tolerance (t_{14} = 2.39; Hedge's *g* = 1.13; 95% CI: 0.07 - 2.18; *P* = 0.032) compared to controls (N = 8 each) (**Fig. 4c**).

DISCUSSION

Our discovery utilizing data on common variants from over 480,000 people of European descent extends our knowledge of the genetic architecture of alcohol intake, increasing the number of identified loci to 46. We found loci involved in neuropsychiatric conditions such as schizophrenia, Parkinson's disease and dementia, as well as *BDNF* where gene expression is affected by alcohol abuse. Our findings illustrate that large-scale studies of genetic associations with alcohol intake in the general population, rather than on alcohol dependency alone, can provide additional insights into genetic mechanisms regulating alcohol consumption.

We highlight the role of the highly pleiotropic *MAPT* and *SLC39A8* genes in the genetics of alcohol consumption. *MAPT* plays a key role in tau-associated dementia[44] and both genes are also implicated in other neuropsychiatric conditions including neuroticism, schizophrenia and Parkinson's disease[16-18]. The *SLC39A8* gene encodes a member of the SLC39 family of metal ion transporters. The encoded protein is glycosylated and found in plasma membrane and mitochondria, and is involved in the cellular transport of zinc, modulation of which could affect microglial inflammatory responses[45]. Our gain- and loss-of-function studies in *Drosophila*

indicate a potential causal role of *SLC39A8* in alcohol drinking behavior, even though results should be interpreted with caution due to small sample size in our experiment. The MRI brain imaging demonstrates a significant association of SNP rs13107325 in the *SLC39A8* gene and putamen volume differences, and these structural differences appear to partially mediate associations of rs13107325 with alcohol consumption. The putamen has been associated with alcohol consumption and the withdrawal syndrome after chronic administration to rodents and non-human primates[46]. Our mediation analysis is suggestive of a plausible causal pathway linking rs13107325 in *SLC39A8* with alcohol intake via an effect on putamen volume, but follow-up work is needed to conclusively demonstrate causal links. Putamen volume differences have also been associated with both schizophrenia and psychosis[47,48] and robust association between SNP rs13107325 in *SLC39A8* and schizophrenia was reported in a previous GWAS[23].

We also report SNP rs7121986 near *DRD2* as a novel alcohol intake variant in GWAS. The gene product of *DRD2*, D2 dopamine receptor, is a G protein-coupled receptor on post-synaptic dopaminergic neurons that has long been implicated in alcoholism[49]. In addition, we identify SNP rs988748 in *BDNF* as a novel alcohol intake variant; BDNF expression is differentially affected by alcohol exposure in animal models[50,51]. Both genes (along with *PPP1R1P*) are centrally involved in reward-mediating mesocortico-limbic pathways and both are implicated in the development of schizophrenia. For example, there is a robust GWAS association between schizophrenia and SNP rs4938021 in *DRD2* (in perfect LD with our novel alcohol intake-related variant rs7121986) and *DRD2* appears to be pivotal in network analyses of genes involved in schizophrenia[52]. Taken together, our results suggest that there are shared genetic mechanisms between the regulation of alcohol intake and susceptibility to schizophrenia, as well as other neuropsychiatric disorders. In this regard, large prospective epidemiological studies report a three-fold risk of schizophrenia in relation to alcohol abuse[53].

We previously reported genome-wide significant associations of alcohol intake with *KLB*, and identified a liver-brain axis linking the liver hormone FGF21 with central regulation of alcohol intake involving β-Klotho receptor (the gene product of *KLB*) in the brain[5]. Here, we identify a significant variant near *FGF21* gene and strongly replicate the previously reported *KLB* gene variant, strengthening the genetic evidence for the importance of this pathway in regulating alcohol consumption.

The LD score regression analysis showed a positive genetic correlation between alcohol consumption, smoking and HDL cholesterol levels. This confirms previous findings that reported an almost identical genetic correlation of alcohol consumption with number of cigarettes per day[54]. Furthermore, the observed genetic correlation with HDL levels is consistent with previous observations of an association between alcohol consumption and HDL[55,56], including results of a Mendelian randomization study that suggested a possible causal role linking alcohol intake with increased HDL levels[57]. Furthermore, we found a genetic correlation (inverse) between sleep duration and alcohol consumption, an association previously reported only in a few small epidemiological studies[58]. We also found a significant genetic correlation with schizophrenia and bipolar disorder, a result that is supported by a recently published trans-ethnic meta-analysis of case-control studies on alcohol dependence[59]. We could not test for a genetic association between alcohol and risk of alcohol-related cancers[60] because of limited availability of summary data. However, our gene-set enrichment analysis showed a significant enrichment for genes related to abdominal as well as other cancers.

Strengths of our study include its size, detailed attention to the alcohol phenotype, dense coverage of the genome through imputation, and incorporation of brain and other imaging data to explore potential mechanisms. Over 80% of the data came from UKB, which combines high-quality phenotypic data and imputed genome-wide genetic data with strict attention to quality control[61]. We adopted a stringent approach to claim novel variants involving a conservative *P*-value threshold, internal replication in UKB and consistent direction of effect with the other studies, to minimize the reporting of false positive signals.

However, since alcohol intake is socio-culturally as well as genetically determined, it is influenced by other lifestyle and environmental factors which may modify or dilute the genetic signal. A key limitation is that assessment of alcohol intake relies on self-report, which is prone to errors and biases including recall bias and systematic under-reporting by heavy drinkers[62,63]. Furthermore, questionnaires on alcohol intake covered a short duration (e.g. day or week) at a single period, which may not be representative of broader drinking patterns of cohort participants. We harmonized data across cohorts by converting alcohol intake into a common metric of g/d, with imputation as necessary in UKB for participants reporting consumption of small amounts of alcohol. Taking this approach, we were able to detect strong genetic associations with alcohol intake that explained 7% of the variance in alcohol in an independent cohort, while our GRS analysis indicates that individuals in the

lower fifth of the GRS distribution were consuming daily approximately one third of a standard drink (2.6 g/d alcohol) less compared with those in the upper fifth.

We should also point out that our eQTL analyses are a first step in the identification of causal genes. Yet, as the most significant eQTLs affected expression of many genes, not necessarily the nearest, there is a need to further prioritize potential causal genes. Unbiased strategies that leverage information from multiple data sets including extensive genomic annotations and high-throughput functional screening in a broad range of tissues will be essential for effective prioritization of genes and uncovering of underlying causal mechanisms[64]. Establishing confidence in the prioritized genes in such a way is a prerequisite for performing functional follow-up studies in appropriate model systems, as demonstrated by the identification of the causal genes and potential disease mechanisms at the obesity- associated *FTO* locus[65].

In summary, in this large study of genetic associations with alcohol consumption, we identified common variants in 46 novel loci, with several of the genes expressed in the brain as well as other tissues. Our findings suggest that there may be shared genetic mechanisms underpinning regulation of alcohol intake and development of a neuropsychiatric disorders including schizophrenia. This may form the basis for greater understanding of observed associations between alcohol consumption, schizophrenia[66] and other disorders.

METHODS

UK Biobank data

We conducted a Genome Wide Association Study (GWAS) analysis among 458,577 UKB participants of European descent, identified from a combination of self-reported and genetic data. The details of the selection of the participants has been described elsewhere[14]. These comprise 408,951 individuals from UKB genotyped at 825,927 variants with a custom Affymetrix UK Biobank Axiom Array chip and 49,626 individuals genotyped at 807,411 variants with a custom Affymetrix UK BiLEVE Axiom Array chip from the UK BiLEVE study, which is a subset of UKB. For our analyses, we used SNPs imputed centrally by UKB using the Haplotype Reference Consortium (HRC) panel.

Alcohol intake

We calculated the alcohol intake as grams of alcohol per day (g/d) based on self-reported alcohol drinking from the touch-screen questionnaire. The quantity of each

type of drink (red wine, white wine, beer/cider, fortified wine, spirits) was multiplied by its standard drink size and reference alcohol content. Drink-specific intake during the reported drinking period (a week for frequent drinkers defined as: daily or almost daily/once or twice a week/three or four times a week; or a month for occasional drinkers defined as: one to three times a month/special occasions only) was summed up and converted to g/d alcohol intake for all participants with complete response to the quantitative drinking questions. The alcohol intake for participants with incomplete response was imputed by bootstrap resampling from the complete responses, stratified by drinking frequency (occasional or frequent) and sex.

Participants were defined as life-time non-drinkers if they reported 'never' on the question on alcohol drinking frequency (UKB field 1558) and 'no' for the question on former drinker (UKB field 3731); they were excluded from further analysis. We considered participants with alcohol consumption > 500 g/d as outliers and they were dropped from the analyses. We also excluded participants with missing covariates, leaving data on 404,732 individuals. We $\log_{10}$ transformed g/d alcohol and sex-specific residuals were derived from the regression of $\log_{10}$ transformed g/d alcohol on age, age^2, genotyping chip and weight.

UKB genetic analysis

We performed linear mixed modeling using BOLT-LMM software[67], under an additive genetic model, for associations of measured and imputed SNPs with alcohol consumption (sex-specific residuals of the $\log_{10}$ transformed g/d variable). Model building was based on SNPs with MAF > 5%, call rate > 98.5% and HWE P > 1 x 10^{-6}. SNPs were imputed using the HRC panel with imputation quality INFO score > 0.1. We estimated the LD score regression (LDSR) intercept to assess the degree of genomic inflation beyond polygenicity as well as the lambda inflation factor λ_{GC}[68].

The Alcohol Genome-Wide Consortium (AlcGen) and the Cohorts for Heart and Aging Research in Genomic Epidemiology Plus (CHARGE+) consortia

We analyzed available GWAS data from 25 independent studies (N=76,111) from the AlcGen and the CHARGE+ consortia. All study participants were of reported European ancestry and data were imputed to either the 1000 Genome Project or the HRC panel. Alcohol intake in g/d was computed and the $\log_{10}$ transformed residuals were analyzed as described above. Study names, cohort information and general study methods are included in **Supplementary Table 2 and 3**.

All studies were centrally quality-controlled using easyQC[69] including filtering for MAF. Finally, we analyzed data on ~7.1 M SNPs at MAF >1% and imputation quality score (Impute [Info score] or Mach [r^2]) > 0.3. Genomic control (GC) was applied at study level. We synthesized the available GWAS using a fixed effects inverse variance weighted meta-analysis and summary estimates were derived for AlcGen and CHARGE+.

One-stage meta-analysis

We performed a one-stage meta-analysis applying a fixed-effects inverse variance weighted meta-analysis using METAL[70] to obtain summary results from the UKB and and the AlcGen plus CHARGE+ GWAS, for up to N=480,842 participants and ~7.1 M SNPs with MAF ≥ 1% for variants present in both the UKB data and AlcGen and CHARGE+ meta-analysis. We assessed the observed heterogeneity using Cochran's Q and we quantified this using the I^2 metric. We considered a Cochran's Q $P < 1 \times 10^{-4}$ as significant. The LDSR intercept (standard error), in the discovery meta-analysis was 1.05 and no further correction was applied. QQ plots of the combined meta-analysis summary results , UK Biobank only as well as AlcGen and CHARGE+ only, are presented in **Supplementary Figure 8**.

Previously reported (known) SNPs

We looked up in the GWAS catalog (http://www.ebi.ac.uk/gwas/) and identified 17 SNPs associated with alcohol consumption at genome-wide significance level ($P < 5 \times 10^{-8}$). We enhanced the list by reference to a recent GWAS by Clarke et al[6] that was not covered by the GWAS catalog at the time of the analysis, reporting 14 additional rare and common SNPs. Together with a SNP in *RASGRF2* shown to be associated with alcohol-induced reinforcement[71], we found 31 previously reported alcohol consumption related SNPs.

Novel loci

According to locus definition of i) SNPs within ±500kb distance of each other; ii) SNPs in linkage disequilibrium LD ($r^2 > 0.1$) calculated with PLINK, we augmented the list of known SNPs with all SNPs present within our data, not contained within the previously published loci. We further excluded SNPs in the HLA region (chromosome 6, 25-34Mb) due to its complex LD structure. We performed LD clumping in PLINK on 4,515 unknown SNPs with $P < 1 \times 10^{-8}$ using an $r^2 > 0.1$ and distance threshold of 500kb. We further grouped the lead SNPs within 500kb from each other into the same loci and selected the SNP with smallest *P*-value from the locus as sentinel SNP.
To report a SNP as novel signal of association with alcohol consumption:

i) the sentinel SNP has $P < 5 \times 10^{-9}$ in the one-stage meta-analysis;
ii) the sentinel SNP is strongly associated ($P < 5 \times 10^{-7}$) in the UKB GWAS alone;
iii) the sentinel SNP has concordant direction of effect between UKB and AlcGen and CHARGE+ datasets;
iv) The sentinel SNP is not located within any of the previously reported loci

We selected the above criteria i) to iii) to minimize false positive findings including use of a conservative one-stage *P*-value threshold that is an order of magnitude more stringent than a genome-wide significance *P*-value. (The threshold of $P < 5 \times 10^{-9}$ has been proposed e.g. for whole-genome sequencing-based studies.) This approach led us to the identification of 46 sentinel SNPs in total. Regional plots for all 46 sentinel SNPs are presented in **Supplementary Figure 9.**

Conditional analysis

We conducted locus-specific conditional analysis using the GCTA (Genome-wide Complex Trait Analysis) software (http://cnsgenomics.com/software/gcta). For each of the 46 novel sentinel SNPs, we obtained conditional analysis results for the SNPs with MAF>1% and within 500kb from the sentinel SNP after conditioning on the sentinel SNP. The meta-analysis results of the GWAS in UKB, AlcGen and CHARGE+ were used as input summary statistics and the individual-level genetic data from UKB were used as the reference sample. Results for a SNP were considered conditionally significant if the difference between the conditional *P*-value and the original *P*-value is greater than 1.5-fold ($-\log_{10}P/-\log_{10}(P\_\text{conditional}) > 1.5$) and the conditional *P*-value is smaller than 5×10^{-8}.

Gene-based analysis

We performed a gene-based analysis using fastBAT, a method that performs a set-based association analysis using summary-level data from GWAS. We used the UKB dataset as a reference set for the LD calculation[72]. Gene-based associations with $P < 5 \times 10^{-9}$ were considered significant.

Gene expression analyses

To analyze the impact of genetic variants on expression of neighboring genes and identify expression quantitative trait loci (*cis*-eQTLs; i.e., SNPs associated with differences in local gene expression), we used two publicly available databases, the Genotype-Tissue Expression (GTEx) database[73] (www.gtexportal.org) and the UK Brain Expression Consortium (UKBEC) dataset[74] (http://www.braineac.org). We

searched these databases for significant variant-transcripts pairs for genes within 1Mb of each input SNP.

With the GTEx database, we tested for *cis*-eQTL effects in 48 tissues from 620 donors. The data described herein were obtained from the GTEx Portal, Release: V7 and used FastQTL[75], to map SNPs to gene-level expression data and calculate q-values based on beta distribution-adjusted empirical *P*-values[76]. A false discovery rate (FDR) threshold of ≤0.05 was applied to identify genes with a significant eQTL. The effect size, defined as the slope of the linear regression, was computed in a normalized space (normalized effect size (NES)), where magnitude has no direct biological interpretation. Here, NES reflects the effects of our GWAS A1 alleles (that are not necessarily the alternative alleles relative to the reference alleles, as reported in the GTEx database). **Supplementary Table 13** lists transcripts-SNPs associations with significant eQTL effects.

With the UKBEC dataset that comprises 134 brains (http://www.braineac.org/), we searched for *cis*-eQTLs in 10 brain regions, including the cerebellar cortex (CRBL), frontal cortex (FCTX), hippocampus (HIPP), medulla (specifically inferior olivary nucleus, MEDU), occipital cortex (specifically primary visual cortex, OCTX), putamen (PUTM), substantia nigra (SNIG), thalamus (THAL), temporal cortex (TCTX) and intralobular white matter (WHMT), as well as across all brain tissues (aveALL). MatrixEQTL[77] generated *P*-values for each expression profile (either exon-level or gene-level) against the respective SNP were obtained for the 10 different tissues and overall (aveALL). **Supplementary Table 14** lists transcripts-SNPs associations with a eQTL *P*-value < 0.0045 in at least one brain tissue. Subsequent data analysis was performed in R (http://www.R-project.org/).

We carried out over-representation enrichment analysis using a list of 146 GTEx eQTL genes that were derived from the single-variant analysis and a list of 160 eQTL genes that were derived from both single-variant and gene-based analysis. Ingenuity pathway analysis (IPA®, QIAGEN Inc.) was performed on these lists using ontology annotations from all available databases except those derived from low-confidence computational predictions.

Magnetic Resonance Imaging Data

We used the most recent release of magnetic resonance imaging (MRI) data on brain, heart and liver for UKB participants to investigate genetic associations with the 46 novel SNPs for alcohol consumption.

Brain imaging

Brain MRI acquisition and pre-processing

We used the T1 data from UKB to elucidate volumetric brain structures, including the cortical and the sub-cortical areas. The T1 data were acquired and pre-processed centrally by UKB. The brain regions were defined by combining the Harvard-Oxford cortical and subcortical atlases[78] (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) and the Diedrichsen cerebellar atlas[79] (http://www.diedrichsenlab.org/imaging/propatlas.htm). FAST (FMRIB's Automated Segmentation Tool)[80] was then used to estimate the grey matter partial volume within each brain region. Subcortical region volumes were also modelled by using FIRST (FMRIB's Integrated Registration and Segmentation Tool). More details about the MRI scanning protocol and pre-processing has been provided in UKB documentation (https://biobank.ctsu.ox.ac.uk/crystal/docs/brain_mri.pdf).

Association Analyses

We performed association analyses on N = 9,702 individuals between all novel SNPs and the grey matter volume of brain regions using Pearson correlation, adjusting for age, age^2, sex, age × sex, age^2 × sex, and head size. All, brain volume features, log transformed alcohol intake data (g/d), and the confounders were firstly transformed by using a rank-based inverse Gaussian transformation. Significance levels were set at $P < 0.05$ adjusted using the false-discovery rate method for multiple comparisons.

Mediation analysis

To assess if the effect of a SNP on alcohol consumption is mediated through a brain region, we performed a single-level mediation analysis based on a standard three-variable path model (SNP-brain region-alcohol consumption) with corrected and accelerated percentile bootstrapping 10,000 times to calculate the significance of the mediation effect. We considered as mediator variable the grey matter volume of brain regions that had a significant association on alcohol consumption. We calculated the significance of path a, path b and a*b mediation (SNP-brain region-alcohol consumption) using a multilevel mediation and moderation (M3) toolbox[81,82]. To exclude the possibility of an inverse causal pathway we performed additional analyses in UKB non-drinkers (N =589). performing 10,000 random permutations, associations of rs13107325 with both left and right putamen.

Cardiac Imaging

Cardiac MRI acquisition and pre-processing

Details of the cardiac image acquisition in UKB are reported previously[83]. Cardiac MRI was acquired using a clinical wide bore 1.5T scanner (MAGNETOM Aera, Syngo Platform VD13A, Siemens Healthcare, Erlangen, Germany) with 48 receiver channels, a 45 mT/m and 200 T/m/s gradient system, an 18-channel anterior body surface coil used in combination with 12 elements of an integrated 32 element spine coil and electrocardiogram gating for cardiac synchronization. A two-dimensional short-axis cardiac MRI was obtained using a balanced steady state free precession to cover the entire left and right ventricle (echo time, 1.10msec; repetition time, 2.6msec; flip angle, 80°; slice thickness, 8mm with 2mm gap; typical field of view, 380×252mm; matrix size, 208×187, acquisition of 1 slice per breath-hold).

The cardiac images were segmented to provide left ventricular mass (LVM), left end-diastolic (LVEDV), left end-systolic volume (LVESV), and right end-diastolic (RVEDV) and right end-systolic volume (RVESV) using a fully convolutional network as described previously[84]. Left (LVEF) and right ventricular ejection fraction (RVEF) were derived from (LVEDV–LVESV)/LVEDV×100 and (RVEDV–RVESV)/RVEDV×100, respectively.

Association Analyses

To test associations between cardiac MRI measures and alcohol consumption-related SNPs, we carried out a regression of LVM, LVEDV, LVEF, RVEDV, and RVEF onto each of the 46 SNPs adjusting for age, sex, height, weight, hypertension (defined as systolic blood pressure >140mmHg and or diastolic blood pressure >90mmHg or under antihypertensive treatment), diabetes, and smoking history on N=10,706 participants. Significance levels were set at $P < 0.05$ adjusted using the false-discovery rate method for multiple comparisons.

Liver Imaging

Liver MRI acquisition and pre-processing

Details of the liver image acquisition protocol have been reported previously[85]. Briefly, all participants were scanned in a Siemens MAGNETOM Aera 1.5-T MRI scanner (Siemens Healthineers, Erlangen, Germany) using a 6-minute dual-echo Dixon Vibe protocol, providing a water and fat separated volumetric data set for fat and muscle covering neck to knees. For liver proton density fat fraction (PDFF) quantification, an additional single multi-echo gradient slice was acquired over the liver. Liver images were analysed by computing specific ROI for water, fat and T2* by

magnitude-based chemical shift technique with a 6-peak lipid model, correcting for T1 and T2*.

Association Analyses

We performed association analyses between 46 alcohol consumption-related SNPs and liver PDFF (%), from 8,479 samples, using a linear regression model adjusting for age, age^2, sex, T2D, BMI, genotyping chip and first three PCs. Liver PDDF was firstly transformed by using a rank-based inverse transformation. Significance levels were set at $P < 0.05$ adjusted using the false-discovery rate method for multiple comparisons.

Drosophila experiments

Flies were kept on standard cornmeal/molasses fly food in a 12:12hr light:dark cycle at 25°C. Transgenc flies were obtained from the Bloomington *Drosophila* Stock Center: *UAS-hZip8* BL#66125, *UAS-dZIP71B-TRiP-RNAi*^{HMC04064} BL#55376, *dZip71B*^{MI13940} BL#59234, and *dZip71B*^{MB11703} BL#29928. For behavioral experiments, crosses were set up such that experimental and control flies were sibling progeny from a cross, and both were therefore in the same hybrid genetic background (*w Berlin / unknown*). Flies aged 1-5 days of adult age were collected, exposed to 100/50 (flowrates) ethanol/air vapor in the Booze-o-Mat 2 days later, and their loss of righting determined by slight tapping, as described[86]. For tolerance, flies were put back onto regular food after a 30-min initial exposure and were then re-exposed to the same vapor 4 hours later. Note that tolerance is not connected to initial sensitivity, and flies naively sensitive to ethanol-induced sedation can have no, or a reduced tolerance phenotype. Flies overexpressing *hZip8* (and their sibling controls) were placed at 28°C for two days to increase the expression levels of the transgene, as we did not detect a phenotype when they were kept at 25°C (data not shown). Data from experimental and control flies were compared by two-sided Student's t-tests. Data were normally distributed according to Shapiro-Wilk testing with Bonferroni adjustment for each of the three experiments.

Effects on other traits and diseases

We queried SNPs against GWAS results included in PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk), to investigate cross-trait effects, extracting all association results with genome-wide significance at $P < 5 \times 10^{-8}$ for all SNPs in high LD ($r^2 \geq 0.8$) with the 46 sentinel novel SNPs, to highlight the loci with strongest evidence of association with other traits. At the gene level,

609 overrepresentation enrichment analysis (ORA) with WebGestalt[41] on the nearest
610 genes to all alcohol consumption loci was carried out.

611 The genetic correlations between alcohol consumption and 235 other traits and
612 diseases were obtained in the online software LD Hub. LD hub is a centralized
613 database of summary-level GWAS results and a web interface for LD score regression
614 analysis

615 To estimate the potential causal effect of alcohol consumption-related variants on
616 schizophrenia, we performed a Mendelian randomization analysis utilizing publicly
617 available GWAS data on schizophrenia and the Mendelian randomization package in
618 R. The effect was estimated using the inverse-variance weighted (IVM) method.
619 Pleiotropy was tested by applying the MR-Egger regression method and
620 heterogeneity statistics were obtained. In presence of heterogeneity the random
621 effects inverse-variance method was applied[87].

622 **Genetic risk scores and percentage of variance explained**
623 We calculated an unbiased weighted GRS in 14,004 unrelated participants in
624 Airwave, an independent cohort with high quality HRC imputed genetic data[33]. All
625 previously reported and novel variants were used for the construction of the GRS.
626 We weighted the alcohol-increasing alleles by the beta coefficients of the meta-
627 analysis. We assessed the association of the GRS with alcohol intake and calculated
628 the alcohol consumption levels for individuals in the top vs the bottom quintiles of
629 the distribution. To calculate the percent of variance of alcohol consumption
630 explained by genetic variants, we generated the residuals from a regression of
631 alcohol consumption in Airwave. We then fit a second linear model for the trait
632 residuals with all novel and known variants plus the top 10 principal components and
633 estimated the percentage variance of the dependent variable explained by the
634 variants.

635 **Statistical analysis**
636 All inferential statistics for the analyses described above are provided in the text or
637 in tables and figures. All performed tests were two-sided.

638 **Data availability statement**
639 The UKB GWAS data can be assessed from the UK Biobank data repository
640 (http://biota.osc.ox.ac.uk/). The genetic and phenotypic UKB data are available upon
641 application to the UK Biobank (https://www.ukbiobank.ac.uk). Summary GWAS data

data can be assessed by request to the corresponding authors and will be available via LDHub (http://ldsc.broadinstitute.org/ldhub/).

References

1. GBD 2016 Alcohol Collaborators. Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **5**, 987-1012 (2018).
2. World Health Organization. Global status report on alcohol and health 2018. Eds: Poznyak V and Rekve D, https://www.who.int/substance_abuse/publications/global_alcohol_report/gsr_2018/en/ (2018).
3. Wood, A.M. *et al.* Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. *Lancet* **391**, 1513-1523 (2018).
4. Verhulst, B., Neale, M.C. & Kendler, K.S. The heritability of alcohol use disorders: a meta-analysis of twin and adoption studies. *Psychol Med* **45**, 1061-72 (2015).
5. Schumann, G. *et al.* KLB is associated with alcohol drinking, and its gene product beta-Klotho is necessary for FGF21 regulation of alcohol preference. *Proc Natl Acad Sci U S A* **113**, 14372-14377 (2016).
6. Clarke, T.K. *et al.* Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112 117). *Mol Psychiatry* **22**, 1376-1384 (2017).
7. Jorgenson, E. *et al.* Genetic contributors to variation in alcohol consumption vary by race/ethnicity in a large multi-ethnic genome-wide association study. *Mol Psychiatry* **22**, 1359-1367 (2017).
8. Baik, I., Cho, N.H., Kim, S.H., Han, B.G. & Shin, C. Genome-wide association studies identify genetic loci related to alcohol consumption in Korean men. *Am J Clin Nutr* **93**, 809-16 (2011).
9. Jackson, B. *et al.* Update on the aldehyde dehydrogenase gene (ALDH) superfamily. *Hum Genomics* **5**, 283-303 (2011).
10. Sudlow, C. *et al.* UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* **12**, e1001779 (2015).
11. McCarthy, S. *et al.* A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet* **48**, 1279-83 (2016).
12. Bulik-Sullivan, B.K. *et al.* LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet* **47**, 291-5 (2015).
13. Evangelou, E. & Ioannidis, J.P. Meta-analysis methods for genome-wide association studies and beyond. *Nat Rev Genet* **14**, 379-89 (2013).

14. Evangelou, E. *et al.* Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat Genet* **50**, 1412-1425 (2018).
15. Desikan, R.S. *et al.* Genetic overlap between Alzheimer's disease and Parkinson's disease at the MAPT locus. *Mol Psychiatry* **20**, 1588-95 (2015).
16. Do, C.B. *et al.* Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genet* **7**, e1002141 (2011).
17. Pankratz, N. *et al.* Meta-analysis of Parkinson's disease: identification of a novel locus, RIT2. *Ann Neurol* **71**, 370-84 (2012).
18. Okbay, A. *et al.* Genetic variants associated with subjective well-being, depressive symptoms, and neuroticism identified through genome-wide analyses. *Nat Genet* **48**, 624-33 (2016).
19. Couch, F.J. *et al.* Genome-wide association study in BRCA1 mutation carriers identifies novel loci associated with breast and ovarian cancer risk. *PLoS Genet* **9**, e1003212 (2013).
20. Ikram, M.A. *et al.* Common variants at 6q22 and 17q21 are associated with intracranial volume. *Nat Genet* **44**, 539-44 (2012).
21. van der Harst, P. *et al.* Seventy-five genetic loci influencing the human red blood cell. *Nature* **492**, 369-75 (2012).
22. Samuel, A. *et al.* Six3 regulates optic nerve development via multiple mechanisms. *Sci Rep* **6**, 20267 (2016).
23. Schizophrenia Working Group of the Psychiatric Genomics, C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421-7 (2014).
24. Liu, J.Z. *et al.* Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet* **47**, 979-986 (2015).
25. International Consortium for Blood Pressure Genome-Wide Association Studies *et al.* Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* **478**, 103-9 (2011).
26. Speliotes, E.K. *et al.* Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* **42**, 937-48 (2010).
27. Teslovich, T.M. *et al.* Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* **466**, 707-13 (2010).
28. Lim, C.S. & Alkon, D.L. Protein kinase C stimulates HuD-mediated mRNA stability and protein expression of neurotrophic factors and enhances dendritic maturation of hippocampal neurons in culture. *Hippocampus* **22**, 2303-19 (2012).
29. Barker, J.M., Taylor, J.R., De Vries, T.J. & Peters, J. Brain-derived neurotrophic factor and addiction: Pathological versus therapeutic effects on drug seeking. *Brain Res* **1628**, 68-81 (2015).

30. Tanaka, T. *et al.* Genome-wide meta-analysis of observational studies shows common genetic variants associated with macronutrient intake. *Am J Clin Nutr* **97**, 1395-402 (2013).
31. Talukdar, S. *et al.* FGF21 Regulates Sweet and Alcohol Preference. *Cell Metab* **23**, 344-9 (2016).
32. Grant, S.F. *et al.* Association analysis of the FTO gene with obesity in children of Caucasian and African ancestry reveals a common tagging SNP. *PLoS One* **3**, e1746 (2008).
33. Elliott, P. *et al.* The Airwave Health Monitoring Study of police officers and staff in Great Britain: rationale, design and methods. *Environ Res* **134**, 280-5 (2014).
34. Elliott, L.T. *et al.* Genome-wide association studies of brain imaging phenotypes in UK Biobank. *Nature* **562**, 210-216 (2018).
35. Stipanovich, A. *et al.* A phosphatase cascade by which rewarding stimuli control nucleosomal response. *Nature* **453**, 879-84 (2008).
36. Yang, B.Z. *et al.* Association of haplotypic variants in DRD2, ANKK1, TTC12 and NCAM1 to alcohol dependence in independent case control and family samples. *Hum Mol Genet* **16**, 2844-53 (2007).
37. Gelernter, J. *et al.* Haplotype spanning TTC12 and ANKK1, flanked by the DRD2 and NCAM1 loci, is strongly associated to nicotine dependence in two distinct American populations. *Hum Mol Genet* **15**, 3498-507 (2006).
38. Treutlein, J. *et al.* Genetic association of the human corticotropin releasing hormone receptor 1 (CRHR1) with binge drinking and alcohol intake patterns in two independent samples. *Mol Psychiatry* **11**, 594-602 (2006).
39. Timpl, P. *et al.* Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nat Genet* **19**, 162-6 (1998).
40. Ruggeri, B. *et al.* Association of Protein Phosphatase PPM1G With Alcohol Use Disorder and Brain Activity During Behavioral Control in a Genome-Wide Methylation Analysis. *Am J Psychiatry* **172**, 543-52 (2015).
41. Wang, J., Vasaikar, S., Shi, Z., Greer, M. & Zhang, B. WebGestalt 2017: a more comprehensive, powerful, flexible and interactive gene set enrichment analysis toolkit. *Nucleic Acids Res* **45**, W130-W137 (2017).
42. Gonzalez, D.A. *et al.* The Arf6 activator Efa6/PSD3 confers regional specificity and modulates ethanol consumption in Drosophila and humans. *Mol Psychiatry* **23**, 621-628 (2018).
43. Ojelade, S.A. *et al.* Rsu1 regulates ethanol consumption in Drosophila and humans. *Proc Natl Acad Sci U S A* **112**, E4085-93 (2015).
44. Rademakers, R., Cruts, M. & van Broeckhoven, C. The role of tau (MAPT) in frontotemporal dementia and related tauopathies. *Hum Mutat* **24**, 277-95 (2004).
45. Higashi, Y. *et al.* Influence of extracellular zinc on M1 microglial activation. *Sci Rep* **7**, 43778 (2017).

46. Chen, G. *et al.* Striatal involvement in human alcoholism and alcohol consumption, and withdrawal in animal models. *Alcohol Clin Exp Res* **35**, 1739-48 (2011).
47. Okada, N. *et al.* Abnormal asymmetries in subcortical brain volume in schizophrenia. *Mol Psychiatry* **21**, 1460-6 (2016).
48. van Erp, T.G. *et al.* Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. *Mol Psychiatry* **21**, 547-53 (2016).
49. Meyers, J.L. *et al.* The association between DRD2/ANKK1 and genetically informed measures of alcohol use and problems. *Addict Biol* **18**, 523-36 (2013).
50. Logrip, M.L., Barak, S., Warnault, V. & Ron, D. Corticostriatal BDNF and alcohol addiction. *Brain Res* **1628**, 60-7 (2015).
51. Boschen, K.E., Criss, K.J., Palamarchouk, V., Roth, T.L. & Klintsova, A.Y. Effects of developmental alcohol exposure vs. intubation stress on BDNF and TrkB expression in the hippocampus and frontal cortex of neonatal rats. *Int J Dev Neurosci* **43**, 16-24 (2015).
52. Monaco, A. *et al.* A complex network approach reveals a pivotal substructure of genes linked to schizophrenia. *PLoS One* **13**, e0190110 (2018).
53. Nielsen, S.M., Toftdahl, N.G., Nordentoft, M. & Hjorthoj, C. Association between alcohol, cannabis, and other illicit substance abuse and risk of developing schizophrenia: a nationwide population based register study. *Psychol Med* **47**, 1668-1677 (2017).
54. Nivard, M.G. *et al.* Connecting the dots, genome-wide association studies in substance use. *Mol Psychiatry* **21**, 733-5 (2016).
55. Gaziano, J.M. *et al.* Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. *N Engl J Med* **329**, 1829-34 (1993).
56. Linn, S. *et al.* High-density lipoprotein cholesterol and alcohol consumption in US white and black adults: data from NHANES II. *Am J Public Health* **83**, 811-6 (1993).
57. Vu, K.N. *et al.* Causal Role of Alcohol Consumption in an Improved Lipid Profile: The Atherosclerosis Risk in Communities (ARIC) Study. *PLoS One* **11**, e0148765 (2016).
58. Chaput, J.P., McNeil, J., Despres, J.P., Bouchard, C. & Tremblay, A. Short sleep duration is associated with greater alcohol consumption in adults. *Appetite* **59**, 650-5 (2012).
59. Walters, R.K. *et al.* Transancestral GWAS of alcohol dependence reveals common genetic underpinnings with psychiatric disorders. *Nat Neurosci* **21**, 1656-1669 (2018).
60. Bagnardi, V. *et al.* Alcohol consumption and site-specific cancer risk: a comprehensive dose-response meta-analysis. *Br J Cancer* **112**, 580-93 (2015).

61. Bycroft, C. *et al.* The UK Biobank resource with deep phenotyping and genomic data. *Nature* **562**, 203-209 (2018).
62. Boniface, S., Kneale, J. & Shelton, N. Drinking pattern is more strongly associated with under-reporting of alcohol consumption than socio-demographic factors: evidence from a mixed-methods study. *BMC Public Health* **14**, 1297 (2014).
63. Greenfield, T.K. & Kerr, W.C. Alcohol measurement methodology in epidemiology: recent advances and opportunities. *Addiction* **103**, 1082-99 (2008).
64 Grotz, A.K., Gloyn, A.L. & Thomsen, S.K. Prioritising Causal Genes at Type 2 Diabetes Risk Loci. *Curr Diab Rep* **17**, 76 (2017).
65. Claussnitzer, M. *et al.* FTO Obesity Variant Circuitry and Adipocyte Browning in Humans. *N Engl J Med* **373**, 895-907 (2015)
66. Hambrecht, M. & Hafner, H. Substance abuse and the onset of schizophrenia. *Biol Psychiatry* **40**, 1155-63 (1996).
67. Loh, P.R. *et al.* Efficient Bayesian mixed-model analysis increases association power in large cohorts. *Nat Genet* **47**, 284-90 (2015).
68. Georgiopoulos, G. & Evangelou, E. Power considerations for lambda inflation factor in meta-analyses of genome-wide association studies. *Genet Res (Camb)* **98**, e9 (2016).
69. Winkler, T.W. *et al.* Quality control and conduct of genome-wide association meta-analyses. *Nat Protoc* **9**, 1192-212 (2014).
70. Willer, C.J., Li, Y. & Abecasis, G.R. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26**, 2190-1 (2010).
71. Stacey, D. *et al.* RASGRF2 regulates alcohol-induced reinforcement by influencing mesolimbic dopamine neuron activity and dopamine release. *Proc Natl Acad Sci U S A* **109**, 21128-33 (2012).
72. Bakshi, A. *et al.* Fast set-based association analysis using summary data from GWAS identifies novel gene loci for human complex traits. *Sci Rep* **6**, 32894 (2016).
73. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* **45**, 580-5 (2013).
74. Ramasamy, A. *et al.* Genetic variability in the regulation of gene expression in ten regions of the human brain. *Nat Neurosci* **17**, 1418-1428 (2014).
75. Ongen, H., Buil, A., Brown, A.A., Dermitzakis, E.T. & Delaneau, O. Fast and efficient QTL mapper for thousands of molecular phenotypes. *Bioinformatics* **32**, 1479-85 (2016).
76. Storey, J.D. & Tibshirani, R. Statistical significance for genomewide studies. *Proc Natl Acad Sci U S A* **100**, 9440-5 (2003).
77. Shabalin, A.A. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. *Bioinformatics* **28**, 1353-8 (2012).

78. Brown, C.A. *et al.* Development, validation and application of a new fornix template for studies of aging and preclinical Alzheimer's disease. *Neuroimage Clin* **13**, 106-115 (2017).
79. Diedrichsen, J. *et al.* Imaging the deep cerebellar nuclei: a probabilistic atlas and normalization procedure. *Neuroimage* **54**, 1786-94 (2011).
80. Zhang, Y., Brady, M. & Smith, S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imaging* **20**, 45-57 (2001).
81. Wager, T.D., Davidson, M.L., Hughes, B.L., Lindquist, M.A. & Ochsner, K.N. Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron* **59**, 1037-50 (2008).
82. Wager, T.D. *et al.* Brain mediators of cardiovascular responses to social threat: part I: Reciprocal dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity. *Neuroimage* **47**, 821-35 (2009).
83. Petersen, S.E. *et al.* UK Biobank's cardiovascular magnetic resonance protocol. *J Cardiovasc Magn Reson* **18**, 8 (2016).
84. Bai, W. *et al.* Automated cardiovascular magnetic resonance image analysis with fully convolutional networks. *J Cardiovasc Magn Reson* **20**, 65 (2018).
85. Linge, J. *et al.* Body Composition Profiling in the UK Biobank Imaging Study. *Obesity (Silver Spring)* (2018).
86. Peru, Y.C.d.P.R.L. *et al.* Adult neuronal Arf6 controls ethanol-induced behavior with Arfaptin downstream of Rac1 and RhoGAP18B. *J Neurosci* **32**, 17706-13 (2012).
87. Dimou, N.L. & Tsilidis, K.K. A Primer in Mendelian Randomization Methodology with a Focus on Utilizing Published Summary Association Data. *Methods Mol Biol* **1793**, 211-230 (2018).

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Table 1: Association results of 46 novel alcohol variants identified through the meta-analysis of UK Biobank and AlcGen and CHARGE+. Results are ordered by *P*-value of combined analysis.

leadSNP						Combined			UKB			AlcGen and CHARGE+		
Nearest_Gene	Annotated Gene	rsID_LEAD_SNP	CP	EA	EAF	BETA	SE	P	BETA	SE	P	BETA	SE	P
MAPT	STH	rs1991556	17:44083402	A	0.22	-0.012	0.001	4.5E-23	-0.013	0.001	2.4E-21	-0.011	0.004	4.0E-03
RP11-89K21.1	SIX3	rs1004787	2:45159091	A	0.54	0.009	0.001	6.7E-17	0.009	0.001	1.1E-15	0.007	0.003	1.4E-02
SLC39A8	SLC39A8	rs13107325	4:103188709	T	0.07	-0.016	0.002	1.3E-15	-0.017	0.002	4.8E-16	-0.006	0.006	3.6E-01
IZUMO1, RASIP1, FUT1	IZUMO1	rs838145	19:49248730	A	0.55	-0.008	0.001	3.2E-15	-0.009	0.001	2.4E-15	-0.004	0.003	1.7E-01
na	PSMD7	rs1104608	16:73912588	C	0.43	-0.008	0.001	1.2E-14	-0.009	0.001	4.9E-15	-0.003	0.003	2.5E-01
MYBPC3	MYBPC3	rs2071305	11:47370957	A	0.69	0.009	0.001	4.5E-14	0.009	0.001	3.9E-13	0.007	0.003	3.1E-02
na	DRD2	rs7121986	11:113355444	T	0.37	-0.008	0.001	6.2E-14	-0.008	0.001	1.3E-13	-0.005	0.003	1.1E-01
na	DPP6	rs6969458	7:153489725	A	0.47	0.008	0.001	6.4E-14	0.008	0.001	1.3E-12	0.007	0.003	1.5E-02
RP11-308N19.1	ZNF462	rs74424378	9:109331094	T	0.76	0.009	0.001	1.7E-13	0.009	0.001	4.5E-13	0.006	0.003	8.4E-02
ARHGAP15, AC096558.1, RP11-570L15.2	ARHGAP15	rs13024996	2:144225215	A	0.37	-0.008	0.001	4.4E-13	-0.008	0.001	6.6E-13	-0.004	0.003	1.4E-01
MLXIPL	MLXIPL	rs34060476	7:73037956	A	0.87	-0.011	0.002	5.0E-13	-0.012	0.002	1.4E-13	-0.004	0.004	4.1E-01
na	FAM178A	rs61873510	10:102626510	T	0.33	-0.008	0.001	5.1E-13	-0.008	0.001	9.8E-12	-0.008	0.003	1.7E-02
FTO	FTO	rs1421085	16:53800954	T	0.60	0.008	0.001	9.2E-13	0.007	0.001	1.7E-10	0.010	0.003	9.2E-04
na	PMFBP1	rs11648570	16:72356964	T	0.89	-0.012	0.002	2.1E-12	-0.011	0.002	1.5E-10	-0.013	0.005	3.4E-03
OTX2, RP11-1085N6.6	OTX2	rs2277499	14:57271127	T	0.34	-0.008	0.001	2.2E-12	-0.007	0.001	2.4E-09	-0.012	0.003	9.1E-05
PDE4B	PDE4B	rs2310752	1:66392405	A	0.43	-0.007	0.001	2.8E-12	-0.008	0.001	1.8E-11	-0.006	0.003	4.2E-02
SERPINA1	SERPINA1	rs112635299	14:94838142	T	0.02	-0.025	0.004	3.7E-12	-0.027	0.004	9.8E-12	-0.017	0.010	9.9E-02
na	AJAP1	rs780569	1:4569436	A	0.71	-0.008	0.001	5.2E-12	-0.008	0.001	1.1E-11	-0.005	0.003	1.2E-01
na	VRK2	rs10496076	2:57942987	T	0.37	-0.007	0.001	9.7E-12	-0.007	0.001	1.3E-09	-0.009	0.003	1.6E-03
ACTR10, C14orf37	ACTR10	rs71414193	14:58685301	A	0.19	-0.009	0.001	1.8E-11	-0.008	0.001	5.8E-09	-0.013	0.004	4.5E-04
BEND4	BEND4	rs16854020	4:42117559	A	0.13	0.010	0.002	2.9E-11	0.010	0.002	5.8E-09	0.016	0.005	6.4E-04
na	SORL1	rs485425	11:121544984	C	0.45	-0.007	0.001	6.1E-11	-0.007	0.001	7.3E-11	-0.004	0.003	1.9E-01
SEZ6L2	SEZ6L2	rs113443718	16:29892184	A	0.31	-0.007	0.001	7.4E-11	-0.008	0.001	4.5E-11	-0.003	0.003	2.9E-01
CBX5, RP11-968A15.2	CBX5	rs57281063	12:54660427	A	0.41	0.007	0.001	7.9E-11	0.007	0.001	1.8E-09	0.007	0.003	1.2E-02
na	TNRC6A	rs72768626	16:24693048	A	0.94	0.014	0.002	9.7E-11	0.015	0.002	1.7E-09	0.014	0.006	1.8E-02
SYT14	SYT14	rs227179	1:210216731	A	0.59	-0.007	0.001	1.1E-10	-0.007	0.001	1.4E-09	-0.006	0.003	2.8E-02
TCF4	TCF4	rs9320010	18:53053897	A	0.60	0.007	0.001	1.1E-10	0.007	0.001	1.6E-09	0.007	0.003	2.2E-02
SBK1	NPIPB6	rs2726034	16:28336882	T	0.68	0.007	0.001	1.4E-10	0.007	0.001	1.1E-09	0.006	0.003	4.7E-02
ANKRD36	ANKRD36	rs13390019	2:97797680	T	0.87	0.010	0.002	1.6E-10	0.011	0.002	7.0E-11	0.004	0.005	4.5E-01
na	ELAVL4	rs7517344	1:50711961	A	0.17	0.009	0.001	1.9E-10	0.008	0.001	2.5E-07	0.016	0.004	2.1E-05
LINC00461	MEF2C	rs4916723	5:87854395	A	0.58	0.007	0.001	2.1E-10	0.007	0.001	5.1E-10	0.005	0.003	1.1E-01
ARPC1B, ARPC1A	ARPC1B	rs10249167	7:98980879	A	0.87	0.010	0.002	2.9E-10	0.009	0.002	8.1E-08	0.015	0.004	3.8E-04
EFNB3, WRAP53	EFNB3	rs7640	17:7606722	C	0.80	0.008	0.001	4.3E-10	0.009	0.001	1.3E-09	0.006	0.004	9.9E-02
RP11-501C14.5	IGF2BP1	rs4794015	17:47067826	A	0.41	0.007	0.001	4.3E-10	0.006	0.001	5.4E-08	0.009	0.003	1.2E-03
TCAP, PNMT, STARD3	TCAP	rs1053651	17:37822311	A	0.27	-0.007	0.001	1.1E-09	-0.008	0.001	8.4E-10	-0.003	0.003	2.8E-01
na	AADAT	rs7698119	4:171070910	A	0.49	-0.006	0.001	1.3E-09	-0.006	0.001	1.6E-07	-0.009	0.003	1.6E-03
STAT6, AC023237.1	STAT6	rs12312693	12:57511734	T	0.55	-0.006	0.001	1.5E-09	-0.006	0.001	9.5E-09	-0.005	0.003	5.6E-02
SCN8A	SCN8A	rs7958704	12:51984349	T	0.41	-0.006	0.001	1.6E-09	-0.006	0.001	1.7E-08	-0.006	0.003	3.5E-02
ACSS3	ACSS3	rs11114787	12:81595700	T	0.27	0.007	0.001	2.0E-09	0.007	0.001	2.7E-08	0.007	0.003	2.4E-02
RP11-32K4.1	BHLHE22	rs2356369	8:64956882	T	0.52	-0.006	0.001	2.0E-09	-0.006	0.001	4.1E-08	-0.007	0.003	1.6E-02
ZRANB2-AS2	ZRANB2	rs12031875	1:71585097	A	0.82	-0.008	0.001	2.2E-09	-0.008	0.001	7.6E-08	-0.010	0.004	8.7E-03
MSANTD1, HTT	MSANTD1	rs12646808	4:3249828	T	0.66	0.007	0.001	2.4E-09	0.007	0.001	1.1E-09	0.002	0.003	4.7E-01
TENM2	TENM2	rs10078588	5:166816176	A	0.52	0.006	0.001	2.5E-09	0.006	0.001	4.3E-08	0.007	0.003	1.9E-02
IGSF9B	IGSF9B	rs748919	11:133783232	T	0.79	0.008	0.001	3.3E-09	0.008	0.001	1.0E-08	0.005	0.003	1.1E-01
AC010967.2	GPR75-ASB3	rs785293	2:53023304	A	0.57	-0.006	0.001	3.3E-09	-0.006	0.001	3.2E-08	-0.006	0.003	3.8E-02
BDNF, RP11-587D21.4	BDNF	rs988748	11:27724745	C	0.21	-0.008	0.001	4.4E-09	-0.007	0.001	1.2E-07	-0.010	0.004	8.3E-03

SNP: Single Nucleotide polymorphism; LocusName: Nearest Gene; rsID_LEAD_SNP: Rs ID number of the lead SNP; CP: Chromosome/Position (build hg19/37); EA: Effect allele of the discovered SNP; EAF: Frequency of the effect allele; BETA_comb: Effect size in meta-analysis; SE_comb; Standard Error of the effect in meta-analysis; P_comb: Meta-analysis P-value; BETA_UKB: Effect size in UK Biobank analysis; SE_UKB: Standard Error of the effect in the UK Biobank analysis; P_UKB: UK Biobank analysis P-value;BETA_AlcGenCHARGE+: Effect size in the AlcGen meta-analysis; SE_AlcGenCHARGE+: Standard Error of the effect in the AlcGen meta-analysis; P_AlcGenCHARGE+: AlcGen meta-analysis P-value

962 **FIGURE CAPTIONS**

963 **Figure 1**. **Manhattan plot showing *P*-values from discovery genome-wide**
964 **association meta-analysis with alcohol intake (log g/d) among 480,842 individuals**
965 **across UK Biobank, AlcGen and CHARGE+, excluding known variants.** The *P*-value
966 was computed using inverse variance fixed effects models. The y axis shows the –
967 $\log_{10}$ *P* values and the x axis shows their chromosomal positions. Horizontal blue line
968 represents the threshold of $P = 5 \times 10^{-9}$.
969
970 **Figure 2**. **Association of alcohol intake loci with other traits**. Plot shows results from
971 associations with other traits which were extracted from the PhenoScanner database
972 for the 46 novel sentinel SNPs including proxies in Linkage Disequilibrium ($r^2 \geq 0.8$)
973 with genome-wide significant associations. Each colored line connects a specific
974 variant with the associated traits and diseases.
975
976 **Figure 3**. **Mediation effect of the grey matter volume of bilateral putamen on the**
977 **relationship between SNP rs13107325 and alcohol intake**. The green is for left
978 putamen, and, the red is for the right one. We use 'a' for the relationship between
979 rs13107325 and putamen, 'b' for the relationship between putamen and alcohol
980 consumption, 'c' for the relationship between rs13107325 and alcohol consumption,
981 'c'' for the relationship between rs13107325 and alcohol consumption after
982 excluding the effect of putamen, and 'ab' as the mediation effect. The significance
983 tests are based on the bootstrapping method (10,000 times). Z- statistics and the
984 corresponding *P* values are provided in parentheses. The brain icon was created
985 using Mango software, version 4.1 (http://ric.uthscsa.edu/mango/).
986
987 **Figure 4. Comparison of *Zip8* alcohol phenotypes in *Drosophila*.** Flies were exposed
988 to 100/50 Ethanol/Air vapor for 30 min for exposure 1, and the time to 50% loss of
989 righting was determined (ST-50, sedation time). After recovery on food for 4 hours,
990 flies were re-exposed to the same vapors, and the second ST-50 recorded (left side).
991 The resulting increase in ST-50, i.e. tolerance, is shown on the right. In a)
992 overexpressed human *hZIP8* in *ics*-expressing cells flies are compared against
993 controls whereas in b) knockdown of the fly ortholog *dZip71B* is compared against
994 controls. In c) flies carrying two transposon insertions in the endogenous *dZip71B*
995 gene are compared against controls. Significance levels: ***P <0.001, **P <0.01, *P
996 <0.05. Exact *P*-values are presented in the text.
997