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# *KLB* is associated with alcohol drinking, and its gene product β-Klotho is necessary for FGF21 regulation of alcohol preference

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**Abstract**

Excessive alcohol consumption is a major public health problem worldwide. While drinking habits are known to be inherited, few genes have been identified that are robustly linked to alcohol drinking. We conducted a genome-wide association meta-analysis and replication study among >105,000 individuals of European ancestry, and identified β-Klotho *(KLB)* as a locus associated with alcohol consumption (rs11940694; $P$=9.2x10$^{-12}$). β-Klotho is an obligate co-receptor for the hormone FGF21, which is secreted from the liver and implicated in macronutrient preference in man. We show that brain-specific β-Klotho knock-out mice have an increased alcohol preference and that FGF21 inhibits alcohol drinking by acting on the brain. These data suggest that a liver-brain endocrine axis may play an important role in the regulation of alcohol drinking behavior and provide a unique pharmacologic target for reducing alcohol consumption.

**Keywords:** alcohol consumption, human, mouse model, brain, β-Klotho, FGF21

## Significance

Alcohol is a widely consumed drug in western societies that can lead to addiction. A small shift in consumption can have dramatic consequences on public health. We performed the largest genome-wide association meta-analysis and replication study to date (>105,000 individuals) and identified a new genetic basis for alcohol consumption during non-addictive drinking. We found a locus in the gene encoding β-Klotho *(KLB)* is associated with alcohol consumption. β-Klotho is an essential receptor component for the endocrine fibroblast growth factors (FGFs) 19 and 21. Using mouse models and pharmacologic administration of FGF21, we demonstrate that β-Klotho in the brain controls alcohol drinking. These findings reveal a mechanism regulating alcohol consumption in humans that may be pharmacologically tractable for reducing alcohol intake.

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## Introduction

Excessive alcohol consumption is a major public health problem worldwide causing an estimated 3.3 million deaths in 2012 (1). Much of the behavioral research associated with alcohol has focused on alcohol-dependent patients. However, the burden of alcohol-associated disease largely reflects the amount of alcohol consumption in a population, not alcohol dependence (2). It has long been recognized that small shifts in the mean of a continuously distributed behavior such as alcohol drinking can have major public health benefits (3). For example, a shift from heavy to moderate drinking could have beneficial effects on cardiovascular disease risk (4).

Alcohol drinking is a heritable complex trait (5). Genetic variants in the alcohol and aldehyde dehydrogenase gene family can result in alcohol intolerance caused by altering peripheral alcohol metabolism, and may thus influence alcohol consumption and dependence (6). However, genetic influences on brain functions affecting drinking behavior have been more difficult to detect because, as for many complex traits, the effect of individual genes is small, so large sample sizes are required to detect the genetic signal (7).

Here we report a genome-wide association (GWAS) and replication study of over 100,000 individuals of European descent. We identify a gene variant in β-Klotho *(KLB)* that associates with alcohol consumption. β-Klotho is a single-pass transmembrane protein that complexes with FGF receptors to form cell surface receptors for the

hormones FGF19 and FGF21 (8, 9). FGF19 is induced by bile acids in the small intestine to regulate bile acid homeostasis and metabolism in the liver (9). FGF21 is induced in liver and released into the blood in response to various metabolic stresses, including high carbohydrate diets and alcohol (10-12). Notably, *FGF21* was recently associated in a human GWAS study with macronutrient preference, including changes in carbohydrate, protein and fat intake (13). Moreover, FGF21 was shown to suppress sweet and alcohol preference in mice (14, 15). Our current findings suggest that the FGF21-β-Klotho signaling pathway regulates alcohol consumption in humans.

## Results

### Association of *KLB* gene SNP rs11940694 with alcohol drinking in humans

We carried out a GWAS of quantitative data on alcohol intake in 70,460 individuals (60.9% women) of European descent from 30 cohorts. We followed up the most significantly associated SNPs (six sentinel SNPs $P<1.0\times10^{-6}$ from independent regions) among up to 35,438 individuals from 14 additional cohorts (Dataset S1; and Appendix 1). We analyzed both continuous data on daily alcohol intake in drinkers (as g/day, log transformed) and a dichotomous variable of heavy versus light or no drinking (Dataset S1). Average alcohol intake in drinkers across the samples was 14.0 g/day in men and 6.0 g/day in women. We performed per cohort sex-specific and combined-sex single SNP regression analyses under an additive genetic model, and conducted meta-analysis across the sex-specific strata and cohorts using an inverse variance weighted fixed effects model.

Results of the primary GWAS for log g/day alcohol are shown in Figures 1 and S1, Dataset S2. We identified five SNPs for replication at $P<1x10^{-6}$: rs11940694 in the *KLB* gene, rs197273 in *TANK,* rs780094 in *GCKR,* rs350721 in *ASB3* and rs10950202 in *AUTS2* (Table 1, Dataset S2). In addition to rs10950202 in *AUTS2* ($P=2.9x10^{-7}$), we took forward SNP rs6943555 in *AUTS2* ($P=1.4x10^{-4}$), which was previously reported in relation to alcohol drinking (7). In both men and women the newly discovered SNPs were all significantly associated with log g/day alcohol at $P<0.005$ (Table S1). When combining discovery and replication data, we observed genome-wide significance for SNP rs11940694 (A/G) in *KLB* ($P=9.2x10^{-12}$) (Table 1 and Figure S1), for which the minor allele A was associated with reduced drinking. *KLB* is localized on human chromosome 4p14 and encodes a transmembrane protein, β-Klotho, which is an essential component of receptors for FGF19 and FGF21 (8, 9). Rs197273 in the TRAF family member-associated NF-kappa-B activator gene (*TANK*) narrowly missed reaching genome-wide significance in the combined sample (Table 1; $P=7.4x10^{-8}$). In the dichotomous analysis of the primary GWAS, SNP rs12599112 in the Cadherin 13 gene (*CDH13*) and rs10927848 in the Transmembrane protein 82 gene (*TMEM82*) were significant at $P=2.3x10^{-8}$ and $P=2.6x10^{-7}$, respectively (Figure S2, Table S2 and Dataset S2), but did not reach genome wide significance in the combined analysis (Table S2).

SNP rs11940694 is localized in intron 1 of the *KLB* gene. The local linkage disequilibrium (LD) structure of the *KLB* gene is shown in Figure S3. The minor allele frequencies of this SNP were generally high (between 0.37 and 0.44) in different ethnic groups (Table S3). We found no significant association of rs11940694 with gene

expression in peripheral blood of 5,236 participants of the Framingham study (Table S4) (16).

**β-Klotho in the brain controls alcohol drinking in mice**

To examine whether β-Klotho affects alcohol drinking in mice, and whether it does so through actions in the brain, we measured alcohol intake and the alcohol preference ratio of brain-specific β-Klotho-knockout ($Klb^{Camk2a}$) mice and control floxed *Klb* ($Klb^{fl/fl}$) mice. We used a voluntary two-bottle drinking assay performed with water and alcohol. Since we previously showed that FGF21-transgenic mice, which express FGF21 at pharmacologic levels, have a reduced alcohol preference (14), we performed these studies while administering either recombinant FGF21 or vehicle by osmotic minipump. Alcohol preference versus water was significantly increased in vehicle-treated $Klb^{Camk2a}$ compared to $Klb^{fl/fl}$ mice at 16 vol. % alcohol (Fig. 2A). FGF21 suppressed alcohol preference in $Klb^{fl/fl}$ mice, but not in $Klb^{Camk2a}$ demonstrating that the effect of FGF21 on alcohol drinking depends on β-Klotho expressed in the brain (Fig. 2A). There was a corresponding decrease in plasma alcohol levels immediately after 16 vol. % alcohol drinking, which reflects the modulation of the drinking behavior (Fig. 2B). However, plasma FGF21 levels were comparable in $Klb^{fl/fl}$ and $Klb^{Camk2a}$ mice administered recombinant FGF21 at the end of the experiment (Fig. 2C). Alcohol bioavailability was not different between FGF21 treated $Klb^{fl/fl}$ and $Klb^{Camk2a}$ mice (Fig. 2D). We have previously shown that FGF21 decreases the sucrose and saccharin preference ratio in $Klb^{fl/fl}$ but not $Klb^{Camk2a}$ mice, and has no effect on the quinine preference ratio (14). To rule out a potential perturbation of our findings as a result of the experimental

procedure, we independently measured preference and consumption of 16 vol. % alcohol in *Klb*$^{fl/fl}$ and *Klb*$^{Camk2a}$ mice without osmotic minipump implantation. Again, *Klb*$^{Camk2a}$ mice showed significantly greater alcohol consumption and increased alcohol preference compared to *Klb*$^{fl/fl}$ mice (Fig. 2E and F), thus replicating our findings above. Alcohol bioavailability after an intraperitoneal injection was not different between *Klb*$^{fl/fl}$ and *Klb*$^{Camk2a}$ mice after 1 and 3 hours (Fig. 2G).

**β-Klotho in brain does not regulate emotional behavior in mice**

Increased alcohol drinking in humans and mice may be motivated by its reward properties or as a means to relieve anxiety and stress (17). In mice, FGF21 increases corticotropin-releasing hormone expression in hypothalamus, circulating glucocorticoid concentrations and sympathetic outflow (18-20), which are linked to heightened anxiety. We therefore tested *Klb*$^{fl/fl}$ and *Klb*$^{Camk2a}$ mice in behavioral paradigms measuring anxiety, including novelty suppressed feeding (Fig. 3A), elevated plus maze (Fig. 3B), and open field activity tests (Fig. 3C). However, we did not find differences between *Klb*$^{fl/fl}$ and *Klb*$^{Camk2a}$ mice in any of these anxiety measures or in general locomotor activity. Our finding of increased alcohol preference in *Klb*$^{Camk2a}$ mice may thus be caused by alteration of alcohol-associated reward mechanisms. While this notion is consistent with our previous results showing *Klb* expression in areas important for alcohol reinforcement, specifically the nucleus accumbens and the ventral tegmental area (14), additional studies will be required to determine precisely where in the brain and how β-Klotho affects alcohol drinking.

## Discussion

Here we report that in a GWAS performed in over 100,000 individuals, SNP rs11940694 in *KLB* associates with alcohol consumption in non-addicts. We further show that mice lacking β-Klotho in the brain have increased alcohol consumption and are refractory to the inhibitory effect of FGF21 on alcohol consumption. These findings reveal a previously unrecognized brain pathway regulating alcohol consumption in humans that may prove pharmacologically tractable for suppressing alcohol drinking.

FGF21 is induced in liver by simple sugars through a mechanism involving the transcription factor carbohydrate response element binding protein (10, 11, 15, 21, 22). FGF21 in turn acts on brain to suppress sweet preference (14, 15). Thus, FGF21 is part of a liver-brain feedback loop that limits the consumption of simple sugars. Notably, FGF21 is also strongly induced in liver by alcohol and contributes to alcohol-induced adipose tissue lipolysis in a mouse model of chronic-binge alcohol consumption (12). Our present data suggest the existence of an analogous feedback loop wherein liver-derived FGF21 acts on brain to limit the consumption of alcohol. However, additional studies will be required to establish the existence of this FGF21 pathway in vivo.

In murine brain, there is evidence that FGF21 suppresses sweet preference through effects on the paraventricular nucleus in the hypothalamus (15). Among its actions in the hypothalamus, FGF21 induces corticotropin-releasing hormone (18, 19), which is a strong modulator of alcohol consumption (23). Notably, β-Klotho is also present in mesolimbic regions of the brain that regulate reward behavior, including the ventral

tegmental area and nucleus accumbens, and FGF21 administration reduced tissue levels of dopamine and its metabolites in the nucleus accumbens (14). Thus, FGF21 may act coordinately on multiple brain regions to regulate the consumption of both simple sugars and alcohol.

In closing, our data linking β-Klotho to alcohol consumption together with previous GWAS data linking FGF21 to macronutrient preference raise the intriguing possibility of a liver-brain endocrine axis that plays an important role in the regulation of complex adaptive behaviors, including alcohol drinking. While our findings support an important role for the *KLB* gene in the regulation of alcohol drinking, we cannot rule out the possibility that *KLB* rs11940694 acts by affecting neighboring genes. Therefore additional genetic and mechanistic studies are warranted. Finally, it will be important to follow up on our findings in more severe forms of alcohol drinking, since our results suggest that this pathway could be targeted pharmacologically for reducing the desire for alcohol.

## Methods

### Alcohol phenotypes

Alcohol intake in grams of alcohol per day was estimated by each cohort based on information about drinking frequency and type of alcohol consumed. For cohorts that collected data in ‘drinks per week’, standard ethanol contents in different types of alcohol drinks were provided as guidance to convert the data to ‘grams per week’, which was further divided by 7 to give intake as ‘grams per day’. Adjustment was made if

cohort-specific drink sizes differed from the standard. For cohorts that collected alcohol use in grams of ethanol per week, the numbers were divided by 7 directly into 'grams per day'. Cohorts with only a categorical response to the question for drinks per week used mid-points of each category for the calculation. All non-drinkers (individuals reporting zero drinks per week) were removed from the analysis. The 'grams per day' variable was then $\log_{10}$ transformed prior to the analysis. Sex-specific residuals were derived by regressing alcohol in $\log_{10}$ (grams per day) in a linear model on age, age-square, weight, and if applicable, study site and principal components to account for population structure. The sex-specific residuals were pooled and used as the main phenotype for subsequent analyses.

Dichotomous alcohol phenotype was created based on categorization of 'drinks per week' variable. Heavy drinking was defined as >=21 drinks per week in men, or >=14 drinks per week in women. Light (or zero) drinking was defined if male participants had <=14 drinks per week, or female participants had <=7 drinks per week. Drinkers having >14 to <21 drinks for men, or >7 to <14 drinks for women were excluded. Where information was available, current non-drinker who was former drinker of >14 drinker per week in men, and >7 drinks per week in women, as well as current non-drinker who was a former drinker of unknown amount were excluded; whereas current non-drinkers who were former drinkers of <=14 for men or <=7 for women were included. Further exclusion was made if there were missing data on alcohol consumption or on the covariates.

The analyses only included participants of European origin and were performed in accordance with the principles expressed in the Declaration of Helsinki. Each cohort's study protocol was reviewed and approved by their respective institutional review board and informed consent was obtained from all study subjects.

**Discovery GWAS in AlcGen and CHARGE+ and replication analyses**

Genotyping methods are summarized in Dataset S1B, S1C and S1F. SNPs were excluded if: HWE $P < 1x10^{-6}$ or based on cohort-specific criteria; MAF < 1%; imputation information score < 0.5; if results were only available from 2 or fewer cohorts, or total N < 10,000. Population structure was accounted for within cohorts via principal components analysis (PCA). Linkage disequilibrium (LD) score regression (24) was conducted on the GWAS summary results to examine the degree of inflation in test statistics, and genomic control correction was considered unnecessary ($\lambda_{GC}$=1.06 and intercept=1.00; λ=0.99 to 1.06 for individual cohorts, Dataset S1B and S1C). SNPs were taken forward for replication from discovery GWAS if they passed the above criteria and if they had $P < 1x10^{-6}$ (one SNP with the smallest *P* taken forward in each region, except for *AUTS2* for which two SNPs were taken forward based on previous results (7)). Meta-analyses were performed by METAL (25) or R (v3.2.2).

**Gene expression profiling in Framingham study**

In the Framingham study, gene expression profiling was undertaken for the blood samples of a total of 5,626 participants from the Offspring (N=2,446) at examination eight and the Third Generation (N=3,180) at examination two. Fasting peripheral whole

blood samples (2.5ml) were collected in PAXgene™ tubes (PreAnalytiX, Hombrechtikon, Switzerland). RNA expression profiling was conducted using the Affymetrix Human Exon Array ST 1.0 (Affymetrix, Inc., Santa Clara, CA) for samples that passed RNA quality control. The expression values for ~ 18,000 transcripts were obtained from the total 1.2 million core probe sets. Quality control procedures for transcripts have been described previously. All data used herein are available online in dbGaP (http://www.ncbi.nlm.nih.gov/gap; accession number phs000007).

**The *cis*-expression quantitative trait loci analysis in the Framingham study**

To investigate possible effects of rs11940694 in *KLB* on gene expression, we performed *cis*-eQTL analysis. The SNP in *KLB* was used as the independent variable in association analysis with the transcript of *KLB* measured using whole blood samples in the FHS (n=5,236). Affymetrix probe 2724308 was used to represent the *KLB* overall transcript levels. Age, sex, BMI, batch effects and blood cell differentials were included as covariates in the association analysis. Linear mixed model was used to account for familial correlation in association analysis.

**Mouse studies**

All mouse experiments were approved by the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center. Male littermates (2 to 4-month-old) maintained on a 12 hr light/dark cycle with *ad libitum* access to chow diet (Harlan Teklad TD2916) were used for all experiments. The *Klb*

gene was deleted from brain by crossing *Klb*$^{fl/fl}$ mice with *Camk2a*-Cre mice on a mixed C57BL/6J;129/Sv background as described (26).

**Alcohol drinking in mice**

For voluntary two-bottle preference experiments, male mice (n=9-13 per group) were given access to two bottles, one containing water and the other containing 2-16% ethanol (vol/vol) in water. After acclimation to the two-bottle paradigm, mice were exposed to each concentration of ethanol for 4 days. Total fluid intake (water + ethanol-containing water), food intake and body weight were measured each day. Alcohol consumption (g) was calculated based on EtOH density (0.789 g/ml). To obtain accurate alcohol intake that corrected for individual differences in littermate size, alcohol consumption was normalized by body weight per day for each mouse. As a measure of relative alcohol preference, the preference ratio was calculated at each alcohol concentration by dividing total consumed alcohol solution (ml) by total fluid volume. Two-bottle preference assays were also performed with sucrose (0.5 and 5%) and quinine (2 and 20 mg/dl) solutions. For all experiments, the positions of the two bottles were changed every two days to exclude position effects.

**Mouse experiments with FGF21**

For FGF21 administration studies, recombinant human FGF21 protein provided by Novo Nordisk was administered at a dose of 0.7 mg/kg/day by subcutaneous osmotic mini-pumps (Alzet 1004). Mice were single caged following mini-pump surgery, which was conducted under isoflurane anesthesia and 24 hour buprenorphine analgesia. Mice were

allowed to recover from mini-pump surgery for 4 days prior to alcohol drinking tests. After experiments, mice were sacrificed by decapitation and plasma was collected using EDTA or heparin after centrifugation for 15 minutes at 3000 rpm. Plasma FGF21 concentrations were measured using the Biovendor FGF21 ELISA Kit according to manufacturer's protocol.

**Plasma ethanol concentration and clearance**

For alcohol bioavailability tests, mice (n=4-5 per group) were injected i.p. with alcohol (2.0 g/kg, 20% w/vol) in saline, and tail vein blood was collected after 1 and 3 hours. Plasma alcohol concentrations were measured using the EnzyChrom™ Ethanol Assay Kit.

**Emotional behavior in mice**

For open field activity assays, naïve mice were placed in an open arena (44 x 44 cm, with the center defined as the middle 14 x 14 cm and the periphery defined as the area 5 cm from the wall), and the amount of time spent in the center versus along the walls and total distance traveled were measured. For elevated plus maze activity assays, mice were placed in the center of a plus maze with 2 dark enclosed arms and 2 open arms. Mice were allowed to move freely around the maze, and the total duration of time in each arm and the frequency to enter both the closed and open arms was measured. For novelty suppression of feeding assays, mice fasted for 12 hours were placed in a novel environment and the time to approach and eat a known food was measured.

## Statistical analysis

All data are expressed as means ± S.E.M. Statistical analysis between the two groups was performed by unpaired two-tailed Student's t test using Excel or GraphPad Prism (GraphPad Software, Inc.). For multiple comparisons, one-way analysis of variance (ANOVA) with post-hoc Tukey was done using SPSS.


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## Author contributions

G.S., P.E., D.L., C.P.M., D.J.M. and S.A.K. designed the study, acquired and analyzed data, and wrote the manuscript; P.S. performed animal experiments, acquired and analyzed data, and contributed to writing the manuscript; C.L., P.F.O'R., H.G. and E.E. analyzed GWAS data and contributed to writing the manuscript; B.X., B.R. and S.D. carried out functional analyses and contributed to writing the manuscript; G.B. and Yu.L. acquired and analyzed epigenetic data; N.A., T.J., S.R.P., M.P.S.L., and K.R. analyzed GWAS data. The following authors contributed to the primary GWAS and replication by participating in (i) study concept/design: D.I. B., J.C.C., D.I.C, T.D., I.J.D., E.J.C. deG., J.G.E., T.E., O.H.F., P.F., C.G., H.J.G., V.G., U.G., T.B.H., A.-L.H., A.C.H., A.H., C.H., M.-R.J., J.W.J., J.K., J.S.K., J.L., C.L., T.L., D.L., Y.L., P.A.F.M., N.G.M., A.C.M., J.A.N.,

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B.Y., W.Z., J.Z.

**Conflict of interest statement:**

Dr. Psaty serves on the DSMB for a clinical trial funded by the manufacturer (Zoll LifeCor) and on the Steering Committee of the Yale Open Data Access project funded by Johnson & Johnson. Dr. Mangelsdorf serves on the scientific advisory board of Metacrine. The other authors report no competing financial interests.

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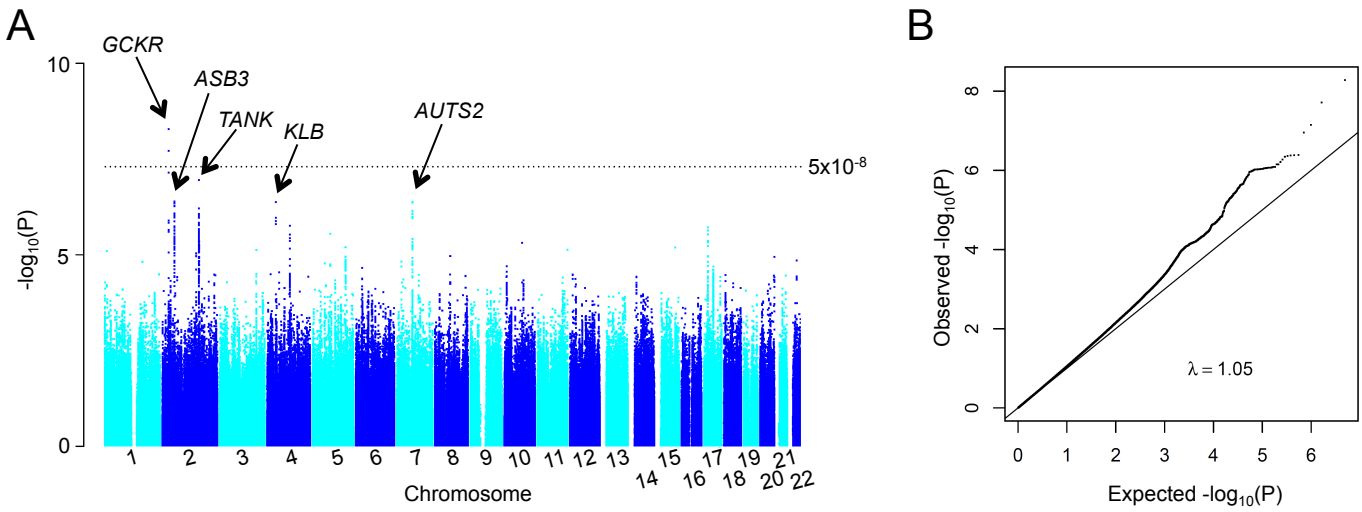
## Figure Legends

**Figure 1.** Genome-wide association results of log g/day alcohol in AlcGen and CHARGE+ consortia. **(A)** Manhattan plot showing the significance of the association (-$\log_{10}$ transformed *P* value on the y axis) for each SNP at chromosomal position shown on the x axis. The dotted line represents the genome-wide significance level at $P=5x10^{-8}$. The genes that were followed up are labelled. **(B)** Quantile-quantile plot comparing the expected *P* value on the x axis and the observed *P* value on the y axis (both were -log10 transformed).

**Figure 2:** FGF21 reduces alcohol preference in mice by acting on β-Klotho in brain. **(A)** Alcohol preference ratios determined by two-bottle preference assays with water and the indicated ethanol concentrations for control ($Klb^{fl/fl}$) and brain-specific β-Klotho knockout (*KlbCamk2a*) mice administered either FGF21 (0.7 mg/kg/day) or vehicle (n=10/ group). **(B)** Plasma ethanol and **(C)** FGF21 concentrations at the end of the 16% ethanol step of the two-bottle assay. **(D)** Plasma ethanol concentrations 1 and 3 hours after i.p. injection of 2 g/kg alcohol (n=4/each group). **(E)** Consumption of 16% ethanol (g/kg/d) and **(F)** alcohol preference ratios in two-bottle preferences assays performed with control ($Klb^{fl/fl}$) and brain-specific β-Klotho-knockout (*KlbCamk2a*) mice. Alcohol preference was measured by volume of ethanol/total volume of fluid consumed (n=13/group). **(G)** Plasma ethanol concentrations 1 and 3 hours after i.p. injection of 2 g/kg alcohol (n=5/group). Values are means ±S.E.M. For **(A-C),** *p<0.05; ***p<0.001 for $Klb^{fl/fl}$ + vehicle versus $Klb^{fl/fl}$ + FGF21 groups; and $^{\#\#}$p<0.01; $^{\#\#\#}$p<0.001 for $Klb^{fl/fl}$ +

FGF21 versus *Klb*$^{Camk2a}$ + FGF21 groups as determined by one-way ANOVA followed by Tukey's post-tests. For **(E, F)**,*p<0.05 and **p<0.01.

**Figure 3:** Behavior tests in brain-specific β-Klotho knockout mice. Results from **(A)** novelty suppressed feeding, **(B)** elevated plus maze and **(C)** open field activity assays performed with control (*Klb*$^{fl/fl}$) and brain-specific β-Klotho-knockout (*Klb*$^{Camk2a}$) mice (n=15/each group). Values are the time (seconds) spent for each step of the assay.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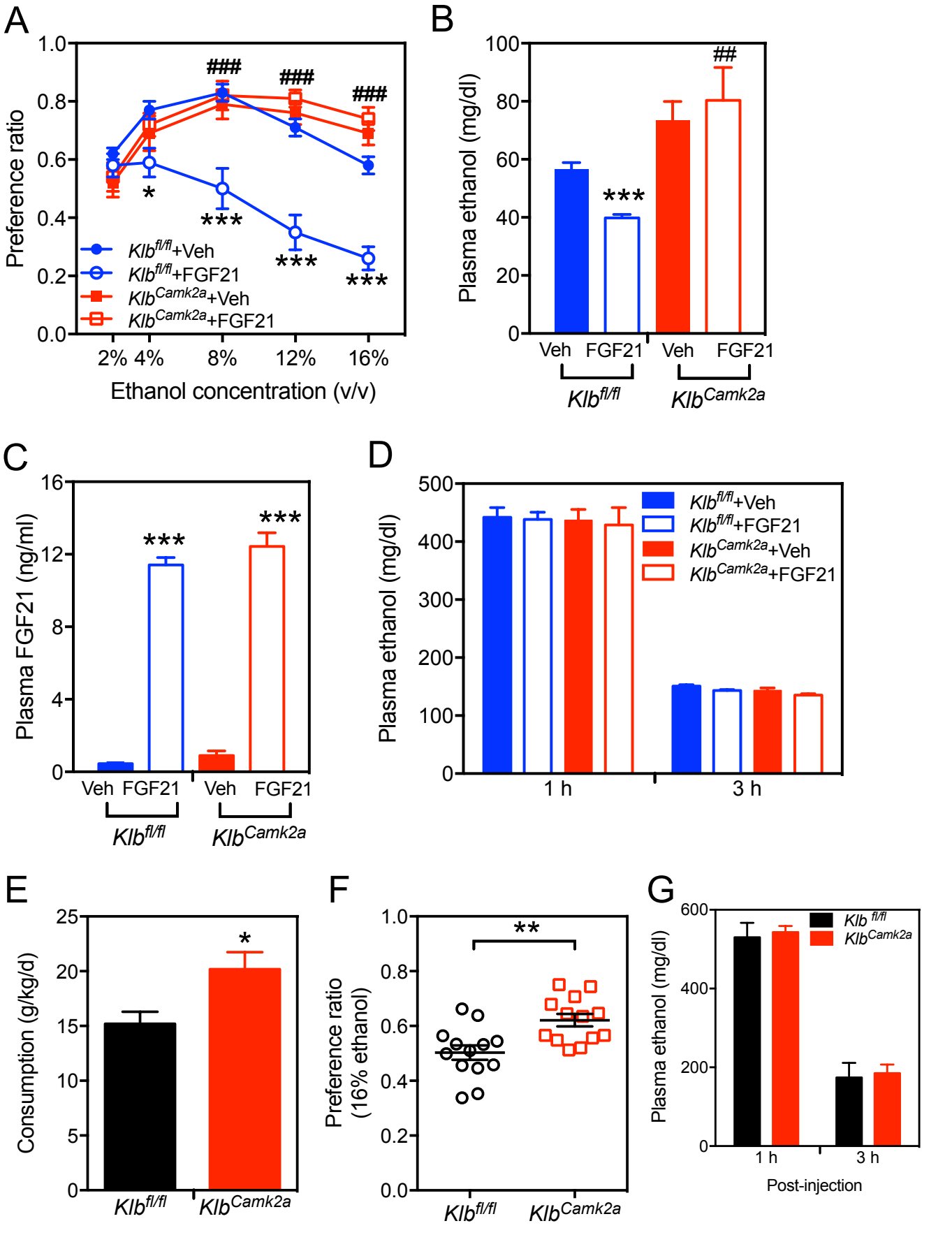
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Preference ratio
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Klb fl/fl +Veh
Klb fl/fl +FGF21
Klb Camk2a +Veh
Klb Camk2a +FGF21
2% 4% 8% 12% 16%
Ethanol concentration (v/v)
B
Plasma ethanol (mg/dl)
100
80
60
40
20
0
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***
Veh FGF21 Veh FGF21
Klb fl/fl
Klb Camk2a
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Plasma FGF21 (ng/ml)
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Veh FGF21 Veh FGF21
Klb fl/fl
Klb Camk2a
D
Plasma ethanol (mg/dl)
500
400
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100
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Klb fl/fl +Veh
Klb fl/fl +FGF21
Klb Camk2a +Veh
Klb Camk2a +FGF21
1 h
3 h
E
Consumption (g/kg/d)
25
20
15
10
5
0
*
Klb fl/fl
Klb Camk2a
F
Preference ratio (16% ethanol)
1.0
0.8
0.6
0.4
0.2
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Klb fl/fl
Klb Camk2a
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Plasma ethanol (mg/dl)
600
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Klb fl/fl
Klb Camk2a
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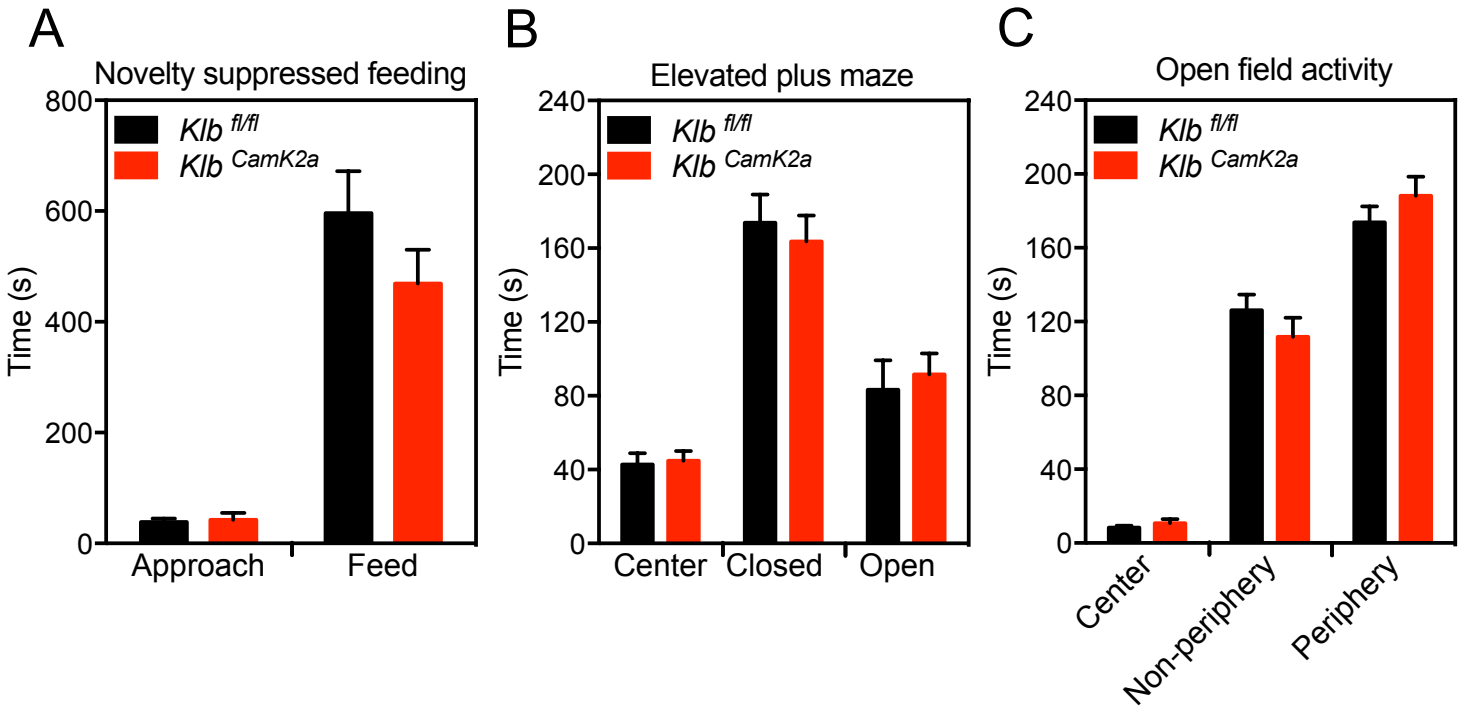
Post-injection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Novelty suppressed feeding
Klb fl/fl
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Approach
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Elevated plus maze
Klb fl/fl
Klb CamK2a
Time (s)
240
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Center
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Open field activity
Klb fl/fl
Klb CamK2a
Time (s)
240
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Periphery

**Appendices**

## 1. Cohort descriptions

### 1.1 Population descriptions for GWAS discovery cohorts in the Alcohol Genome-wide Association (AlcGen) consortium

**Cohorte Lausannoise study (CoLaus)**
The cohort is a random population sample of the city of Lausanne aged 35-75 years. Recruitment began in June 2003 and ended in May 2006. The CoLaus study was approved by the Institutional Ethics Committee of the University of Lausanne and informed consent was appropriately obtained by all participants. All participants attended the outpatient clinic of the University Hospital of Lausanne in the morning after an overnight fast. Data were collected by trained field interviewers in a single visit lasting about 60 min. Alcohol consumption was assessed by questionnaire and measured in units per week. In total 3,121 individuals were included in the analysis.

**The Estonian Biobank Cohort (EGCUT)**
The Estonian Biobank Cohort is a population-based cohort of 52000 Estonian residents (81% ethnic Estonians), recruited on volunteer-basis in 2002-2010 (www.biobank.ee), managed by the Estonian Genome Center, University of Tartu.

**The European Prospective Investigation of Cancer - Norfolk study (EPIC-Norfolk)**
The EPIC-Norfolk sample includes 2,566 participants randomly selected from the EPIC-Norfolk Study, a population-based cohort study of 25,663 men and women of European descent aged 39-79 years recruited in Norfolk, UK between 1993 and 1997.

**The Erasmus Rucphen Family study (ERF)**
The ERF (1) is a family based study that includes over 3,000 participants descending from 22 couples living in the Rucphen region in the 19th century. All living descendants of these couples and their spouses were invited to take part in the study. The medical ethics committee of Erasmus MC constituted according to the WMO (National Act Medical-scientific research in human beings) approved the Study (MEC 213.575/2002/114). The genotyping for the ERF study was supported by EUROSPAN (European Special Populations Research Network) through the European Commission FP6 STRP grant (018947; LSHG-CT-2006-01947). The ERF study was further supported by grants from the Netherlands Organisation for Scientific Research (NWO), Erasmus MC, the Centre for Medical Systems Biology (CMSB1 and CMSB2) and the Netherlands Genomics Initiative (NGI) and also received funding from the European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413 by the European Commission under the program “Quality of Life and Management of the Living Resources” of 5th Framework Programme (no. QLG2-CT-2002-01254). High-throughput analysis of the ERF data was supported by joint grant from Netherlands Organisation for Scientific Research and the Russian Foundation for Basic Research (NWO-RFBR 047.017.043). Exome sequencing analysis in ERF was supported by the ZonMw grant (project 91111025).

**The Fenland study (Fenland)**
The Fenland study is a population-based cohort study that uses objective measures

of disease exposure, such as accurate methods of body composition and energy expenditure, to study the interactions between genetic and lifestyle factors that cause obesity and diabetes. The volunteers are recruited from general practice lists in and around Cambridgeshire (Cambridge, Ely, and Wisbech) in the United Kingdom from birth cohorts from 1950–1975.

**The Younger Finnish Twin Cohort (FinnTwin12)**
The FinnTwin12 cohort is composed of twins born in Finland during 1983-87. The study has a two-stage sampling design. The larger, first-stage study is an epidemiological investigation of five consecutive and complete birth cohorts of Finnish twin children, including questionnaire assessments of both twins and parents at baseline, starting with a family questionnaire (returned by 2724 families, 87% participation rate) that was mailed late in the year before the twins reach age 12, with follow-up of all twins at age 14, 17.5 years and ~22. Nested within this epidemiological, population-based study, is the second-stage of FinnTwin12, an intensive assessment of a sub-sample of twin families. Most of the sub-sample was selected at random, but this random sample (~72%) was then enriched with twins at elevated familial risk for alcoholism. Genome-wide genotyping was performed on the subjects of the intensive sub-sample.

**The Older Finnish Twin Cohort (FinnTwinOld)**
This sample originates from the Older Finnish Twin Cohort. The 1975 , 1981 and 1990 questionnaires for the same-sex twins and the 1996-97 questionnaires for opposite-sex twins requested identical information on the frequency and quantity of alcohol used during an average week (or month), the frequency of passouts experienced during the preceding year, and required a yes/no response to a question on drinking density. Frequency of alcohol use, measured as days' use per month on 5-point scales (“never” to “over 16 days a month”) was assessed separately for beer, wine, and spirits. Similarly, quantity was measured on three 7-point scales, with the upper limits defined as consuming >48 bottles of beer (or 10 bottles of wine) per week, or >20 bottles of spirits per month. Wine use did contribute to the consumption measure. For each type of beverage, consumption was converted into grams of absolute alcohol and summed to yield an estimate of total consumption in grams per month using the class midpoints of the categories and the average alcohol content of each beverage type. The script for computing alcohol amount is available from Jaakko Kaprio on request.

**The Helsinki Birth Cohort Study (HBCS)**
The Helsinki Birth Cohort Study (HBCS) is composed of 8,760 individuals born between the years 1934-44 in one of the two main maternity hospitals in Helsinki, Finland. Between 2001 and 2003, a randomly selected sample of 928 males and 1075 females participated in a clinical follow-up study with a focus on cardiovascular, metabolic and reproductive health, cognitive function and depressive symptoms.

**The population-based Cooperative Health Research in the Region of Augsburg F3 Study (KORA F3)**
The population-based Cooperative Health Research in the Region of Augsburg (KORA) F3 Study was carried out in 2004-2005 as a follow-up of the MONICA/KORA S3 baseline study (1994-1995). In S3, 4,856 participants were recruited out of a randomized two-stage cluster sample of 6,640 subjects, with equal-sized sex- and

age-strata, from the target population of all German residents in the region of Augsburg aged 25–74 years. The F3 Study included 3,007 participants aged 35–84 years. 1,644 randomly drawn participants aged 35–79 with Affymetrix genotype data and data on alcohol intake were included in the investigations reported.

**The population-based Cooperative Health Research in the Region of Augsburg F4 Study (KORA F4)**

The population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 Study was carried out in 2006–2008 as a follow-up of the KORA S4 baseline study (1999–2001). In S4, 4,261 participants were recruited out of a randomized two-stage cluster sample of 6,640 subjects, with equal-sized sex- and age-strata, from the target population of all German residents in the region of Augsburg aged 25-74 years. The F4 Study included 3,080 participants aged 32-81 years. 1814 randomly drawn participants aged 32-81 with Affymetrix genotype data and data on alcohol intake were included in the investigations reported.

**LifeLines Cohort Study & Biobank (Lifelines)**

LifeLines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 165,000 persons living in the North East region of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics.

**The London Life Sciences Prospective Population Study (LOLIPOP)**

LOLIPOP is a population based prospective study of 17,606 Indian Asian and 7,766 European men and women aged 35-75 years, recruited from the lists of 58 General Practitioners in West London, United Kingdom between 2003 and 2008 (2, 3). Europeans were of self-reported white ancestry. Assessments of participants were carried out by trained research nurses with an interviewer-administered questionnaire. Anthropometric measurements and blood samples were taken on site. Alcohol consumption was measured in units per week. One unit is equivalent to: a small glass of wine, a single pub measure of spirits, or half a pint of beer/lager. Aliquots of whole blood were stored at -80C and DNA was extracted and genotyping was carried out thereafter. The LOLIPOP study is approved by the local Research Ethics Committees (3). All participants provided written consent for the study.

**The Netherlands Study of Depression and Anxiety (NESDA)**

The Netherlands Study of Depression and Anxiety (NESDA) (4), an ongoing cohort study into the long-term course and consequences of depressive and anxiety disorders. Briefly, in 2004-2007 participants aged 18 to 65 years were recruited from the community (19%), general practice (54%) and secondary mental health care (27%), reflecting therefore various settings and developmental stages of psychopathology in order to obtain a full and generalizable picture of the course of psychiatric disorders. A total of 2,981 participants were included, consisting of persons with a current or past depressive and/or anxiety disorder and healthy controls. The research protocol was approved by the ethical committee of participating universities, and all respondents provided written informed consent.

**The Northern Finland Birth Cohort 1966 (NFBC1996)**
The North Finland Birth Cohort of 1966 (NFBC1966, n=12,058 live born) was designed to study factors affecting preterm birth, low birth weight, and subsequent morbidity and mortality (http://kelo.oulu.fi/NFBC/). The longitudinal data collection includes clinical examination and blood sampling at age 31 years, from which data in the current study are drawn. The attendees in the follow-up (71% response rate) were adequately representative of the original cohort as is the final study sample in the present analyses. A total of 4,763 genotyped samples were available from the NFBC1966.

**Netherlands Twin Register cohort (NTR)**
Netherlands Twin Register (NTR) (5, 6) participants are ascertained based of the presence of twins or triplets in the family and consist of multiples, their parents, siblings and spouses. Twins are born in all strata of society and NTR represents a general sample from the Dutch population.

**The Australian twin-family study of alcohol use disorder (OZALC)**
This twin/family cohort was based on two groups of twins, born before 1964 and born 1964-71, enrolled in a voluntary Australia-wide twin registry. Twins, their spouses, and first-degree relatives were recruited for a study on alcohol dependence and related phenotypes (7). Alcohol intake in the week preceding blood collection was self-reported, and history of alcohol use and dependence was obtained through structured telephone interviews.

**The Prevention of REnal and Vascular ENd-stage Disease study (PREVEND)**
The PREVEND study is an ongoing prospective study investigating the natural course of increased levels of urinary albumin excretion and its relation to renal and cardiovascular disease. Inhabitants 28 to 75 years of age (n=85,421) in the city of Groningen, The Netherlands, were asked to complete a short questionnaire, 47% responded, and individuals were then selected with a urinary albumin concentration of at least 10 mg/L (n = 7,768) and a randomly selected control group with a urinary albumin concentration less than 10 mg/L (n = 3,395).

**The Study of Health in Pomerania (SHIP)**
The Study of Health in Pomerania (SHIP) is a population-based project in West Pomerania, the north-east area of Germany (8, 9). A sample from the population aged 20 to 79 years was drawn from population registries. First, the three cities of the region (with 17,076 to 65,977 inhabitants) and the 12 towns (with 1,516 to 3,044 inhabitants) were selected, and then 17 out of 97 smaller towns (with less than 1,500 inhabitants), were drawn at random. Second, from each of the selected communities, subjects were drawn at random, proportional to the population size of each community and stratified by age and gender. Only individuals with German citizenship and main residency in the study area were included. Finally, 7,008 subjects were sampled, with 292 persons of each gender in each of the twelve five-year age strata. In order to minimize drop-outs by migration or death, subjects were selected in two waves. The net sample (without migrated or deceased persons) comprised 6,267 eligible subjects. Selected persons received a maximum of three written invitations. In case of non-response, letters were followed by a phone call or by home visits if contact by phone was not possible. The SHIP population finally comprised 4,308 participants (corresponding to a final response of 68.8%). Alcohol

intake was assessed by questionnaire, including drink-specific quantity-frequency over 30 days (10).

**TwinsUK**
TwinsUK is based on a sample of 5,654 individuals from the UK. Among these, 3,471 have been genotyped and have data on alcohol intake assessed by self-reported questionnaire, and 1,204 represent one co-twin per family which have been genotyped and have data on alcohol intake assessed by self-reported questionnaire.

**The Cardiovascular Risk in Young Finns Study (YFS)**
The YFS is a population-based follow up-study started in 1980. The main aim of the YFS is to determine the contribution made by childhood lifestyle, biological and psychological measures to the risk of cardiovascular diseases in adulthood. In 1980, over 3,500 children and adolescents all around Finland participated in the baseline study. The follow-up studies have been conducted mainly with 3-year intervals. The 27-year follow-up study was conducted in 2007 (ages 30-45 years) with 2,204 participants. The study was approved by the local ethics committees (University Hospitals of Helsinki, Turku, Tampere, Kuopio and Oulu) and was conducted following the guidelines of the Declaration of Helsinki. All participants gave their written informed consent.

## 1.2 Population descriptions for GWAS discovery cohorts in the Heart and Aging Research in Genomic Epidemiology Plus (CHARGE+) Consortium

**Age, Gene/Environment Susceptibility–Reykjavik (AGES-Reykjavik)**
The AGES-Reykjavik Study (11) is a single center prospective cohort study based on the Reykjavik Study. The Reykjavik Study was initiated in 1967 by the Icelandic Heart Association to study cardiovascular disease and risk factors. The cohort included men and women born between 1907 and 1935 who lived in Reykjavik at the 1967 baseline examination. Re-examination of surviving members of the cohort was initiated in 2002 as part of the AGES-Reykjavik Study.

**The Atherosclerosis Risk in Communities Study** (**ARIC**)
The ARIC study (12) consists of a prospective cohort designed to identify the causes and outcomes of cardiovascular disease in 15,792 individuals from 4 communities (Forsyth County, NC; Jackson, MS; suburbs of Minneapolis, MN; and Washington County, MD). ARIC study participants underwent interviews, fasting venipuncture, and measurement of anthropometrics at the baseline and follow-up examinations. Trained interviewers ascertained basic demographic data, medical history, and information about personal diet habits. A full description of study design is available on the ARIC website (http://www2.cscc.unc.edu/aric/). In total, 4,106 individuals had both genotyping and alcohol phenotype. Alcohol consumption was ascertained by means of an interviewer-administered dietary questionnaire. Frequency of alcohol consumption was determined as usual weekly intake, with the amount of alcohol consumed in grams per week calculated assuming different serving sizes and alcohol content for beer, wine, and hard liquor. Serving sizes and alcohol content were defined as follows: ‘one beer' (12 oz. bottles or cans of beer, 13.2 g), 'one glass of wine' (4 oz. glass, 10.8 g), or ‘one shot of liquor or one mixed drink' (1.5 oz. shot

of hard liquor, 15.1 g). The total amount of absolute alcohol ingested weekly for past alcohol consumption was determined by multiplying the number of servings by the amount of alcohol in one serving of the type of alcohol ordinarily drunk. If more than one type was ordinarily drunk, the calculation was made assuming an equal number of drinks of each type. The total amount of absolute alcohol ingested weekly for present alcohol consumption resulted from the addition of absolute alcohol consumed for wine, beer, and hard liquor. The total amount of absolute alcohol drunk during the 24 hours prior to the clinic interview was determined by multiplying the number of drinks by the amount of absolute alcohol in the type of drink consumed. For a drinker who reported less than one drink per week, the alcohol consumption was recorded as zero grams per week. All questions were closed-ended and designed for direct data entry by a trained interviewer. In order to ensure standardization, exact wording and order of questions were followed. Questions were skipped only if specified in the questionnaire instructions.

**The Cardiovascular Health Study (CHS)**

The CHS is a population-based cohort study of risk factors for coronary heart disease and stroke in adults ≥65 years conducted across four field centers (13). The original predominantly European ancestry cohort of 5,201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists; subsequently, an additional predominantly African-American cohort of 687 persons was enrolled for a total sample of 5,888. The CHS GWAS, which had the primary aim of studying incident cardiovascular events, focused on 3,980 participants who were free of clinical cardiovascular disease at study baseline, consented to genetic testing, and had DNA available for genotyping. A total of 1,908 persons were excluded from the GWAS study sample due to the presence at study baseline of coronary heart disease, congestive heart failure, peripheral vascular disease, valvular heart disease, stroke, or transient ischemic attack. Because the other cohorts were predominantly of European descent, the African American participants were excluded from this analysis. In total, 3009 participants with both genotype and alcohol phenotype were included in the analyses.

At the baseline visit and annually, participants separately reported their usual frequency of consumption of beer, wine, and liquor, and the usual number of 12-ounce cans or bottles of beer, 6-ounce glasses of wine, and shots of liquor that they drank on each occasion. The full text of the CHS nutritional questionnaire is publicly available (http://www.chs-nhlbi.org/forms/r25p3.htm). At baseline, participants also reported whether they changed their pattern of consumption during the past 5 years and whether they ever regularly consumed 5 or more drinks daily.

**The Framingham Heart Study (FHS)**

The FHS sample includes the Framingham Heart Study Offspring (14) and the third generation (15) cohorts. In 1971, children and spouses of children of the original FHS cohort participants were recruited into the Framingham offspring cohort, which consists of 5,124 men and women. The FHS offspring participants have been examined every four to eight years unless specified otherwise, common clinical phenotypes from all examinations were available for this investigation. From 2002 to 2005, a third generation cohort of 4,095 individuals was recruited to the FHS. The third generation cohort (n=4,095) includes children and spouses of children of the Offspring cohort. In total, 8,955 individuals had both genotyping and alcohol phenotype.

Alcohol consumption was assessed via questionnaire at the study examination closest to the time point of DNA collection.

**The Health, Aging, and Body Composition (HABC)**
The Health ABC study (16) is a prospective cohort study investigating the associations between body composition, weight-related health conditions, and incident functional limitation in older adults. Health ABC enrolled well-functioning, community-dwelling black (n=1281) and white (n=1794) men and women aged 70-79 years between April 1997 and June 1998. Participants were recruited from a random sample of white and all black Medicare eligible residents in the Pittsburgh, PA, and Memphis, TN, metropolitan areas. Participants have undergone annual exams and semi-annual phone interviews. The current study sample consists of 1559 white participants who attended the second exam in 1998-1999 with available genotyping data.
Alcohol consumption at baseline was assessed by asking the participant how many alcoholic drinks he/she consumed in a typical week, during the past 12 months. Furthermore, it was asked whether a person ever drank more than what he/she typically drank in the past 12 months.

**The Multi-Ethnic Study of Atherosclerosis (MESA**)
The MESA is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease (17). MESA researchers study a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84. Thirty-eight percent of the recruited participants are White, 28 percent African-American, 22 percent Hispanic, and 12 percent Asian, predominantly of Chinese descent. Participants were recruited from six field centers across the United States: Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University and University of California, Los Angeles. The current analysis was limited to n=1596 White participants with data available on alcohol consumption through the Food-frequency questionnaire. Data on alcoholic beverage consumption (drinks/day) were obtained on 2,382 Caucasian individuals with genotypes available through MESA SHARe. As a part of a 120-item food frequency questionnaire, participants were asked the frequency they consumed each beer, wine, and liquor or mixed drinks (9 frequency options ranging from rarely/never to six or more drinks/day) (18, 19). Responses to these three line items were summed to estimate total alcoholic drinks consumed each day.

**The Rotterdam Study (RS)**
The RS (20) is a prospective, population-based study from the well-defined district of Ommoord within the city of Rotterdam, designed to investigate the occurrence and determinants of diseases in the elderly. The cohort was initially defined in 1990 among 7 983 persons who underwent a home interview and extensive physical examination at baseline and during follow-up examinations occurring every 3-4 years (RS-I). The cohort was further extended in 2000 (RS-II) and 2005 (RS-III), establishing a total of 14926 participants.

**The Women's Genome Health Study (WGHS)**

The WGHS (21) is a prospective cohort of initially healthy, female North American health care professionals at least 45 years old at baseline in 1992-1994, representing participants in the Women's Health Study (WHS) who provided a blood sample at baseline and consent for blood-based analyses. These WHS was 2x2 randomized, placebo controled trial of aspirin and vitamin E in prevention of cardiovascular disease and cancer over 10 years. Since the end of the trial, follow-up in the WHS/WGHS has continued in observational mode.

### 1.3 Population descriptions for replication cohorts

**Airwave Health Monitoring Study (Airwave)**
The Airwave Health Monitoring Study (22) was established to evaluate possible health risks associated with use of TETRA, a digital communication system used by police forces and other emergency services in Great Britain since 2001. The study has been broadened to investigate more generally the health of the work force. From 2004, participants from each force who agreed to participate were enrolled either with an enrolment questionnaire or a comprehensive health screening performed locally. This includes questionnaire, 7-day food diaries, anthropometry, measurements of cardiovascular and cognitive function, blood chemistry, coagulation and hematology. By March 2015, the study had recruited 53,606 participants, of whom 45,433 had attended the health screening. 12,930 participants with genotype data were included in this analysis.

**The Austrian Stroke Prevention Study (ASPS)**
The ASPS study is a single center prospective follow-up study on the effects of vascular risk factors on brain structure and function in the normal elderly population of the city of Graz, Austria. The procedure of recruitment and diagnostic work-up of study participants has been described previously (23, 24). A total of 2,007 participants were randomly selected from the official community register stratified by gender and 5 year age groups. Individuals were excluded from the study if they had a history of neuropsychiatric disease, including previous stroke, transient ischemic attacks, and dementia, or an abnormal neurologic examination determined on the basis of a structured clinical interview and a physical and neurologic examination. During 2 study periods between September 1991 and March 1994 and between January 1999 and December 2003 an extended diagnostic work-up including neuropsychological testing was done in 1,076 individuals aged 45 to 85 years randomly selected from the entire cohort: 509 from the first period and 567 from the second. In 1992, blood was drawn from all study participants for DNA extraction. They were all European Caucasians. Genotyping was performed in 996 participants, and those 829 who passed genotyping quality control and have data on alcohol intake were available for these analyses.

**The British 1958 birth cohort (B58C)**
The British 1958 birth cohort (25) is a follow-up study of persons born throughout England, Scotland and Wales one week in March 1958. Alcohol consumption was self-reported at a biomedical examination at age 44-45 years, at which blood sampling was performed with consent for DNA extraction and creation of immortalized cell lines. Genotyping of three non-overlapping subsets of the cohort

was performed by the Wellcome Trust Case-Control Consortium, the Type 1 Diabetes Genetics Consortium and the GABRIEL Asthma Genetics Consortium. The three subsets were combined for imputation using the 1000-genomes phase 1 reference panel, and for subsequent statistical analysis.

**Data from an Epidemiological Study on the Insulin Resistance syndrome (DESIR)**
General population from ten French Social Security Health Examination Centers.

**The Finnish Twin Cohort replication sample (FinnTwin replication)**
Sample used for replication consists of subjects from the Older Finnish Twin Cohort and the Younger Finnish Twin Cohorts (non-overlapping with the discovery sample). Please see cohort descriptions of the discovery sample.

**Genetic Regulation of Arterial Pressure of Humans in the Community Study (GRAPHIC)**
The GRAPHIC Study comprises 2024 individuals from 520 nuclear families recruited from the general population in Leicestershire, UK between 2003-2005 for the purpose of investigating the genetic determinants of blood pressure and related cardiovascular traits. Families were included if both parents aged 40-60 years and two offspring ≥18 years wished to participate. A detailed medical and lifestyle history including alcohol intake was obtained from study subjects by standardized questionnaires and clinical examination was performed by research nurses following standard procedures.

**Generation Scotland: Scottish Family Health Study (GS:SFHS)**
GS:SFHS (26) consists of 23,960 individuals recruited at random from general medical practices across Scotland, 21,516 of these attended the research clinic. Eligibility criteria specified that participants were over 18 years of age and had one first-degree relative also willing to participate. Genome-wide SNP data were ascertained for 10,000 individuals, and after quality control, genotype data were available for 9,863 participants, which are the participants used in this study. 7,281 of these individuals self-reported as currently consuming alcohol. Alcohol consumption was assessed using a pre-clinical questionnaire. Participants were identified as current drinkers, former drinkers or never drinkers. Consumption was measured in self-reported units of alcohol consumed in the previous week. The cohort has been described in further detail elsewhere (26).

**The INGI - Carlantino study (INGI_CARL)**
This cohort comprises the samples coming from a small village from the southern region of Italy Puglia. For all samples a wide range of information are available including alcohol intake and anthropometric measurements. Moreover for all samples a DNA sample was acquired and was used for genotyping with high density SNP arrays.

**The INGI - Friuli Venezia Giulia study (INGI_FVG)**
This cohort comprises the samples coming from a 6 small villages from the northern region of Italy Friuli Venezia Giulia. For all samples a wide range of information are available including alcohol intake and anthropometric measurements. Moreover for all samples a DNA sample was acquired and was used for genotyping with high

density SNP arrays.

**The INGI-Val Borbera study (INGI_VB)**
The INGI-Val Borbera population is a collection of 1,785 genotyped samples collected in the Val Borbera Valley, a geographically isolated valley located within the Appenine Mountains in Northwest Italy.

**The Lothian Birth Cohort 1921 (LBC1921)**
LBC1921 consists of 550 (234 male) relatively healthy individuals, assessed on cognitive and medical traits at a mean age of 79.1 years (SD = 0.6). They were born in 1921, most took part in the Scottish Mental Survey of 1932, and almost all lived independently in the Lothian region (Edinburgh City and surrounding area) of Scotland. Data on alcohol intake is available.

**The Lothian Birth Cohort 1936 (LBC1936)**
LBC1936 consists of 1091 (548 male) relatively healthy individuals who underwent cognitive and medical testing at a mean age of 69.6 years (SD = 0.8). They were born in 1936, most took part in the Scottish Mental Survey of 1947, and almost all lived independently in the Lothian region of Scotland. Data on alcohol intake is available.

**The Northern Swedish Population Health Study (NSPHS)**
The NSPHS was initiated in 2006 to provide a health survey of the population in the parish of Karesuando, county of Norrbotten, Sweden, and to study the medical consequences of lifestyle and genetics. This parish has about 1,500 inhabitants who meet the eligibility criteria in terms of age (≥15 years), of which 1066 individuals participated in the study.

**The Orkney Complex Disease Study (ORCADES)**
The Orkney Complex Disease Study (ORCADES) is a family-based study of 2078 individuals with ancestry from the isolated Scottish archipelago of Orkney. Fasting blood samples were collected and over 300 health-related phenotypes and environmental exposures were measured in each individual. All participants gave informed consent and the study was approved by Research Ethics Committees in Orkney and Aberdeen.

**The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)**
All data come from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). A detailed description of the study has been published elsewhere. PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. A total number of 5,804 subjects were randomly assigned to pravastatin or placebo. A large number of prospective tests were performed including Biobank tests and cognitive function measurements.

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| LOLIPOP | The LOLIPOP study was supported by the National Institute for Health Research (NIHR) Comprehensive Biomedical Research Centre Imperial College Healthcare NHS Trust. The work was carried out in part at the NIHR/Wellcome Trust Imperial Clinical Research Facility. We thank the participants and research staff who made the study possible. The LOLIPOP study was funded by the British Heart Foundation (SP/04/002), the Medical Research Council (G0601966, G0700931), the Wellcome Trust (084723/Z/08/Z), the NIHR (RP-PG-0407-10371), European Union FP7 (EpiMigrant, 279143) and Action on Hearing Loss (G51). |
| NESDA | Studies were supported by Netherlands Organization for Scientific Research (Geestkracht program grant 10-000-1002); the Center for Medical Systems Biology (CSMB, NWO Genomics); Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL); VU University's Institutes for Health and Care Research (EMGO+) and Neuroscience Campus Amsterdam, University Medical Center Groningen, Leiden University Medical Center, National Institutes of Health (NIH, R01D0042157-01A, MH081802, Grand Opportunity grants 1RC2 MH089951 and 1RC2 MH089995). Genotyping and analyses were partly funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health. Computing was supported by BiG Grid, the Dutch e-Science Grid, which was supported by NWO. |
| NFBC1966 | We thank the late Professor Paula Rantakallio for launching NFBCs), and Ms. Outi Tornwall and Ms. Minttu Jussila for DNA biobanking. The authors also acknowledge the contribution of the late Academian of Science Leena Peltonen. NFBC1966 received financial support from the Academy of Finland (grants 104781, 120315, 129269, 1114194, 24300796, Center of Excellence in Complex Disease Genetics and SALVE), University Hospital Oulu, Biocenter, University of Oulu, Finland (75617), NHLBI grant 5R01HL087679-02 through the STAMPEED program (1RL1MH083268-01), NIH/NIMH (5R01MH63706:02), ENGAGE project and grant agreement HEALTH-F4-2007-201413, EU FP7 EurHEALTHAgeing-277849, the Medical Research Council, UK (G0500539, G0600705, G1002319, PrevMetSyn/SALVE) and the MRC, Centenary Early Career Award. The program is funded by the H2020-633595 DynaHEALTH action and academy of Finland EGEA-project (285547). The DNA extractions, sample quality controls, biobank up-keeping and aliquotting was performed in the National Public Health Institute, Biomedicum Helsinki, Finland and supported financially by the Academy of Finland and Biocentrum Helsinki. |
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| | Council ERC-230374 and ERC-284167; Centre for Medical Systems Biology (NWO Genomics); Netherlands Bioinformatics Center/BioAssist/RK/2008.024; National Institute of Mental Health (NIH) MH081802, 1RC2 MH089951, 1RC2 MH089995; National Institute of Mental Health, Grant number RC2 MH089951. |
| OZALC | We acknowledge the contribution of the participants in this study, and the work of the interviewers and laboratory staff at QIMR Berghofer Medical Research Institute in obtaining the phenotype information and genotypes. We also acknowledge the contributions of Dr. Grant Montgomery and Ms Anjali Henders to project management, sample acquisition and genotyping. Aspects of the data collection and genotyping were made possible by grants to Dr. Pamela AF Madden and to the late Dr. Richard Todd. The study was supported by National Institutes of Health grants AA07535,AA07728, AA13320, AA13321, AA14041, AA11998, AA17688,DA012854, and DA019951; by grants from the Australian National Health and Medical Research Council (241944, 339462, 389927,389875, 389891, 389892, 389938, 442915, 442981, 496739, 552485, and 552498) and the Australian Research Council(A7960034, A79906588, A79801419, DP0770096, DP0212016, and DP0343921); and by the 5th Framework Programme (FP-5) GenomEUtwin Project (QLG2-CT-2002-01254). Genome-wide genotyping at the Center for Inherited Disease Research was supported by a grant to the late Richard Todd, M.D., Ph.D. GWM was supported by the National Health and Medical Research Council Fellowship Scheme. |
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| SHIP | SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which was funded by the Federal Ministry of Education and Research (grants 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network 'Greifswald Approach to Individualized Medicine (GANI_MED)' funded by the Federal Ministry of Education and Research (grant 03IS2061A). Genome-wide data have been supported by the Federal Ministry of Education and Research (grant 03ZIK012) and a joint grant from Siemens Healthcare, Erlangen, Germany and the Federal State of Mecklenburg- West Pomerania. The University of Greifswald is a member of the Caché Campus program of the InterSystems GmbH. |

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| TwinsUK | We are grateful to all the twins who took part in this study, the midwives for recruiting them and the whole TwinsUK team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. SNP Genotyping was performed by The Wellcome Trust Sanger Institute and National Eye Institute via NIH/CIDR. The study was funded by the Wellcome Trust; European Community's Seventh Framework Programme (FP7/2007-2013). The study also received support from the National Institute for Health Research (NIHR)-funded BioResource, Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London. |
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| AGES | The researchers are indebted to the participants for their willingness to participate in the study. This study was funded by NIH contracts N01-AG-1-2100 and 271201200022C, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament). The study was approved by the Icelandic National Bioethics Committee, VSN: 00-063. |
| ARIC | The Atherosclerosis Risk in Communities Study was carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. |
| CHS | Infrastructure for the CHARGE Consortium was supported in part by the National Heart, Lung, and Blood Institute grant R01HL105756. This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, and R01HL120393 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National |

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| | Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. |
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| | supports the databank of the ASPS. |
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| GRAPHIC | We thank all the participants in the study for their contribution and support. The GRAPHIC Study was funded by the British Heart Foundation (BHF). CPN and NJS were both funded by the BHF and NJS is a NIHR Senior Investigator. |
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| INGI_CARL | We thank all the participants in the study for their contribution and support. The INGI-FVG study was funded through the Italian Ministry of Health |
| INGI_FVG | We thank all the participants in the study for their contribution and support. The INGI-CARL study was funded through the Italian Ministry of Health. |
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| LBC1921 | We thank the cohort participants and team members who contributed to these studies. Phenotype collection in the LBC1921 was supported by the BBSRC, The Royal Society, and The Chief Scientist Office of the Scottish Government. Genotyping was funded by the BBSRC. The work was undertaken by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (MR/K026992/1). Funding from the BBSRC and MRC is gratefully acknowledged. |
| LBC1936 | We thank the cohort participants and team members who contributed to these studies. Phenotype collection in the LBC1936 was supported by Age UK (The Disconnected Mind project). Genotyping of the cohorts was funded by the BBSRC. The work was undertaken by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross |

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| PROSPER | The PROSPER study was supported by an investigator initiated grant obtained from Bristol-Myers Squibb. Prof. Dr. J. W. Jukema is an Established Clinical Investigator of the Netherlands Heart Foundation (grant 2001 D 032). Support for genotyping was provided by the seventh framework program of the European commission (grant 223004) and by the Netherlands Genomics Initiative (Netherlands Consortium for Healthy Aging grant 050-060-810). |

**Table S1. Sex-specific associations of SNPs taken forward for replication from discovery GWAS.**

| | | | | | | | | Men | | | Women | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Alcohol phenotype** | **SNP** | **Gene** | **Chr** | **Position** | **Effect allele** | **Other allele** | **EAF** | **Effect*** | **SE** | ***P*-value** | **Effect** | **SE** | ***P*-value** |
| log g/day | rs780094 | *GCKR* | 2 | 27594741 | T | C | 0.403 | -0.015 | 0.004 | $4.0 \times 10^{-4}$ | -0.015 | 0.003 | $9.6 \times 10^{-6}$ |
| log g/day | rs197273 | *TANK* | 2 | 161602909 | A | G | 0.487 | -0.018 | 0.004 | $4.8 \times 10^{-5}$ | -0.010 | 0.003 | $2.1 \times 10^{-3}$ |
| log g/day | rs10950202 | *AUTS2* | 7 | 69568034 | C | G | 0.842 | 0.020 | 0.006 | $5.7 \times 10^{-4}$ | 0.018 | 0.005 | $5.1 \times 10^{-4}$ |
| log g/day | rs11940694 | *KLB* | 4 | 39091388 | A | G | 0.422 | -0.017 | 0.004 | $8.7 \times 10^{-5}$ | -0.011 | 0.003 | $9.8 \times 10^{-4}$ |
| log g/day | rs350721 | *ASB3* | 2 | 52833931 | C | G | 0.175 | 0.024 | 0.007 | $4.0 \times 10^{-4}$ | 0.021 | 0.005 | $2.8 \times 10^{-5}$ |
| log g/day | rs6943555 | *AUTS2* | 7 | 69806023 | A | T | 0.289 | -0.012 | 0.005 | $9.9 \times 10^{-3}$ | -0.012 | 0.004 | $3.3 \times 10^{-3}$ |
| dichotomous | rs12599112 | *CDH13* | 16 | 81276212 | A | C | 0.957 | 0.053 | 0.091 | 0.560 | -0.063 | 0.015 | $2.4 \times 10^{-5}$ |
| dichotomous | rs10927848 | *TMEM82* | 1 | 15948493 | A | G | 0.321 | 0.027 | 0.036 | 0.450 | -0.023 | 0.008 | $2.5 \times 10^{-3}$ |

* Effect refers to beta coefficient from linear regression for log g/day alcohol phenotype, and log(OR) from logistic regression for dichotomous alcohol phenotype.

**Table S2. Dichotomous trait replication results**

| SNP | Chr | Position (hg19) | Gene* | Discovery *P* | Replication *P* | Overall *P* | Overall N |
|---|---|---|---|---|---|---|---|
| rs12599112 | 16 | 82718711 | *CDH13* | $2.3 \times 10^{-8}$ | 0.895 | $5.0 \times 10^{-8}$ | 86,213 |
| rs10927848 | 1 | 16075906 | *TMEM82* | $2.6 \times 10^{-7}$ | 0.291 | $1.9 \times 10^{-7}$ | 103,219 |

Cohorts - Airwave, ASPS, B58C, FinnTwin_replication, GRAPHIC, GS:SFHS, INGI_CARL, INGI_FVG, INGI_VB, LBC1921, LBC1936, PROSPER
The most significant SNP per locus is displayed in the tables.

* Loci are named according to the closest gene based on the position of the most significant SNP.

**Table S3. Allele frequencies of the *KLB* SNP rs11940694 in different ethnic groups**

| Ethnicity | Sample (2N) | Major Allele Frequency | Minor Allele frequency |
|---|---|---|---|
| Ad-mixture American | 694 | A=0.565 | G=0.435 |
| African | 1322 | G=0.570 | A=0.430 |
| East Asian | 1008 | A=0.541 | G=0.459 |
| South Asian | 978 | A=0.630 | G=0.370 |
| European | 1006 | G=0.612 | A=0.388 |

**Table S4. Gene expression in peripheral blood in the Framingham Heart Study**

***(A) Demographics for gene expression analysis in Framingham Heart study***

| **Phenotypes/Covariates** | **Offspring Cohort (examination cycle 8: 2005-2008)** | **Third Generation Cohort (examination cycle 2: 2008-2011)** |
|---|---|---|
| *Gene Expression Analysis* | n=2,222 | n=3,014 |
| Female (%) | 1,221 (54.95) | 1,603 (53.10) |
| Age (yrs), mean (SD) | 66.41 (8.95) | 46.88 (8.79) |
| BMI (kg/m2), mean (SD) | 28.04 (5.87) | 28.31 (5.5.30) |

BMI: Body mass index

***(B) Association of KLB SNP rs11940694 with gene expression***

| | **chr** | **position** | **Effect allele** | **Beta** | ***P*-value** |
|---|---|---|---|---|---|
| rs11940694 | 4 | 39414993 | A | 0.00409 | 0.165 |

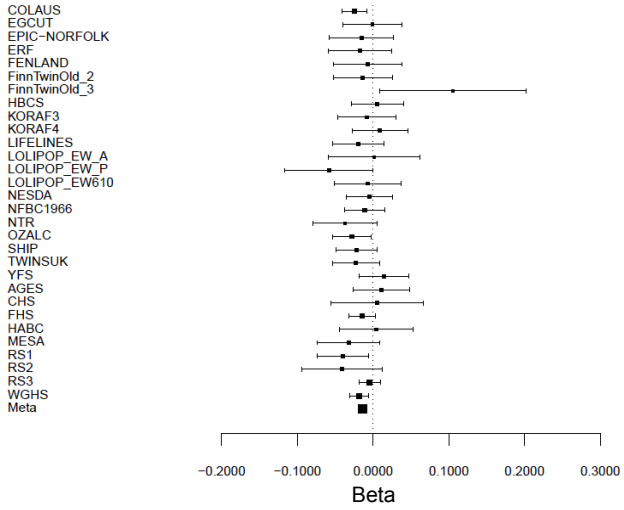
**Supplementary Figure legends**

**Figure S1**. **Forest plot for the association of rs11940694 in *KLB* with log g/day alcohol in the discovery GWAS and replication cohorts.** (A) rs11940694 in *KLB* in discovery GWAS cohorts. Discovery GWAS cohorts - AlcGen: Colaus, EGCUT, EPIC-Norfolk, ERF, Fenland, FinnTwinOld_2, HBCS, KORA F3 and F4, Lifelines, LOLIPOP (EW A, EW P, EW610), FinnTwinOld_3, NESDA, NFBC1966, NTR, OZALC, SHIP, TwinsUK, YFS; CHARGE+: AGES, CHS, FHS, HABC, MESA, RS1, RS2, RS2, and WGHS. In rs11940694, the coded allele was A, the non-coded allele was G. The allele frequency for A was ~ 0.42 in the entire sample. The beta/SE estimates were for A allele. (B) rs11940694 in *KLB* in discovery + replication cohorts. The coded allele was A, the non-coded allele was G. The beta/SE estimates were for A allele.

**Figure S2. Genome-wide association results of dichotomous alcohol in AlcGen and CHARGE+ consortia.** (A) Manhattan plot showing the significance of the association ($-\log_{10}$ transformed *P* value on the y axis) for each SNP at chromosomal position shown on the x axis. The dotted line represents the genome-wide significance level at $P = 5 \times 10^{-8}$. The genes that were followed up are labelled. (B) Quantile-quantile plot comparing the expected *P* value on the x axis and the observed *P* value on the y axis (both were –log10 transformed).

**Figure S3. Illustration of common SNPs (minor allele frequency > 0.01) and linkage disequilibrium (LD) structure in the genomic regions around the *KLB* gene.** The target SNP rs11940694 is highlighted with black background and pointed to by a red arrow in the LD structure plot. LD is measured by r-square and the darker the red color, the higher the r-square value.

A


COLAUS
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ERF
FENLAND
FinnTwinOld_2
FinnTwinOld_3
HBCS
KORAF3
KORAF4
LIFELINES
LOLIPOP_EW_A
LOLIPOP_EW_P
LOLIPOP_EW610
NESDA
NFBC1966
NTR
OZALC
SHIP
TWINSUK
YFS
AGES
CHS
FHS
HABC
MESA
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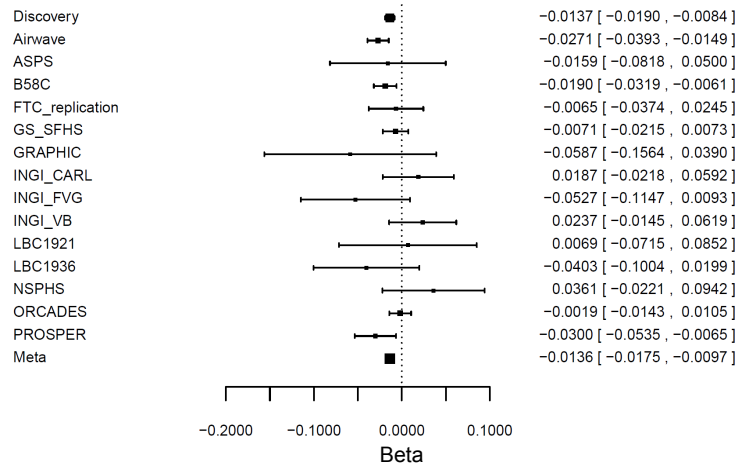
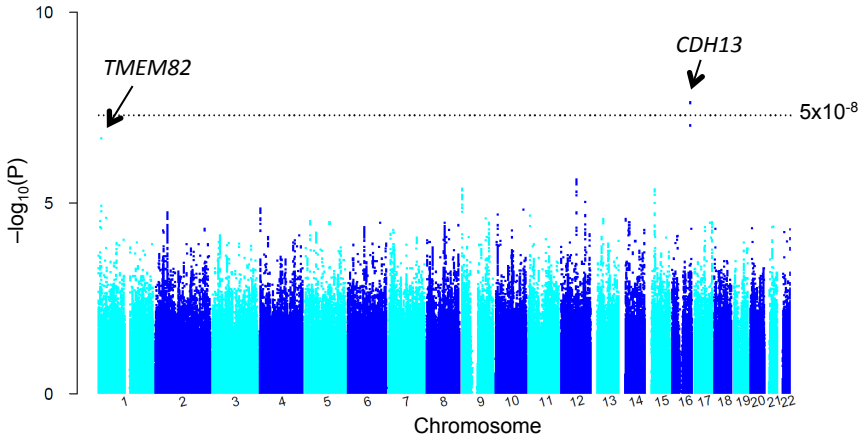
Beta

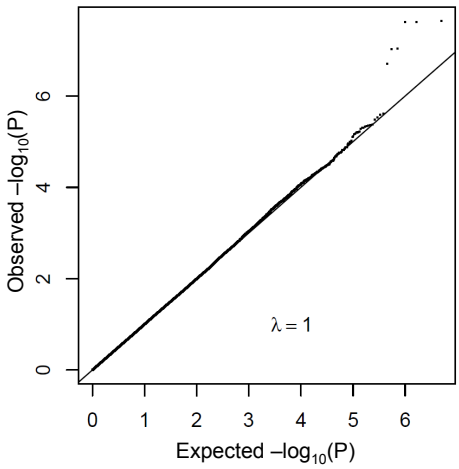
B

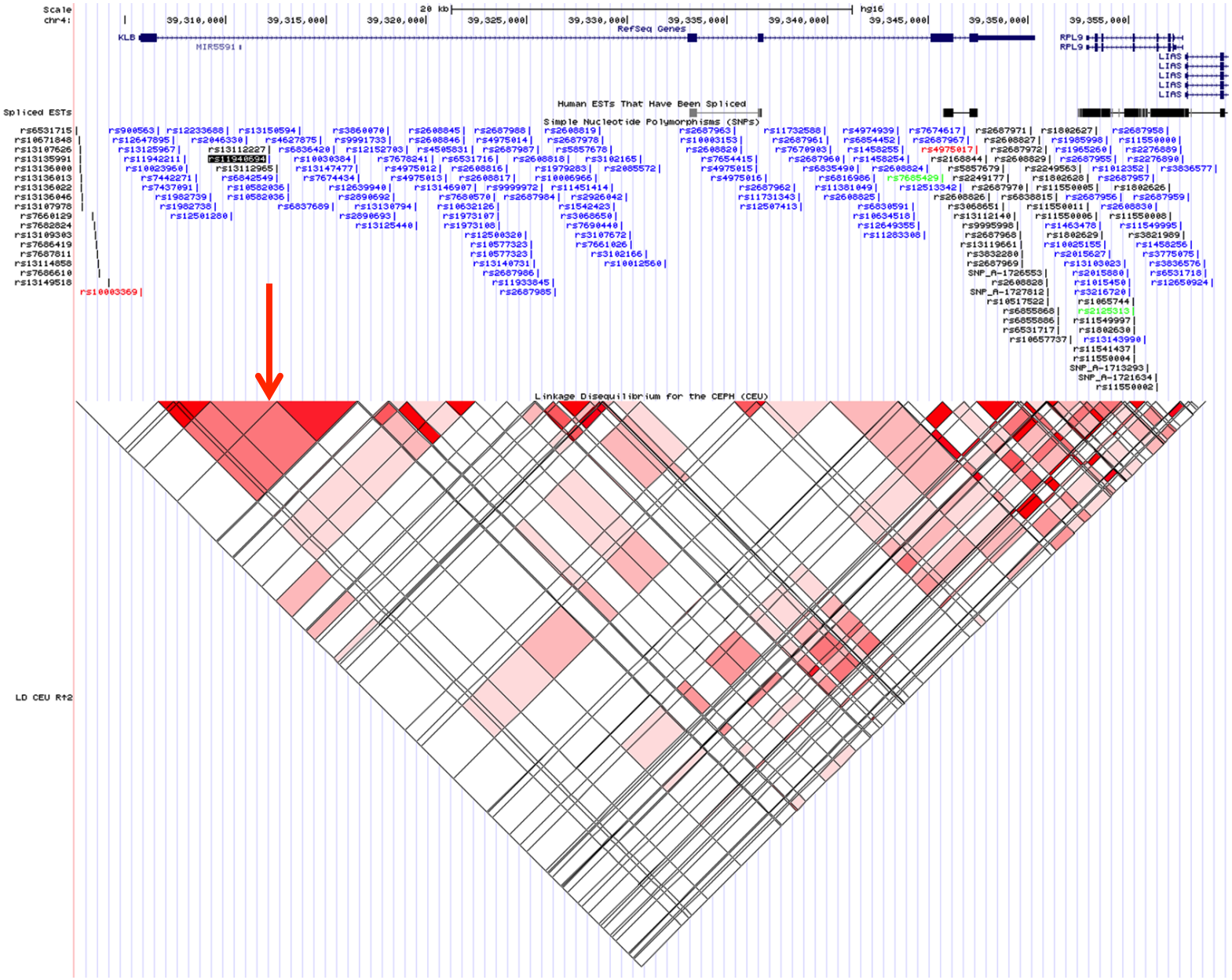

Discovery −0.0137 [ −0.0190 , −0.0084 ]
Airwave −0.0271 [ −0.0393 , −0.0149 ]
ASPS −0.0159 [ −0.0818 , 0.0500 ]
B58C −0.0190 [ −0.0319 , −0.0061 ]
FTC_replication −0.0065 [ −0.0374 , 0.0245 ]
GS_SFHS −0.0071 [ −0.0215 , 0.0073 ]
GRAPHIC −0.0587 [ −0.1564 , 0.0390 ]
INGI_CARL 0.0187 [ −0.0218 , 0.0592 ]
INGI_FVG −0.0527 [ −0.1147 , 0.0093 ]
INGI_VB 0.0237 [ −0.0145 , 0.0619 ]
LBC1921 0.0069 [ −0.0715 , 0.0852 ]
LBC1936 −0.0403 [ −0.1004 , 0.0199 ]
NSPHS 0.0361 [ −0.0221 , 0.0942 ]
ORCADES −0.0019 [ −0.0143 , 0.0105 ]
PROSPER −0.0300 [ −0.0535 , −0.0065 ]
Meta −0.0136 [ −0.0175 , −0.0097 ]
−0.2000
−0.1000
0.0000
0.1000
Beta

A


TMEM82
CDH13
5x10-8
–log10(P)
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Chromosome

B


Observed –log10(P)
Expected –log10(P)
λ = 1
0 1 2 3 4 5 6


Scale
chr4:
20 kb
hg16
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39,315,000
39,320,000
39,325,000
39,330,000
39,335,000
39,340,000
39,345,000
39,350,000
39,355,000
RefSeq Genes
KLB
MIR5591
RPL9
RPL9
LIAS
LIAS
LIAS
LIAS
LIAS
Spliced ESTs
Human ESTs That Have Been Spliced
Simple Nucleotide Polymorphisms (SNPs)
rs6531715
rs10671848
rs13107626
rs13135991
rs13136000
rs13136013
rs13136022
rs13136046
rs13107978
rs7660129
rs7682824
rs13109303
rs7686419
rs7687811
rs13114858
rs7686610
rs13149518
rs10003369
rs900563
rs12233688
rs13150594
rs3860070
rs2608845
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rs2608819
rs2687963
rs11732588
rs4974939
rs7674617
rs2687971
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rs12647895
rs2046330
rs4627875
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rs2608817
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rs4975016
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rs6830591
rs3068651
rs11550011
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rs3068650
rs10634518
rs13112140
rs11550006
rs11550008
rs13125440
rs1973108
rs7690440
rs12649355
rs9995998
rs1463478
rs11549995
rs12500320
rs3107672
rs11283308
rs2687968
rs1802629
rs3821989
rs10577323
rs7661026
rs13119661
rs10025155
rs1458256
rs10577323
rs3102166
rs3832280
rs2015627
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rs13140731
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rs2687969
rs13103023
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rs2687986
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Linkage Disequilibrium for the CEPH (CEU)
LD CEU Rt2