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

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Eumil Partinen53, Rajesh Rawal54, Antonietta Robinetti55, Linda Roselli56, Cinzia Sala57,58,59, Takashi Satoh60, Reinhold Schmid61,62, Jaspal S. Kooner63,64,65,66,67, Zoltan Kutalik68,69, Jarri Lahis57, Christopher M. Munroe70,71, Daniel Levy4,7,72,1, Peter K. Joshi73,74,75,76, Jennifer Nettleton67,68,69, Jennifer Nettleton77,78,79, Steven A. Kliewer80,81,82, David J. Mangelsdorff2,8,9,10, Christian P. Munn95,96,97, Daniel Levy4,1,1,1,1, Paul Elliott1,1,1,1

Contributed by David J. Mangelsdorf, October 18, 2016 (sent for review July 11, 2016; reviewed by Robert Adron Harris and Victor Hesselbrock)

Excessive alcohol consumption is a major public health problem worldwide. Although 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 metaanalysis and replication study among >105,000 individuals of European ancestry and identified β-Klotho (KLB) as a locus associated with alcohol consumption (rs11940694; P = 9.2 × 10−15). β-Klotho is an obligate coreceptor for the hormone FGF21, which is secreted from the liver and implicated in macronutrient preference in humans. We show that brain-specific β-Klotho KO 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.

## Excessive alcohol consumption

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 consumed 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, and therefore, large sample sizes are required to detect the genetic signal (7).


Conflict of interest statement: B. Psaty serves on the Data and Safety Monitoring Board for a clinical trial funded by the manufacturer (Zoll LifeCor) and the Steering Committee of the Yale Open Data Access project funded by Johnson & Johnson. D.I.M. serves on the scientific advisory board of Metacrine. The other authors report no competing financial interests.

Data deposition: The database information reported in this paper has been supplied in Datasets S1 and S2. The gene expression profiling data from the Framingham study used herein are available online in dbGaP (www.ncbi.nlm.nih.gov/gap; accession no. phs000007). The full meta-analysis data for the continuous and dichotomous alcohol consumption traits are available online at the dbGaP CHARGE Summary site (accession no. phs000093).
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 metaanalysis and replication study to date (>105,000 individuals) and identified a genetic basis for alcohol consumption during nonaddictive drinking. We found that a locus in the gene encoding β-Klotho is associated with alcohol consumption. β-Klotho is an essential receptor component for the endocrine FGFs, FGF19 and FGF21. Using mouse models and pharmacologic administration of FGF21, we show 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.

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. We identified 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 findings suggest that the FGF21-β-Klotho signaling pathway regulates alcohol consumption in humans.

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 KO (KlbCamk2a−/−) mice and control floxed Klb (Klb+/−) mice. We used a voluntary two-bottle drinking assay performed with water and alcohol. Because we previously showed that FGF21-transgenic mice, which express FGF21 at pharmacologic levels, reduce alcohol preference (14), we performed these studies while administering either recombinant FGF21 or vehicle by osmotic minipump. Alcohol preference vs. water was significantly increased in vehicle-treated KlbCamk2a−/− compared with Klb+/− mice at 16 vol % alcohol (Fig. 2A). FGF21 suppressed alcohol preference in Klb+/− mice but not in KlbCamk2a−/− mice, showing 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+/− and KlbCamk2a−/− mice administered recombinant FGF21 at the end of the experiment (Fig. 2C). Alcohol bioavailability was not different between FGF21-treated Klb+/− and KlbCamk2a−/− mice (Fig. 2D). We have previously shown that FGF21 decreases the sucrose and saccharin preference ratio in Klb+/− but not KlbCamk2a−/− 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+/− and KlbCamk2a−/− mice without osmotic minipump implantation. Again, KlbCamk2a−/− mice showed significantly greater alcohol consumption and increased alcohol preference compared with Klb+/− mice (Fig. 2 E and F), thus replicating our findings above. Alcohol bioavailability after an i.p. injection was not different between Klb+/− and KlbCamk2a−/− mice after 1 and 3 h (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, regulating glucocorticoid concentrations, and sympathetic outflow (18–20), which are linked to heightened anxiety. We, therefore, tested Klb+/− and KlbCamk2a−/− 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+/− and KlbCamk2a−/− mice in any of these anxiety measures or general locomotor activity. Our finding of increased alcohol preference in KlbCamk2a−/− mice may thus be caused by alteration of alcohol-associated reward mechanisms. Although 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 nonaddicts. 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 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. Although 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, because 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.

**Table 1. Associations of SNPs with alcohol intake (log grams per day) in the GWAS analysis**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Position (hg 19)</th>
<th>Nearest gene</th>
<th>Effect/other alleles</th>
<th>EAF</th>
<th>Discovery GWAS</th>
<th>Replication</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs780094</td>
<td>2</td>
<td>27,741,237</td>
<td>GCKR</td>
<td>T/C</td>
<td>0.40</td>
<td>-0.0155 (0.0026)</td>
<td>3.6 x 10^-3</td>
<td>0.0035 (0.0029)</td>
</tr>
<tr>
<td>rs350721</td>
<td>2</td>
<td>52,980,427</td>
<td>ASB3</td>
<td>C/G</td>
<td>0.18</td>
<td>0.0206 (0.0040)</td>
<td>3.2 x 10^-7</td>
<td>-0.0000 (0.0042)</td>
</tr>
<tr>
<td>rs197273</td>
<td>2</td>
<td>161,894,663</td>
<td>TANK</td>
<td>A/G</td>
<td>0.49</td>
<td>-0.0141 (0.0026)</td>
<td>9.8 x 10^-8</td>
<td>-0.0058 (0.0028)</td>
</tr>
<tr>
<td>rs11940694</td>
<td>4</td>
<td>39,414,993</td>
<td>KLB</td>
<td>A/G</td>
<td>0.42</td>
<td>-0.0137 (0.0027)</td>
<td>3.2 x 10^-7</td>
<td>-0.0135 (0.0030)</td>
</tr>
<tr>
<td>rs6943555</td>
<td>7</td>
<td>698,060,23</td>
<td>AUTS2</td>
<td>A/T</td>
<td>0.29</td>
<td>-0.0115 (0.0030)</td>
<td>1.4 x 10^-3</td>
<td>-0.0070 (0.0033)</td>
</tr>
<tr>
<td>rs10950202</td>
<td>7</td>
<td>69,930,098</td>
<td>AUTS2</td>
<td>G/C</td>
<td>0.16</td>
<td>-0.0194 (0.0038)</td>
<td>2.9 x 10^-7</td>
<td>-0.0015 (0.0042)</td>
</tr>
</tbody>
</table>

One SNP with the smallest P value was taken forward per region. Chr, chromosome; EAF, effect allele frequency in the discovery GWAS.
by seven 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 seven
directly into grams per day. Cohorts with only a categorical response to the
question for drinks per week used midpoints of each category for the cal-
culation. All nondrinkers (individuals reporting zero drinks per week) were
removed from the analysis. The grams per day variable was then log_10
transformed before the analysis. Sex-specific residuals were derived by
subtracting sex-adjusted mean consumption from individual consumption.
These residuals were then transformed to normality by seven to give intake as grams per day. 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 the Alcohol Genome-Wide Association (AlcGen) and Cohorts for Heart and Aging Research in Genomic Epidemiology Plus (CHARGE+) Consortia and Replication Analyses. Genotyping methods are summarized in Dataset 1B, C, and F. SNPs were excluded if the Hardy-Weinberg equilibrium (HWE) P value was < 1 x 10^-6 or based on cohort-specific criteria, minor allele frequency (MAF) was <1%; imputation information score was <0.5; results were only available from two or fewer cohorts; or total n <10,000. Population structure was accounted for within cohorts via principal components analysis. 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 (λGC = 1.06 and intercept = 1.00; λ = 0.99–1.06 for individual cohorts) (Dataset 1B and C). SNPs were taken forward for replication from discovery GWASs if they passed the above criteria and had P < 1 x 10^-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 the Framingham Heart Study. In the Framingham
Heart Study, gene expression profiling was undertaken for the blood samples of
a total of 5,626 participants from the offspring cohort (n = 2,446) at

12 drinks for men or >7 to <14 drinks for women were excluded. Where information was available, current nondrinkers who were former drinkers of >14 drinks per week in men and >7 drinks per week in women as well as current nondrinkers who were former drinkers of unknown amount were excluded, whereas current nondrinkers who were former drinkers of ≤14 drinks for men or ≤7 for women were included. Additional exclusion was made if there were missing data on alcohol consumption or the covariates.

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...act on β-Klotho in brain. (A) Alcohol preference ratios determined by two-bottle preference assays with water and the indicated ethanol concentrations for control (Klbfl/fl) and brain-specific KlbCamk2a mice administered either FGF21 (0.7 mg/kg per day) or vehicle (n = 10 per group). (B) Plasma ethanol and (C) FGF21 concentrations at the end of the 16% (vol/vol) ethanol step of the two-bottle assay. For A–C, ***p < 0.001 for Klbfl/fl vs. KlbCamk2a groups; ***p < 0.001 for Klbfl/fl vs. FGF21 vs. KlbCamk2a + FGF21 groups as determined by one-way ANOVA followed by Tukey’s posttests. (D) Plasma ethanol concentrations 1 and 3 h after i.p. injection of 2 g/kg alcohol (n = 4 per each group). (E) Consumption of 16% (vol/vol) ethanol (grams per kilogram per day) and (F) alcohol preference ratios in two-bottle preferences assays performed with control (Klbfl/fl) and brain-specific KlbCamk2a mice. Alcohol preference was measured by volume of ethanol/total volume of fluid consumed (n = 13 per group). (G) Plasma ethanol concentrations 1 and 3 h after i.p. injection of 2 g/kg alcohol (n = 5 per group). Values are means ± SEM. For E and F, *p < 0.05; **p < 0.01.
To investigate possible effects of rs11940694 in procedures for transcripts have been described previously.

**Mouse Experiments with FGFR2.** For FGFR2 administration studies, recombinant human FGFR2 protein provided by Novo Nordisk was administered at a dose of 0.7 mg/kg per day by s.c. osmotic minipumps (Alzet 1004). Mice were single caged after minipump surgery, which was conducted under isoflurane anesthesia and 24 h of buprenorphine analgesia. Mice were allowed to recover from minipump surgery for 4 d before alcohol drinking tests. After experiments, mice were killed by decapitation, and plasma was collected using EDTA or heparin after centrifugation for 15 min at 4,697 × g. Plasma FGFR2 concentrations were measured using the Biovendor FGFR2 ELISA Kit according to the 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% v/v) in saline, and tail vein blood was collected after 1 and 3 h. 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 × 44 cm, with the center defined as the middle 14 × 14 cm and the periphery defined as the area 5 cm from the wall), and the amount of time spent in the center vs. 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 two dark enclosed arms and two open arms. Mice were allowed to move freely around the maze, and the total duration of time in each arm and the frequencies of entering both the closed and open arms were measured. For novelty suppression of feeding assays, mice fasted for 12 h 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 ± SEM. 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 ANOVA with post hoc Tukey was done using SPSS.

**ACKNOWLEDGMENTS.** Funding sources and acknowledgments for contributing authors and consortia can be found in *SI Appendix*. Part of this work used computing resources provided by Medical Research Council-funded UK Medical Bioinformatics Partnership Programme MR/L01632X/1.
22. Poeggel GE, et al. (2010) Genomics of alcoholism and Molecular Medicine, Ministry of Education, 200032 Shanghai, People's Republic of China. Department of Biostatistics, University of Washington, Seattle, WA 98195; and Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390.
**Fig. S1.** Forest plot for the association of rs11940694 in KLB with log grams per day alcohol in the discovery GWAS and replication cohorts. (A) rs11940694 in KLB in discovery GWAS cohorts: the Alcohol Genome-Wide Association (AlcGen) Consortium: the Cohorte Lausannoise study (COLAUS), the Estonian Biobank Cohort (EGCUT), the European Prospective Investigation of Cancer–Norfolk study (EPIC-NORFOLK), the Erasmus Rucphen Family study (ERF), the Fenland study (FENLAND), the Older Finnish Twin Cohort_2 (FinnTwinOld_2), the Helsinki Birth Cohort Study (HBCS), 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 F4 Study (KORA-F4), the LifeLines Cohort Study & Biobank (LIFELINES), the London Life Sciences Prospective Population Study (LOLIPOP_EW_A, LOLIPOP_EW_P, and LOLIPOP_EW610), the Older Finnish Twin Cohort_3 (FinnTwinOld_3), the Netherlands Study of Depression and Anxiety (NESDA), the Northern Finland Birth Cohort 1966 (NFB1966), the Netherlands Twin Register cohort (NTR), the Australian twin-family study of alcohol use disorder (OZALC), the Study of Health in Pomerania (SHIP), the TwinsUK study (TWINSUK), and the Cardiovascular Risk in Young Finns Study (YFS). 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 and replication cohorts. The coded allele was A, and the noncoded allele was G. The beta/SE estimates were for A allele.
Fig. S2. Genome-wide association results of dichotomous alcohol in the Alcohol Genome-Wide Association (AlcGen) and Cohorts for Heart and Aging Research in Genomic Epidemiology Plus (CHARGE+) consortia. (A) Manhattan plot showing the significance of the association (−log_{10}-transformed P value on the y axis) for each SNP at the 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 labeled. (B) Quantile-quantile plot comparing the expected P value on the x axis and the observed P value on the y axis (both were −log_{10} transformed).
Fig. S3. Illustration of common SNPs (minor allele frequency > 0.01) and LD structure in the genomic regions around the KLB gene. The target SNP rs11940694 is highlighted with the black background and indicated by a red arrow in the LD structure plot. LD is measured by $r^2$, and the darker the red color, the higher the $r^2$ value.
Table S1. Sex-specific associations of SNPs taken forward for replication from discovery GWAS

<table>
<thead>
<tr>
<th>Alcohol phenotype</th>
<th>SNP</th>
<th>Gene</th>
<th>Chr</th>
<th>Position</th>
<th>Effect allele</th>
<th>Other allele</th>
<th>Men Effect*</th>
<th>SE</th>
<th>P value</th>
<th>Women Effect*</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log g/d</td>
<td>rs780094</td>
<td>GCKR</td>
<td>2</td>
<td>27,594,741</td>
<td>T</td>
<td>C</td>
<td>-0.016</td>
<td>0.004</td>
<td>2.8 × 10^-4</td>
<td>-0.014</td>
<td>0.003</td>
<td>2.0 × 10^-5</td>
</tr>
<tr>
<td>Log g/d</td>
<td>rs197273</td>
<td>TANK</td>
<td>2</td>
<td>161,602,909</td>
<td>A</td>
<td>G</td>
<td>-0.018</td>
<td>0.004</td>
<td>6.5 × 10^-5</td>
<td>-0.010</td>
<td>0.003</td>
<td>2.1 × 10^-3</td>
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<tr>
<td>Log g/d</td>
<td>rs10950202</td>
<td>AUTS2</td>
<td>7</td>
<td>69,568,034</td>
<td>C</td>
<td>G</td>
<td>0.022</td>
<td>0.006</td>
<td>5.7 × 10^-4</td>
<td>0.017</td>
<td>0.005</td>
<td>5.1 × 10^-4</td>
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<tr>
<td>Log g/d</td>
<td>rs11940694</td>
<td>KLB</td>
<td>4</td>
<td>39,091,388</td>
<td>A</td>
<td>G</td>
<td>-0.019</td>
<td>0.004</td>
<td>2.3 × 10^-5</td>
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<td>8.9 × 10^-4</td>
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<td>Log g/d</td>
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<td>ASB3</td>
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<td>52,833,931</td>
<td>C</td>
<td>G</td>
<td>0.023</td>
<td>0.007</td>
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<td>0.005</td>
<td>5.7 × 10^-5</td>
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<td>Log g/d</td>
<td>rs6943555</td>
<td>AUTS2</td>
<td>7</td>
<td>69,806,023</td>
<td>A</td>
<td>T</td>
<td>-0.013</td>
<td>0.005</td>
<td>9.9 × 10^-3</td>
<td>-0.011</td>
<td>0.004</td>
<td>3.3 × 10^-3</td>
</tr>
<tr>
<td>Dichotomous</td>
<td>rs12599112</td>
<td>CDH13</td>
<td>16</td>
<td>81,276,212</td>
<td>A</td>
<td>G</td>
<td>0.053</td>
<td>0.091</td>
<td>0.561</td>
<td>-0.063</td>
<td>0.015</td>
<td>2.4 × 10^-5</td>
</tr>
<tr>
<td>Dichotomous</td>
<td>rs10927848</td>
<td>TMEM82</td>
<td>1</td>
<td>15,948,493</td>
<td>A</td>
<td>G</td>
<td>0.027</td>
<td>0.036</td>
<td>0.452</td>
<td>-0.023</td>
<td>0.008</td>
<td>2.5 × 10^-3</td>
</tr>
</tbody>
</table>

Chr, chromosome; EAF, effect allele frequency; CDH13, Cadherin 13; TMEM82, Transmembrane protein 82.

*Effect refers to beta coefficient from linear regression for log grams per day alcohol phenotype and log(odds ratio) from logistic regression for dichotomous alcohol phenotype.

Table S2. Dichotomous trait replication results

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Position (hg19)</th>
<th>Gene*</th>
<th>Discovery P</th>
<th>Replication P</th>
<th>Overall P</th>
<th>Overall N</th>
</tr>
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<tbody>
<tr>
<td>rs12599112</td>
<td>16</td>
<td>82,718,711</td>
<td>CDH13</td>
<td>2.3 × 10^-8</td>
<td>0.895</td>
<td>5.0 × 10^-8</td>
<td>86,213</td>
</tr>
<tr>
<td>rs10927848</td>
<td>1</td>
<td>16,075,906</td>
<td>TMEM82</td>
<td>2.6 × 10^-7</td>
<td>0.291</td>
<td>1.9 × 10^-7</td>
<td>103,219</td>
</tr>
</tbody>
</table>

Cohorts: the Airwave Health Monitoring Study (Airwave), the Austrian Stroke Prevention Study (ASPS), the British 1958 birth cohort (B58C), the Finnish Twin Cohort replication sample (FinnTwin_replication), the Genetic Regulation of Arterial Pressure of Humans in the Community Study (GRAPHC), the Generation Scotland: Scottish Family Health Study (GS:SFHS), the INGI–Carlantino study (INGI_CARL), the INGI–Friuli Venezia Giulia study (INGI_FVG), the INGI–Val Borbera study (INGI_VB), the Lothian Birth Cohort 1921 (LBC1921), the Lothian Birth Cohort 1936 (LBC1936), and the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). The most significant SNP per locus is displayed.

Chr, chromosome; CDH13, Cadherin 13; TMEM82, Transmembrane protein 82.

*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

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Sample (2N)</th>
<th>Major allele frequency</th>
<th>Minor allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admixture American</td>
<td>694</td>
<td>0.565</td>
<td>0.435</td>
</tr>
<tr>
<td>African</td>
<td>1,322</td>
<td>0.570</td>
<td>0.430</td>
</tr>
<tr>
<td>East Asian</td>
<td>1,008</td>
<td>0.541</td>
<td>0.459</td>
</tr>
<tr>
<td>South Asian</td>
<td>978</td>
<td>0.630</td>
<td>0.370</td>
</tr>
<tr>
<td>European</td>
<td>1,006</td>
<td>0.612</td>
<td>0.388</td>
</tr>
</tbody>
</table>

Table S4. Gene expression in peripheral blood in the Framingham Heart Study: Demographics for gene expression analysis

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene expression analysis</td>
<td>n = 2,222</td>
<td>n = 3,014</td>
</tr>
<tr>
<td>Female (%)</td>
<td>1,221 (54.95)</td>
<td>1,603 (53.10)</td>
</tr>
<tr>
<td>Age (y), mean (SD)</td>
<td>66.41 (8.95)</td>
<td>46.88 (8.79)</td>
</tr>
<tr>
<td>BMI (kg/m²), mean (SD)</td>
<td>28.04 (5.87)</td>
<td>28.31 (5.5.30)</td>
</tr>
</tbody>
</table>

BMI, body mass index.

Table S5. Gene expression in peripheral blood in the Framingham Heart Study: Association of KLB SNP rs11940694 with gene expression

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Position</th>
<th>Effect allele</th>
<th>Beta</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11940694</td>
<td>16</td>
<td>39,414,993</td>
<td>A</td>
<td>0.00409</td>
<td>0.165</td>
</tr>
</tbody>
</table>

Chr, chromosome.
Other Supporting Information Files

Si Appendix (PDF)
Dataset S1 (XLSX)
Dataset S2 (XLSX)
Appendices

1. Cohort descriptions

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

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

1.3. Population descriptions for replication cohorts

2. Funding and acknowledgements
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 52,000 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 on 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 controlled 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.


## 2. Funding and acknowledgements

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<tr>
<th>Human GWAS studies</th>
<th>This work received support from the following sources: the European Union-funded FP6 Integrated Project IMAGEN (Reinforcement-related behaviour in normal brain function and psychopathology) (LSHM-CT-2007-037286), the FP7 projects IMAGEMEND(602450; IMAging GENetics for MENtal Disorders), MATRICS (603016), the Innovative Medicine Initiative Project EU-AIMS (115300-2), the Medical Research Council Grants “Developmental pathways into adolescent substance abuse” (93558) and Consortium on Vulnerability to Externalizing Disorders and Addictions [c-VEDA] (MR/N000390/1), the Swedish funding agencies VR, FORTE and FORMAS, the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King’s College London, the Bundesministerium für Bildung und Forschung (BMBF grants 01GS08152; 01EV0711; eMED SysAlc01ZX1311A; Forschungsnetz AERIAL), the Deutsche Forschungsgemeinschaft (DFG grants SM 80/7-1, SM 80/7-2, SFB 940/1). Further support was provided by NIH Consortium grant U54 EB020403, supported by a cross-NIH alliance that funds Big Data to Knowledge Centres of Excellence.</th>
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<td>Mouse Klb studies</td>
<td>We thank Yuan Zhang for assistance with animal experiments. This work was supported by National Institutes of Health grants R01DK067158 (S.A.K. and D.J.M.); the Robert A. Welch Foundation (grant I-1558 to S.A.K. and grant I-1275 to D.J.M.); and the Howard Hughes Medical Institute (D.J.M.).</td>
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<tr>
<td>CoLaus</td>
<td>The authors are grateful to the participants in the Lausanne CoLaus study and to the investigators who have contributed to the study in particular Gérard Waebber, Vincent Mooser and Dawn Waterworth, and the research nurses for data collection. ZK received financial support from Swiss National Science Foundation (31003A-143914) and the Leenaards Foundation. The CoLaus study was supported by research grants from GlaxoSmithKline, the Faculty of Biology and Medicine of Lausanne, and the Swiss National Science Foundation (grants 33CSCO-122661, 33CS30-139468 and 33CS30-148401).</td>
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<td>EPIC-Norfolk</td>
<td>We thank all participants who contributed the study, and colleagues and collaborators who performed the genotyping and facilitated the analysis. The EPIC Norfolk Study was funded by Cancer Research United Kingdom and the Medical Research Council.</td>
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<td>ERF</td>
<td>We are grateful to all study participants and their relatives, general practitioners and neurologists for their contributions and to P. Veraart for her help in genealogy, J. Vergeer for the supervision of the laboratory work and P. Snijders for his help in data collection. 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</td>
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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 (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). Najaf Amin was supported by the Netherlands Brain Foundation (project number F2013(1)-28). Exome sequencing analysis in ERF was supported by the ZonMw grant (project 91111025).

**Fenland**

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**FinnTwin12**

We warmly thank the participating twin pairs and their family members for their contribution. We express our appreciation to the skilled study interviewers A-M Iivonen, K Karhu, H-M Kuha, U Kulmala-Gråhn, M Mantere, K Saanakorpi, M Saarinen, R Sipilä, L Viljanen and E Voipio. Anja Häppölä and Kauko Heikkilä are acknowledged for their valuable contribution in recruitment, data collection, and data management. Antti-Pekka Sarin and Samuli Ripatti are acknowledged for genotype data quality controls and imputation. Phenotyping and genotyping of the Finnish twin cohorts was supported by the Academy of Finland Center of Excellence in Complex Disease Genetics (grants 213506, 129680), the Academy of Finland (grants 100499, 205585, 118555, 141054, 265240, 263278 and 264146 to J. Kaprio), National Institute of Alcohol Abuse and Alcoholism (grants AA-12502, AA-00145, and AA-09203 to R. J. Rose and AA15416 and K02AA018755 to D. M. Dick), and the Welcome Trust Sanger Institute, UK.

**FinnTwinOld_1**

We warmly thank the participating twin pairs and their family members for their contribution. We express our appreciation to the skilled study interviewers A-M Iivonen, K Karhu, H-M Kuha, U Kulmala-Gråhn, M Mantere, K Saanakorpi, M Saarinen, R Sipilä, L Viljanen and E Voipio. Anja Häppölä and Kauko Heikkilä are acknowledged for their valuable contribution in recruitment, data collection, and data management. Antti-Pekka Sarin and Samuli Ripatti are acknowledged for genotype data quality controls and imputation. Phenotyping and genotyping of the Finnish twin cohorts was supported by the Academy of Finland Center of
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<td>We warmly thank the participating twin pairs and their family members for their contribution. We express our appreciation to the skilled study interviewers A-M Iivonen, K Karhu, H-M Kuha, U Kulmala-Gråhn, M Mantere, K Saanakorpi, M Saarinen, R Sipilä, L Viljanen and E Voipio. Anja Häppölä and Kauko Heikkilä are acknowledged for their valuable contribution in recruitment, data collection, and data management. The DNA extractions, sample quality controls, biobank upkeep and aliquotting were performed in the National Public Health Institute, Helsinki, Finland. The Finnish Twin Cohort study received financial support from the Academy of Finland Center of Excellence in Complex Disease Genetics, ENGAGE project and grant agreement HEALTH-F4-2007-201413 and the GenomeEUtwin project, which was supported by the European Commission under the program 'Quality of Life and Management of the Living Resources' of 5th Framework Program (QLG2-CT-2002-01254).</td>
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<td>FinnTwinOld_3</td>
<td>We warmly thank the participating twin pairs and their family members for their contribution. We express our appreciation to the skilled study interviewers A-M Iivonen, K Karhu, H-M Kuha, U Kulmala-Gråhn, M Mantere, K Saanakorpi, M Saarinen, R Sipilä, L Viljanen and E Voipio. Anja Häppölä and Kauko Heikkilä are acknowledged for their valuable contribution in recruitment, data collection, and data management. Antti-Pekka Sarin and Samuli Ripatti are acknowledged for genotype data quality controls and imputation. Phenotyping and genotyping of the Finnish twin cohorts was supported by the Academy of Finland Center of Excellence in Complex Disease Genetics (grants 213506, 129680), the Academy of Finland (grants 100499, 205585, 118555, 141054, 265240, 263278 and 264146 to J. Kaprio), and the Welcome Trust Sanger Institute, UK.</td>
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<td>HBCS</td>
<td>We thank all study participants as well as everybody involved in the Helsinki Birth Cohort Study. The Helsinki Birth Cohort Study was supported by grants from the Academy of Finland, the Finnish Diabetes Research Society, Folkhälsan Research Foundation, Novo Nordisk Foundation, Finska Läkaresällskapet, Signe and Ane Gyllenberg Foundation, University of Helsinki, Ministry of Education, Ahokas Foundation, Emil Aaltonen Foundation.</td>
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<tr>
<td>KORA F3 and F4</td>
<td>The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which was funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ.</td>
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<tr>
<td><strong>LOLIPOP</strong></td>
<td>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).</td>
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<td><strong>NESDA</strong></td>
<td>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.</td>
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<td><strong>NFBC1966</strong></td>
<td>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.</td>
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<td><strong>NTR</strong></td>
<td>The study was supported by Netherlands Scientific Organization (NWO), grant 480-05-003; Netherlands Organization for Scientific Research, grants ZonMW (Addiction program) 31160008, ZonMW 940-37-024, NWO/SPI 56-464-14192, NWO-400-05-717, NWO-MW 904-61-19, NWOMagW 480-04-004; European Research</td>
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<table>
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<td>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.</td>
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<tr>
<td>YFS</td>
<td>We thank Ville Aalto for expert technical assistance in the statistical analyses and Irina Lisinen. The Young Finns Study was supported by the Academy of Finland: grants 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117778 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; Kuopio, Tampere and Turku University Hospital Medical Funds; Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation of Cardiovascular Research; Finnish Cultural Foundation; Tampere Tuberculosis Foundation; Emil Aaltonen Foundation; and Yrjö Jahnsson Foundation.</td>
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<td>AGES</td>
<td>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 Althing (the Icelandic Parliament). The study was approved by the Icelandic National Bioethics Committee, VSN: 00-063.</td>
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<td>ARIC</td>
<td>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.</td>
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<td>CHS</td>
<td>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</td>
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<td>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.</td>
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| **FHS**
| We thank Dr. Curtis Ellison at Boston University School of Medicine for defining phenotypes. The Framingham Heart Study was funded by National Institutes of Health contract N01-HC-25195. The laboratory work for this investigation was funded by the Division of Intramural Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD. The analytical component of this project was funded by the Division of Intramural Research, National Heart, Lung, and Blood Institute, and the Center for Information Technology, National Institutes of Health, Bethesda, MD. |
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| **RS 1, 2 and 3**
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**FTC_replication**

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<td>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.</td>
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<td>GS:SFHS</td>
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<td>INGI_VB</td>
<td>We thank all the participants to the project, the San Raffaele Hospital MDs who contributed to clinical data collection, Prof. Clara Camaschella who coordinated the data collection, Corrado Masciullo and Massimiliano Cocca for help in the database informatics. The research was supported by funds from Compagnia di San Paolo, Torino, Italy; Fondazione Cariplo, Italy; Telethon Italy; Ministry of Health, Ricerca Finalizzata 2008 and 2011-2012 and Public Health Genomics Project 2010.</td>
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<tr>
<td>LBC1921</td>
<td>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.</td>
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| 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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**NSPHS**
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