Clinical Epigenetics

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Association analysis between an epigenetic alcohol risk score and blood pressure

Helena Bui[1,2†], Amena Keshawarz[1,2†], Mengyao Wang[3†], Mikyeong Lee[4], Scott M. Ratliff[5], Lisha Lin[5], Kira S. Birditt[6], Jessica D. Faul[6], Annette Peters[7,8,9], Christian Gieger[9,10], Thomas Delerue[10], Sharon L. R. Kardia[5], Wei Zhao[5], Xiuqing Guo[11], Jie Yao[11], Jerome I. Rotter[11], Yi Li[3], Xue Liu[3], Dan Liu[12], Juliana F. Tavares[12], Gökhan Pehlivan[12], Monique M. B. Breteler[12,13], Irma Karabegovic[14], Carolina Ochoa-Rosales[14,15], Trudy Voortman[14,16], Mohsen Ghanbari[14], Joyce B. J. van Meurs[17], Mohamed Kamal Nasr[18,19], Marcus Dörr[19,20], Hans J. Grabe[21,22], Stephanie J. London[4], Alexander Teumer[18,19,23], Melanie Waldenberger[9,10], David R. Weir[6], Jennifer A. Smith[5,6], Daniel Levy[1,2*], Jiantao Ma[24*] and Chunyu Liu[2,3*]

Abstract

Background Epigenome-wide association studies have identified multiple DNA methylation sites (CpGs) associated with alcohol consumption, an important lifestyle risk factor for cardiovascular diseases. This study aimed to test the hypothesis that an alcohol consumption epigenetic risk score (ERS) is associated with blood pressure (BP) traits.

Results We implemented an ERS based on a previously reported epigenetic signature of 144 alcohol-associated CpGs in meta-analysis of participants of European ancestry. We found a one-unit increment of ERS was associated with eleven drinks of alcohol consumed per day, on average, across several cohorts ($p < 0.0001$). We examined the association of the ERS with systolic blood pressure (SBP), diastolic blood pressure (DBP), and hypertension (HTN) in 3,898 Framingham Heart Study (FHS) participants. Cross-sectional analyses in FHS revealed that a one-unit increment of the ERS was associated with 1.93 mm Hg higher SBP ($p = 4.64E{-}07$), 0.68 mm Hg higher DBP ($p = 0.006$), and an odds ratio of 1.78 for HTN ($p < 2E{-}16$). Meta-analysis of the cross-sectional association of the ERS with BP traits in eight independent external cohorts ($n = 11{,}544$) showed similar relationships with BP levels, i.e., a one-unit increase in ERS was associated with 0.74 mm Hg ($p = 0.002$) higher SBP and 0.50 mm Hg ($p = 0.0006$) higher DBP, but not with HTN. Longitudinal analyses in FHS ($n = 3260$) and five independent external cohorts ($n = 4021$) showed that the baseline ERS was not associated with a change in BP over time or with incident HTN.

Conclusions Our findings demonstrate that the ERS has potential clinical utility in assessing lifestyle factors related to cardiovascular risk, especially when self-reported behavioral data (e.g., alcohol consumption) are unreliable or unavailable.

Keywords Epigenetic risk score, DNA methylation, Blood pressure, Hypertension, Alcohol

†Helena Bui, Amena Keshawarz and Mengyao Wang shared first authorship.

*Correspondence:
Daniel Levy
levyd@nhlbi.nih.gov
Jiantao Ma
jiantao.ma@tufts.edu
Chunyu Liu
liuc@bu.edu
Full list of author information is available at the end of the article

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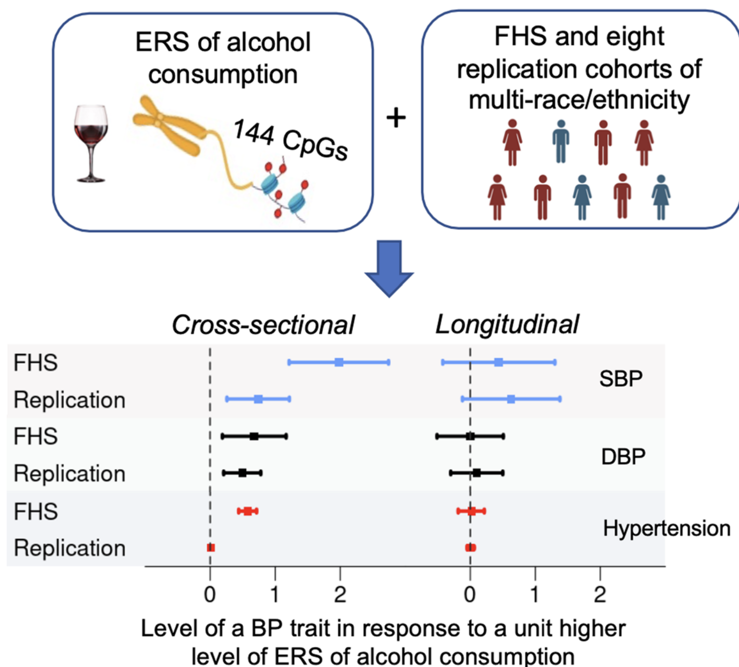
Graphic Abstract



Introduction

Approximately 178,307 people die annually from alcohol-related causes, making alcohol consumption one of leading preventable causes of death in the United States [1]. Alcohol has complex effects on multiple biological processes and systems, including the cardiovascular system. Several studies suggest that habitual, heavy alcohol use can lead to cardiovascular sequelae such as dilated cardiomyopathy and heart failure [2]. The benefits and potential harms of moderate drinking, however, have been a subject of controversy. A few studies have indicated a J-shaped relation between alcohol consumption and a cardiovascular disease (CVD) risk [3–6]. Causal inference analyses using Mendelian randomization have suggested a nonlinear and increased risk of CVD with any dose of alcohol intake [3, 5]. Additionally, multiple studies support a causal relation of alcohol consumption to blood pressure (BP) and the risk of hypertension (HTN) [2, 7]. Furthermore, HTN is one of the leading risk factors for CVD [8, 9]. Therefore, understanding the molecular changes underlying alcohol consumption is crucial to comprehend the relationship between alcohol consumption, HTN, and CVD.

One of the most studied epigenetic modifications, DNA methylation, regulates gene expression through the transfer of a methyl group onto DNA cytosine–phosphate–guanine (CpG) sites [10]. The extent of DNA methylation at certain CpG sites is associated with phenotypic variation in numerous CVD-related traits including body mass index (BMI) [11], blood lipids [12], glycemic traits [13], BP [14], and inflammatory biomarkers [15]. DNA methylation has also been linked to lifestyle behaviors such as alcohol consumption. A large-scale meta-analysis of data from thirteen population-based cohorts including the Framingham Heart Study (FHS) identified 144 differentially methylated CpG sites associated with heavy alcohol intake [16].

A standardized biomarker of alcohol consumption may correct for limitations of self-reported alcohol consumption, such as impression management bias [17]

or faulty recall of drinking history [18, 19], and reveal alcohol-related disease risks that otherwise might not be apparent. In this study, we used 144 alcohol-related, differentially methylated CpGs [16] to generate an alcohol consumption epigenetic risk score (ERS) and examine the association of the ERS with BP traits in cross-sectional and longitudinal analyses. We hypothesized that a DNA methylation-based alcohol consumption ERS would be associated with BP, cross-sectionally and longitudinally. We tested our hypothesis by analyzing the association of our alcohol-associated ERS with BP traits, including systolic BP (SBP), diastolic BP (DBP), and HTN in 3898 FHS participants. In addition, we carried out replication analyses of these findings in eight independent cohorts using meta-analysis (Fig. 1). The alcohol consumption ERS provides an opportunity to investigate the relations of alcohol intake to health outcomes in situations where self-reported intake data are unavailable or unreliable.

Methods

Study population

Data from nine population-based cohort studies were used in the analysis. In addition to the FHS [20], our investigation included the Agricultural Lung Health Study (ALHS) [21], the Cooperative Health Research in the Region Augsburg (KORA) [22], the Genetic Epidemiology Network on Arteriopathy (GENOA) Study [23], the Health and Retirement Study (HRS) [24], the Multi-Ethnic Study of Atherosclerosis (MESA) Study [25], the Rhineland Study [26], the Rotterdam Study [27], and the Study of Health in Pomerania (SHIP) [28]. Institutional review committees of all cohorts approved this study, and all study participants provided written informed consent.

In each cohort, participants with prevalent CVD, heart failure, and atrial fibrillation were excluded. Prevalent CVD includes the following conditions: angina pectoris, coronary insufficiency, cerebrovascular accident, atherothrombotic infarction of the brain, transient ischemic attack, cerebral embolism, intracerebral hemorrhage, subarachnoid hemorrhage, or intermittent claudication. After excluding participants without DNA methylation data, 3,898 participants in FHS and 11,544 participants in eight independent external cohorts were included in cross-sectional association analyses, while 3260 participants in FHS and 3910 participants in five external cohorts were included in longitudinal association analyses (Fig. 1).

Clinical and behavioral data collection

Overall, clinical data for traits such as age, BMI, SBP, DBP, and the use of antihypertensive medication were collected at in-person examinations. Stage 2 HTN was defined as SBP $\geq$ 140 mm Hg, DBP $\geq$ 90 mm Hg, or use of antihypertensive medication for treating HTN at the examination. We added 15 mm Hg and 10 mm Hg to a measured SBP and DBP values, respectively, for participants currently using antihypertensive medication.

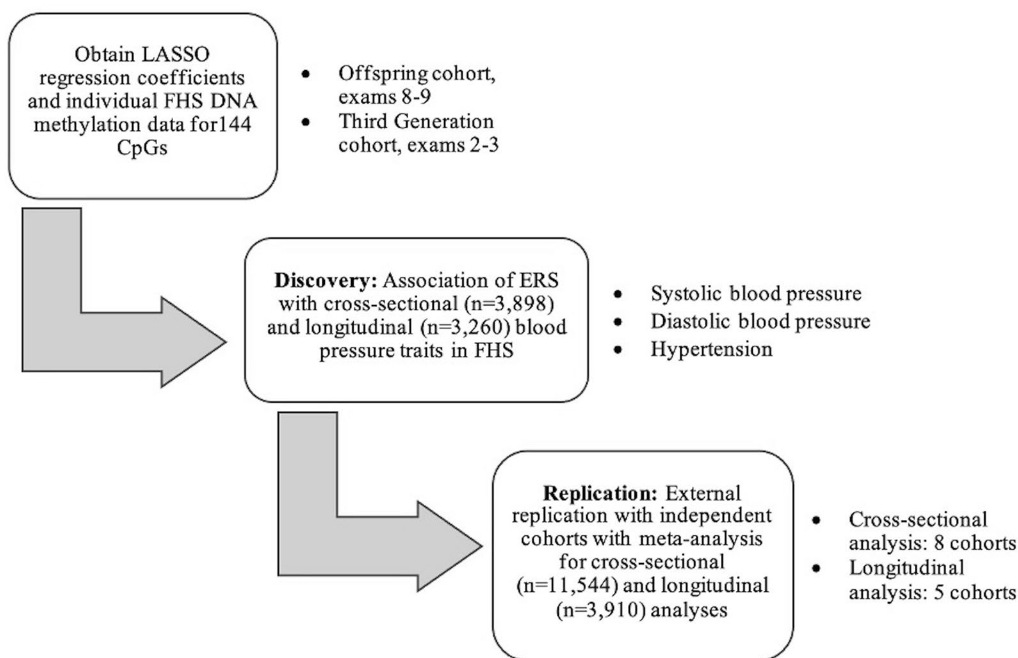


Fig. 1 Study design

Cigarette smoking status was determined based on self-reported smoking behavior. Current smokers were participants who smoked on average at least one cigarette per day in the past year. Self-reported alcohol intake was captured via questionnaires wherein the participants reported the frequency with which they consumed various alcoholic beverages (i.e., beer, liquor, or wine). A standard drink is 12 oz of beer, 4–5 oz of wine, or 1.5 oz of liquor, which is equivalent to appropriately 14 g of ethanol [16, 29]. We summed the total alcohol consumption across all alcoholic beverages and utilized "drink" (i.e., one drink = 14 g of ethanol) as the unit for the alcohol consumption. This study included nine population-based cohorts, and therefore, we focused on habitual alcohol consumption in general populations rather than examining specifically for alcohol disorder. Study-specific methods for clinical data collection are presented in Supplemental Text and Supplemental Table 1.

DNA methylation data collection and processing

DNA methylation was measured using blood samples collected at the same time when alcohol consumption data were assessed in all cohorts. Whole blood samples were assayed for DNA methylation via the Infinium Human Methylation 450 BeadChip platform or Infinium MethylationEPIC platform (San Diego, CA) (Supplemental Text). The methylated probe intensity and total probe intensities were extracted using the Illumina Genome Studio (version 2011.1) with the methylation module (version 1.9.0). Preprocessing of the methylated (M) signal and unmethylated signal (U) was conducted; methylation beta-value (β_M) was defined as $\beta = \frac{M}{U+M}$. Further information regarding DNA extraction and processing has been outlined [16] and described in Supplemental Text.

Derivation of epigenetic risk score (ERS) for alcohol consumption

We implemented an ERS score based on 144 alcohol-associated CpGs previously reported in a meta-analysis of 6926 participants of European ancestry [15]. The previous study generated the regression coefficients (β_i, $i = 1–144$) for these 144 CpGs using the Least Absolute Shrinkage and Selection Operator (LASSO) method. This method performs both variable selection and regularization to enhance the prediction accuracy and interpretability of the resulting statistical model by shrinking the coefficients of some variables to exactly zero [30]. An ERS score was calculated for each participant by summing the regression coefficient-weighted DNA methylation levels of the 144 CpGs: ERS score $= \sum_{i=1}^{144} \beta_i \times CpG_i$. The ERS score represents personal DNA methylation levels in response to alcohol consumption. Across five cohorts, one drink of alcohol consumption was associated with 0.09 higher unit of ERS (Supplemental Table 2). Methods for calculating the ERS for cohorts missing certain CpGs can be found in Supplemental Text.

Discovery association analysis of ERS with BP traits in FHS

We performed both cross-sectional and longitudinal regression analyses in FHS to examine the association between the ERS (independent variable) and BP traits: SBP (continuous), DBP (continuous), and HTN (dichotomous) (dependent variables). Linear mixed regression models were used to evaluate the association of the ERS with the two continuous BP traits. Generalized estimating equations (GEE) were used to evaluate the association of the ERS with dichotomous HTN. A total of 3,898 participants were included in the cross-sectional analysis from the FHS Offspring cohort ($n = 2393$; examination 8) and FHS Third Generation cohort ($n = 1505$; examination 2) participants. All models were adjusted for age, age squared, sex, BMI, and current smoking status. Covariates were selected based on their significant correlation with BP traits and/or DNA methylation based on previous studies [31–35] and our own data (Supplemental Fig. 1–3). Age, sex, and BMI are important risk factors for BP traits [31–33]. The age-squared term was included as a covariate due to the quadratic relationship between age and BP traits (Supplemental Fig. 1–2). Current smoking (versus current none smokers) status was included for its association with BP [34] and DNA methylation [35]. The familial correlation (for family data) was further adjusted for the random effect in models [36]. The familial correlation, or genetic correlation, was calculated based on the self-reported pedigree file to quantify the proportion of shared genetic material or the degree of trait similarity due to genetic factors [37]. Correlation is on average 0.5 for a parent–child relationship, 0.25 for a grandparent–grandchild relationship, and 0.125 for the first-degree siblings.

Longitudinal analyses of all BP traits included FHS Offspring cohort participants ($n = 1932$) who attended both examinations 8 and 9 and third-generation participants ($n = 1328$) who attended both examinations 2 and 3. Our linear mixed regression models evaluated the association of change in BP over time (i.e., ΔSBP and ΔDBP) with the baseline ERS after adjusting for baseline age, baseline age-squared, sex, baseline BMI, baseline smoking status, baseline SBP/DBP (i.e., if the model's outcome was ΔDBP, we adjusted for baseline DBP), and time between baseline and the follow-up examination. Our GEE models evaluated the association of incident HTN with the baseline ERS after adjusting for baseline age, baseline age-squared, baseline BMI, baseline smoking status, and time between

baseline and follow-up examination; in addition, these GEE models excluded all participants with HTN at baseline examination. In the sensitivity analysis, we defined participants with stage 1 HTN using the 2017 guideline (i.e., ≥ 130/80 SBP/DBP mm Hg or with antihypertension treatment) [38]. We performed cross-sectional and longitudinal GEE models to investigate the associations of ERS with prevalent and incident HTN using the new definition.

Replication association analysis of ERS with BP traits in external cohorts

For replication, independent external participants from eight cohorts ($n = 11{,}544$) were included in cross-sectional association analyses, while participants from five cohorts ($n = 3910$) were included for longitudinal association analyses, using the same methods described for the discovery stage in the FHS. We summarized the results from the association analyses using an inverse-variance weighted, fixed-effects meta-analysis, assuming a single true effect between the ERS and a BP trait.

Analysis of epigenetic risk score with BP traits in participants without antihypertension medication

To minimize the possible effects of antihypertension medication on DNA methylation, we conducted a sensitivity analysis among participants without antihypertension medication in five cohorts (i.e., FHS, GENOA, HRS, Rhineland Study, and SHIP). Similar to the primary analysis, we conducted the cross-sectional analysis using linear mixed effects model with ERS as the independent variable and BP traits as the dependent variables in each cohort. The priori power analysis was conducted with a range of assumed effect sizes (i.e., 0.001, 0.0025, 0.005, 0.01, 0.1, 0.2, 0.5), default type I error rate ($\alpha = 0.05$), and actual sample sizes to check if the type-II error was consistent between primary and sensitivity analyses.

Association analysis of ERS with alcohol consumption

We used a linear mixed regression model to test the cross-sectional association between the ERS (outcome) and self-reported alcohol intake (exposure) in each of the five cohorts (i.e., FHS, GENOA, HRS, Rhineland Study, and SHIP). The change in the ERS associated with one drink of alcohol consumption per day was calculated with adjustment for age, age-squared, sex, BMI, current smoking status, and familial correlation.

Association analysis of alcohol consumption with BP traits

To compare the association of BP traits with ERS and questionnaire-based alcohol consumption, we performed cross-sectional (i.e., FHS, GENOA, HRS, Rhineland Study, and SHIP) and longitudinal (i.e., FHS, GENOA, and SHIP) analyses between BP traits and alcohol consumption. We used linear mixed effects or GEE models to quantify the associations between SBP/DBP/HTN (outcome variables) and alcohol consumption (predictor). All models were adjusted for age, age-squared, sex, BMI, current smoking status, and familial correlation.

Association analysis of ERS with biochemical biomarkers of alcohol intake

We tested the association of the ERS with two established biomarkers of chronic alcohol consumption: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations. Separate linear mixed regression models were used with each enzyme as the dependent variable. Serum AST and ALT were measured on fasting morning samples using the kinetic method (Beckman Liquid-State Reagent Kit) [39]. Model 1 (i.e., the reduced model) quantified the association between the self-reported alcohol intake and liver enzyme concentrations after adjusting for age, sex, BMI, and current smoking status. Model 2 (i.e., the full model) further adjusted for the ERS. To compare the two models, we also performed a likelihood ratio test (LRT) to gauge whether the addition of the ERS significantly improved model fit.

Association analysis of individual alcohol-associated cpgs with BP traits in FHS

We examined the cross-sectional association of 144 CpGs that were used to calculate ERS with BP traits in the FHS. We applied the linear mixed effect model to account for the pedigree with each CpG probe as the predictor variable and SBP/DBP as the outcome variable. Covariates included age, age-squared, sex, BMI, and current smoking status.

All statistical analyses were conducted using the R (version 4.0.3) software package [40]. Meta-analyses were conducted with the *metafor* package (version 3.0.2) [41]. The priori power analysis was performed using 'pwr.f2.test' function in the *pwr* R package (version 1.3–0) [42]. LRT was performed using the 'lrtest' function in the *lmtest* R package (version 0.9.39) [43]. Statistical significance was defined as two-sided $p < 0.05$.

Results

Participant characteristics

This present study included discovery and replication analyses in the FHS and independent cohort studies, respectively (Fig. 1). At the baseline examination, FHS participants ($n = 3898$) were, on average, 58 years old (SD = 13 years) and consisted of slightly more women (55%) than men (45%) (Supplemental Table 3). In addition, approximately 42% of FHS participants had stage 2 HTN at baseline. Furthermore, women consumed

less alcohol than men (mean alcohol intake 0.3 drinks per day vs. 0.7 drinks per day). The FHS participants (n = 3260) were followed up for six years and used for longitudinal association analyses with BP traits (Supplemental Table 2).

The average age of participants in the eight independent cohorts (n = 11,544) ranged from 49 years (SHIP) to 68 years (HRS) (Supplemental Tables 3–5). Women also reported a lower average amount of alcohol consumed per day compared to men. For longitudinal analysis, BP traits were measured six to ten years after the baseline measurements (Supplemental Table 1). Additionally, the mean values of the ERS ranged from − 15.35 (SD = 0.74) to − 3.85 (SD = 0.61) across all cohorts at the baseline examination (Supplemental Table 6).

ERS was cross-sectionally associated with BP traits in FHS

The alcohol intake showed a significant association with ALT (p = 2.9E−9) and AST (p = 1.3E−10) in cross-sectional analyses, but not significantly associated with the AST/ALT ratio (p = 0.054) in Model 1 (Supplemental Table 7). The addition of the ERS in Model 2 (i.e., full model) improved model fit with ALT (p = 2.8E−7, LRT between Model 1 and Model 2), AST (p = 2.3E−12, LRT between Model 1 and Model 2), and the AST/ALT ratio (p = 0.0076, LRT between Model 1 and Model 2) (Supplemental Table 7).

Cross-sectional analyses in FHS participants revealed significant association of the ERS with SBP, DBP, and HTN. A one-unit increment of the ERS was associated with a 1.98 mm Hg higher SBP (SE = 0.39, p = 4.6E−7), a 0.68 mm Hg higher DBP (SE = 0.25, p = 0.006), and an odds ratio (OR) of 1.78 for HTN (95% CI [1.55, 2.04], p < 2E−16) (Table 1). In contrast, longitudinal analyses did not reveal significant associations of the ERS with ΔSBP, ΔDBP, or incident HTN (p > 0.3 for all) (Table 1).

In the sensitivity analysis with the definition for stage 1 HTN (i.e., ≥ 130/80 mm Hg or with the antihypertension treatment), we observed consistent results as the stage 2 HTN definition (i.e., ≥ 140/90 mm Hg or with the antihypertension treatment). One-unit higher ERS was positively associated with the prevalent HTN (OR 1.70, 95% CI [1.54, 1.98], p < 2E−16) but not significantly associated with the incident HTN (p = 0.98) (Supplemental Table 8).

Meta-analysis in 11,544 external participants confirmed that ERS was cross-sectionally associated with SBP and DBP

Meta-analysis of eight independent external cohorts (n = 11,544) revealed significant cross-sectional associations. A one-unit greater ERS was associated with a 0.74 (95% CI [0.26, 1.22], p = 0.002) mm Hg higher SBP (Fig. 2) and a 0.50 (95% CI [0.21, 0.78], p = 0.0006) mm Hg higher DBP (Fig. 3). We observed heterogeneity in the meta-analysis for SBP (Q = 17.27, p = 0.008) (Fig. 2), but not for DBP (Q = 5.16, p = 0.52) (Fig. 3). The sensitivity analysis for cross-sectional SBP and DBP was conducted by excluding the ALHS cohort and showed the consistent directionality with the primary analysis. The ERS was not associated with SBP (β = 0.44, 95% CI [−0.06, 0.95]) (Supplemental Fig. 4), but was significantly associated with DBP (β = 0.41, 95% CI [0.12, 0.71]) (Supplemental Fig. 5). No significant association was observed between the ERS and HTN in meta-analysis (OR 1.02, 95% CI [0.83, 1.24], p = 0.10; Supplemental Fig. 6). As a sensitivity analysis for cross-sectional HTN, a meta-analysis was conducted excluding the Rhineland Study, which accounted for 73% of the pooled effect size. However, this exclusion did not significantly alter the results (Supplemental Fig. 7).

No significant associations of the ERS with BP traits in longitudinal meta-analysis were observed

Table 1 Cross-sectional and longitudinal association analyses of epigenetic risk score and blood pressure traits in the Framingham Heart Study

Continuous variable	*β*	SE	*p*
SBP	1.98	0.39	4.64E−07
DBP	0.68	0.25	0.006
ΔSBP	0.44	0.44	0.32
ΔDBP	− 0.0012	0.26	0.99
Binary variable	**OR**	**95% CI**	***p***
HTN	1.78	1.55, 2.04	< 2E−16
Incident HTN	1.02	0.83, 1.24	0.85

The cross-sectional and longitudinal analyses were performed on 3898 and 3260, respectively, Framingham Heart Study (FHS) participants

SBP/DBP systolic/diastolic blood pressure; *ΔSBP/ΔDBP* longitudinal change in systolic/diastolic blood pressure; *SE* standard error; *HTN* hypertension; *OR* odds ratio; *95% CI* 95% confidence interval

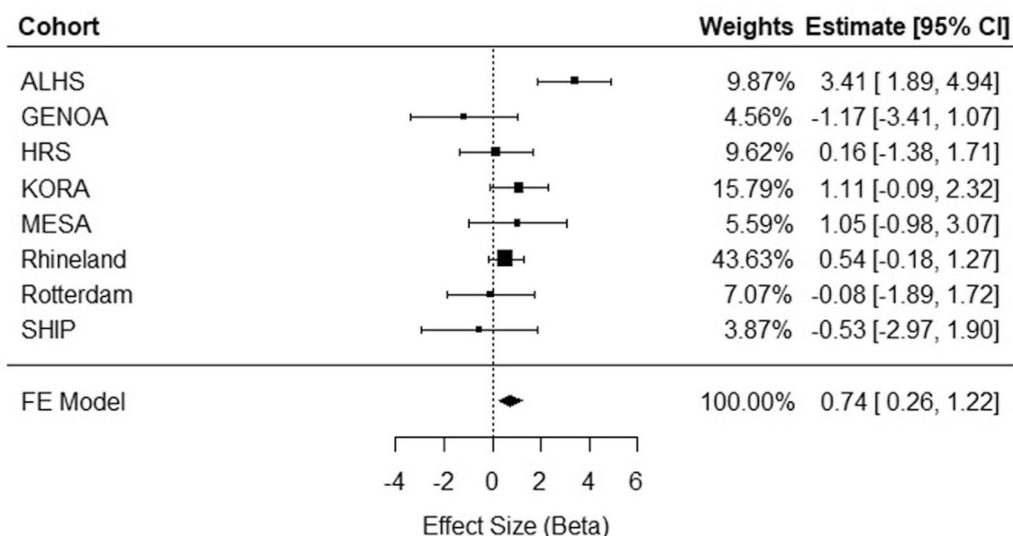


Fig. 2 Meta-analysis of cross-sectional association analyses of ERS in relation to systolic blood pressure in eight independent external cohorts ($n = 11{,}544$). ALHS, Agricultural Lung Health Study; GENOA, Genetic Epidemiology Network of Arteriopathy; HRS, Health and Retirement Study; KORA, Cooperative Health Research in the Region Augsburg; MESA, Multi-Ethnic Study of Atherosclerosis; SHIP, Study of Health in Pomerania; FE, Fixed Effect; 95% CI, Confidence Interval

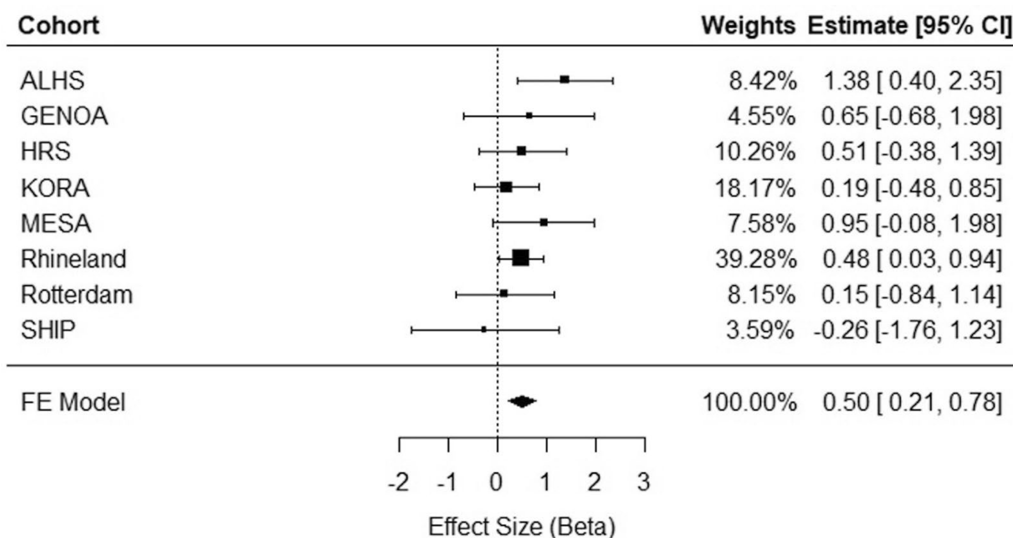


Fig. 3 Cross-sectional meta-analysis of ERS in relation to diastolic blood pressure in eight independent external cohorts ($n = 11{,}544$)**.** ALHS, Agricultural Lung Health Study; GENOA, Genetic Epidemiology Network of Arteriopathy; HRS, Health and Retirement Study; KORA, Cooperative Health Research in the Region Augsburg; MESA, Multi-Ethnic Study of Atherosclerosis; SHIP, Study of Health in Pomerania; FE, Fixed Effect; 95% CI, Confidence Interval

(ΔSBP $\beta = 0.63$, 95% CI [− 0.12, 1.38], $p = 0.10$; ΔDBP $\beta = 0.10$. 95% CI [− 0.30, 0.50], $p = 0.61$; incident HTN β (log OR) = 0.003, 95% CI [− 0.05, 0.06], $p = 0.92$). These analyses included five cohorts (GENOA, KORA, MESA, the Rotterdam Study, and SHIP) that had follow-up examination data available (for ΔSBP, ΔDBP total sample size $n = 3910$; for incident HTN, $n = 3228$). Supplemental Figs. 8, 9, and 10 display the

meta-analysis results for ΔSBP, ΔDBP, and incident HTN, respectively. Cross-sectional and longitudinal analysis results for individual cohort are shown in Supplemental Tables 9 and 10, respectively.

Sensitivity analyses

Association of ERS with BP traits in participants not receiving antihypertension medication yielded consistent findings

We conducted sensitivity analyses between ERS and SBP/DBP in 2577 FHS participants (66.1% of the entire study sample) who were not receiving antihypertension medication. We observed stronger results in the participants without antihypertension treatment compared to the results in all participants (Supplemental Table 11). One-unit higher ERS was associated with a 2.6 mm Hg higher SBP ($p=1.5E{-}7$) and a 1.54 mm Hg higher DBP ($p=1.1E{-}6$), which were stronger than the estimates obtained from all FHS participants (SBP: $\beta=1.98$, $p=4.6E{-}7$; DBP: $\beta=0.68$, $p=0.006$). However, associations were not significant between ERS and SBP/DBP among untreated participants ($p>0.05$) in the four external independent cohorts, except a marginally significant association between ERS and DBP ($\beta=0.40$, $p=0.09$) in Rhineland untreated participants (Supplemental Table 11). Of note, most of the cross-sectional associations between ERS and BP traits were nonsignificant in these four cohorts before participants receiving HTN treatment were excluded (Supplemental Table 9). We further calculated the priori type-II error rates in association analyses between ERS and BP traits in participants with and without antihypertensive medication. Among all cohorts, the priori type-II error tends to 0 with the effect size greater than 0.1 (Supplemental Table 12). The primary analysis in FHS showed an effect size = 0.31/(1–0.31) = 0.45 with SBP and an effect size = 0.15/(1–0.15) = 0.18 with DBP. Meanwhile, the sensitivity analysis in FHS also showed effect sizes greater than 0.1 (SBP: effect size = 0.37/(1–0.37) = 0.59; DBP: effect size = 0.23/(1–0.23) = 0.30). Therefore, results were consistent between the primary analysis using all participants and the sensitivity analysis using participants without antihypertensive treatment in the FHS.

ERS was positively associated with alcohol consumption

We conducted association between ERS and alcohol consumption in FHS. Each self-reported drink of alcohol per day was associated with a 0.25-units higher ERS ($p<0.0001$) in FHS. Consistent positive associations were also observed in three independent cohorts, GENOA, HRS, and Rhineland Study (Supplemental Table 2). One self-reported drink of alcohol per day was associated with a 0.32-unit higher ERS in GENOA ($p<0.0001$), with a 0.26-unit higher ERS in HRS ($p<0.0001$), and with a 0.01-unit higher ERS in the Rhineland Study ($p=0.02$). No significant association was observed in SHIP. In the meta-analysis ($n=10{,}684$), one self-reported drink of alcohol per day was associated with a 0.01-unit increase in the ERS ($p<0.0001$) (Supplemental Table 2). This indicates that a one-unit increment in ERS corresponds to the consumption of approximately 11 drinks per day.

Alcohol consumption was positively associated with BP traits

Alcohol consumption was associated with SBP in both cross-sectional and longitudinal analyses in the FHS (Supplemental Tables 13–14). One additional drink per day of alcohol consumption was associated with a 0.88 mm Hg higher SBP ($p=3.7e{-}4$) in cross-sectional analysis and with a 0.92 mm Hg increase in SBP ($p=2.6e{-}4$) between two exams in longitudinal analysis. We also found that one additional drink/day of alcohol was associated with on OR of 1.13 of HTN (95% CI [1.04, 1.22]; $p=0.006$) in cross-sectional analysis. Associations of alcohol consumption with DBP were not significant ($p>0.05$ in both cross-sectional and longitudinal analyses) in FHS (Supplemental Tables 13–14). In Rhineland and SHIP, we observed that higher alcohol consumption was significantly associated with SBP (Rhineland: $\beta=0.38$, $p=0.006$; SHIP: $\beta=2.94$, $p=0.008$) and DBP (Rhineland: $\beta=0.38$, $p=1.6e{-}6$; SHIP: $\beta=2.06$, $p=0.002$) (Supplemental Table 13). However, self-reported alcohol consumption was not associated with prevalent HTN in either cohort, whereas in HRS, higher alcohol intake was associated with higher DBP ($\beta=0.72$, $p=0.04$) and higher odds of HTN ($\beta=0.17$, $p=0.02$) in cross-sectional analysis. No significant longitudinal association was observed between alcohol consumption and any of the BP traits in GENOA or SHIP participants (Supplemental Table 14).

Additionally, we investigated whether the DNA methylation CpGs that were used for constructing the ERS were associated with BP traits in cross-sectional analysis in FHS. When applying a relaxed threshold by false discovery rate (FDR) < 0.05, 26 and 17 of 144 CpG probes were significantly associated with SBP and DBP, respectively (Supplemental Figs. 11a-c, Supplemental Table 15–16).

Discussion

We conducted extensive analyses to test whether an ERS for alcohol consumption is associated with BP traits. This investigation builds on our previous findings that alcohol consumption behavior can be captured through epigenetic modifications [16]. Using an ERS calculated from 144 CpGs that were identified in this previous work [16], we found that the score was robustly associated with SBP and DBP in cross-sectional analyses, supported by both discovery and replication cohorts. However, the ERS was not associated with longitudinal changes in BP or

incident HTN in either discovery or replication studies. These findings enhance our understanding of the molecular mechanisms underlying the relationship between alcohol consumption, DNA methylation, and BP.

DNA methylation, an epigenetic mechanism, involves adding a methyl group to the fifth carbon of the cytosine DNA base, forming 5-methylcytosine [10]. This modification regulates gene expression by recruiting proteins that primarily inhibit the binding of transcription factors to DNA [10], leading to a stable, tissue-specific gene expression patterns. Our previous study demonstrated that DNA methylation marker is a novel, reliable biomarker of alcohol use, particularly for heavy alcohol intake [16]. DNA methylation has proved to be a reliable measure of heavy alcohol intake and addresses a critical need. Further research is warranted to explore the biological mechanisms, e.g., gene expression changes, inflammation related biological pathways, and metabolic consequences, linking the identified CpGs to alcohol consumption and cardiovascular risk.

Our analyses demonstrate that a risk score comprised of 144 CpGs is associated with current alcohol intake and BP. However, it does not show an association with longitudinal change in BP. Several factors may explain these findings. The follow-up periods in these cohorts are 5–9 years apart (Supplemental Tables 3–4), during which participants may change their lifestyle behaviors such as diet and antihypertension medication use, which may affect methylation patterns. For example, a longitudinal study examining smoking and DNA methylation demonstrated that the most differentially methylated CpG sites associated with smoking reverted to the levels of non-smokers within five years of smoking cessation. Alcohol consumption tends to reduce with older age; therefore, DNA methylation levels may also change toward to the levels of non-drinkers. Thus, we observed that the baseline ERS did not predict blood pressure change over time [35]. The dynamic nature of DNA methylation suggests it may show stronger associations with cross-sectional behaviors and clinical phenotypes than with changes in traits over time. A recent longitudinal study identified 1414 alcohol-related CpGs in cross-sectional analysis, of which only about a third of CpGs ($n=513$) displayed associations between the changes in the methylation levels and the change of alcohol consumption between two exams [44]. This previous study and findings from the present data analysis suggest that DNA methylation sites used to calculate ERS may be more reflective of current alcohol consumption behaviors rather than changes over time.

The meta-analysis of cross-sectional associations for SBP showed significant heterogeneity, which may be attributable to a number of factors such as participants' country or origin, ethnicity, age, sex, diet, and differences in sample collection methodology [45]. For example, GENOA recruited participants with a family history of HTN [23], potentially making them more susceptible to HTN than the general population. In contrast, the SHIP recruited subjects from Northeast Germany, an area with the lowest life expectancy in the country at the time of recruitment [28]. These different recruitment criteria may influence the relationship between alcohol consumption, DNA methylation, and BP traits. Measurement error and higher SBP and DBP in GENOA and Rotterdam Study may partly explained the differential associations between the ERS and alcohol consumption, compared to other cohorts. Despite this heterogeneity, which tends to result in false negatives rather than false positives, our meta-analysis findings remain consistent with those from the FHS. Further, sensitivity meta-analyses with exclusion of ALHS or Rhineland Study reduced heterogeneity and showed consistent results with the primary meta-analysis, supporting the robustness of our findings.

Another key finding was the improved model fit when adding the ERS to models examining the relationship between self-reported alcohol intake and liver enzymes (ALT and AST) in FHS. While the association between self-reported alcohol intake and these liver enzymes was significant, the ERS captured additional interindividual variations in elevated liver enzymes (Supplemental Table 7). However, it remains to be determined whether this is because the ERS more accurately gauges alcohol consumption levels or if it also reflects other biological or environmental factors.

This study has several limitations. Most participants were of European ancestry, potentially limiting the applicability of our findings to other racial/ethnic populations. While we validated the utility of the risk score using ALT and AST, common clinical indicators of chronic alcohol consumption, these enzyme levels can be influenced by other factors such as prescription drugs [46] and conditions like autoimmune hepatitis. In addition, the FHS did not have other biomarkers, such as gamma-glutamyl transferase [47], available for validation at the time of this study. There was also a potential self-fulfilling prophecy in the association between the ERS and liver enzymes, as the ERS was calculated from CpGs identified using cohorts that included the FHS [16]. Despite these limitations, the study has several strengths. We have performed extensive statistical analyses, demonstrating significantly associations between the ERS, self-reported alcohol intake, and clinically useful biomarkers of alcohol consumption. Furthermore, the cross-sectional relationship between the ERS and BP traits observed in the FHS

was replicated in a meta-analysis of eight independent external cohorts.

Conclusion

Our study applied an ERS score for alcohol consumption based on a previous large meta-analysis. This score was robustly associated with cross-sectional measurements of blood pressure and prevalent hypertension among middle-aged and older participants. These findings demonstrate that ERS, a methylation-based score, could effectively assess alcohol consumption and its potential relationship with blood pressure traits and cardiovascular health. Our findings also support that the epigenetic score may have clinical utility in assessing specific lifestyle risk factors contributing to an individual's cardiovascular and health profile, especially in situations where self-reported behavioral data are unavailable, susceptible to recall bias, or subject to significant data loss. However, further study is warranted to establish causal relationships between DNA methylation and human health profile.

Abbreviations

ERS Epigenetic risk score
SBP Systolic blood pressure
DBP Diastolic blood pressure
HTN Hypertension
AST Aspartate aminotransferase
ALT Alanine aminotransferase

Supplementary Information

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Additional file 1.

Additional file 2.

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Author contributions

D Levy, JM, and CL designed the study. HB, AK, M Wang, ML, SMR, LL, KSB, JDF, AP, CG, TD, SLRK, WZ, XG, JY, JIR, YL, XL, D Liu, JFT, GP, MMBB, IK, COR, TV, MG, JBJvM, MKN, MD, HJG, SJL, AT, M Waldenberger, DRW, JAS, D Levy, JM, and CL were involved in the data collection, data preparation, and statistical analyses. HB, AK, and M Wang drafted the manuscript. D Levy, JM, and CL supervised the study and provided critical review. All authors read and approved this manuscript.

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Availability of data and materials

Data and analytical codes that support our findings are available from the corresponding authors upon request.

Declarations

Ethics approval and consent to participate

Institutional review committees of all cohorts approved this study. All study participants provided written informed consent. The FHS was reviewed and approved by The Boston Medical Center Institutional Review Boards. The ALHS was approved by the Institutional Review Board at National Institute of Environmental Health Sciences. The KORA cohort ethical approval was granted by the ethics committee of the Bavarian Medical Association (REC reference numbers: F4: #06068, FF4: #06068), and all were carried out in accordance with the principles of the Declaration of Helsinki. The GENOA was approved by Institutional Review Boards at the University of Michigan, University of Mississippi Medical Center, and Mayo Clinic. The HRS was approved by the University of Michigan. The MESA was approved by the Institutional Review Board of each study site, and written informed consent was obtained from all participants (IRB: #00009029). The Rhineland Study was approved by the ethics committee of the University of Bonn, Medical Faculty. The Rotterdam Study was approved by the medical ethics committee of the Erasmus University Medical Center, Rotterdam, the Netherlands. The SHIP was approved by the medical ethics committee of the University of Greifswald.

Consent for publication

Not applicable.

Competing interests

All first and corresponding authors of this manuscript have no conflicts of interest to disclose.

Author details

[1]Population Sciences Branch, Division of Intramural Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA. [2]Framingham Heart Study, 73 Mt. Wayte Avenue, Framingham, MA 01702, USA. [3]Department of Biostatistics, Boston University School of Public Health, Boston University, 715 Albany Street, Boston, MA 02118, USA. [4]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA. [5]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, USA. [6]Survey Research

Center, Institute for Social Research, University of Michigan, Ann Arbor, MI, USA. [7]Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany. [8]Institute for Medical Informatics, Biometrics and Epidemiology, Ludwig-Maximilians-Universität München, Munich, Germany. [9]German Center for Cardiovascular Research (DZHK), Partner Site Munich Heart Alliance, Munich, Germany. [10]Research Unit Molecular Epidemiology, Institute of Epidemiology, Helmholtz Munich, Neuherberg, Germany. [11]The Institute for Translational Genomics and Population Sciences, Department of Pediatrics, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, USA. [12]Population Health Sciences, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. [13]Institute for Medical Biometry, Informatics and Epidemiology (IMBIE), Faculty of Medicine, University of Bonn, Bonn, Germany. [14]Department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, The Netherlands. [15]Centro de Vida Saludable de La Universidad de Concepción, Concepción, Chile. [16]Division of Human Nutrition and Health, Wageningen University and Research, Wageningen, The Netherlands. [17]Department of Internal Medicine, Erasmus MC University Medical Center, Rotterdam, The Netherlands. [18]Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany. [19]German Center for Cardiovascular Research (DZHK), Partner Site Greifswald, Greifswald, Germany. [20]Department of Internal Medicine B, University Medicine Greifswald, Greifswald, Germany. [21]Department of Psychiatry and Psychotherapy, University Medicine, Greifswald, Germany. [22]German Center of Neurodegenerative Diseases (DZNE), Rostock/Greifswald, Germany. [23]Department of Population Medicine and Lifestyle Diseases Prevention, Medical University of Bialystok, Bialystok, Poland. [24]Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA.

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