Gene-Age Interactions in Blood Pressure Regulation: A Large-Scale Investigation with the CHARGE, Global BPgen, and ICBP Consortia

Jeannette Simino,[1,96,*] Gang Shi,[1,96] Joshua C. Bis,[2,96] Daniel I. Chasman,[3,4,96] Georg B. Ehret,[5,6,96] Xiangjun Gu,[7,96] Xiuqing Guo,[8,96] Shih-Jen Hwang,[9,10,96] Eric Sijbrands,[11,96] Albert V. Smith,[12,13,96] Germaine C. Verwoert,[11,14,96] Jennifer L. Bragg-Gresham,[15] Gemma Cadby,[16,17,18] Peng Chen,[19,20] Ching-Yu Cheng,[19,20,21,22,23,24] Tanguy Corre,[25,26] Rudolf A. de Boer,[27] Anuj Goel,[28,29] Toby Johnson,[30] Chiea-Chuen Khor,[19,20,21,22,31,32,33] LifeLines Cohort Study, Carla Lluís-Ganella,[34] Jian'an Luan,[35] Leo-Pekka Lyytikäinen,[36,37] Ilja M. Nolte,[38] Xueling Sim,[15,39] Siim Sõber,[40] Peter J. van der Most,[38] Niek Verweij,[27] Jing Hua Zhao,[35] Najaf Amin,[14] Eric Boerwinkle,[41] Claude Bouchard,[42] Abbas Dehghan,[14] Gudny Eiriksdottir,[12] Roberto Elosua,[34,43] Oscar H. Franco,[14] Christian Gieger,[44] Tamara B. Harris,[45] Serge Hercberg,[46] Albert Hofman,[14] Alan L. James,[47,48] Andrew D. Johnson,[9,49] Mika Kähönen,[50,51] Kay-Tee Khaw,[52] Zoltan Kutalik,[25,26] Martin G. Larson,[9,53] Lenore J. Launer,[45] Guo Li,[2] Jianjun Liu,[19,20,31] Kiang Liu,[54] Alanna C. Morrison,[41] Gerjan Navis,[55] Rick Twee-Hee Ong,[19,20] George J. Papanicolau,[56] Brenda W. Penninx,[57,58,59] Bruce M. Psaty,[2,60,61,62] Leslie J. Raffel,[63] Olli T. Raitakari,[64,65] Kenneth Rice,[66] Fernando Rivadeneira,[11,14] Lynda M. Rose,[3] Serena Sanna,[67] Robert A. Scott,[35] David S. Siscovick,[2,60] Ronald P. Stolk,[38] Andre G. Uitterlinden,[11,14,68] Dhananjay Vaidya,[69] Melanie M. van der Klauw,[70] Ramachandran S. Vasan,[9,71] Eranga Nishanthie Vithana,[21,22,23,72] Uwe Völker,[73] Henry Völzke,[74] Hugh Watkins,[28,29] Terri L. Young,[75,76] Tin Aung,[21,22,23] Murielle Bochud,[77] Martin Farrall,[28,29] Catharina A. Hartman,[78] Maris Laan,[40] Edward G. Lakatta,[79] Terho Lehtimäki,[36,37] Ruth J.F. Loos,[35,80,81,82] Gavin Lucas,[34] Pierre Meneton,[83] Lyle J. Palmer,[17,18] Rainer Rettig,[84] Harold Snieder,[38] E. Shyong Tai,[19,20,85,86] Yik-Ying Teo,[19,20,87,88,89] Pim van der Harst,[27,90,91] Nicholas J. Wareham,[35] Cisca Wijmenga,[90] Tien Yin Wong,[21,22,23] Myriam Fornage,[7,41,96] Vilmundur Gudnason,[12,13,96] Daniel Levy,[9,10,92,96] Walter Palmas,[93,96] Paul M. Ridker,[3,4,96] Jerome I. Rotter,[8,96] Cornelia M. van Duijn,[14,68,94,96] Jacqueline C.M. Witteman,[14,96] Aravinda Chakravarti,[5,96,*] and Dabeeru C. Rao[1,95,96]

Although age-dependent effects on blood pressure (BP) have been reported, they have not been systematically investigated in large-scale genome-wide association studies (GWASs). We leveraged the infrastructure of three well-established consortia (CHARGE, GBPgen, and ICBP) and a nonstandard approach (age stratification and metaregression) to conduct a genome-wide search of common variants with age-dependent effects on systolic (SBP), diastolic (DBP), mean arterial (MAP), and pulse (PP) pressure. In a two-staged design using 99,241 individuals of European ancestry, we identified 20 genome-wide significant ($p \leq 5 \times 10^{-8}$) loci by using joint tests of the SNP main effect and SNP-age interaction. Nine of the significant loci demonstrated nominal evidence of age-dependent effects on BP by tests of the interactions alone. Index SNPs in the *EHBP1L1* (DBP and MAP), *CASZ1* (SBP and MAP), and *GOSR2* (PP) loci exhibited the largest age interactions, with opposite directions of effect in the young versus the old. The changes in the genetic effects over time were small but nonnegligible (up to 1.58 mm Hg over 60 years). The *EHBP1L1* locus was discovered through gene-age interactions only in whites but had DBP main effects replicated ($p = 8.3 \times 10^{-4}$) in 8,682 Asians from Singapore, indicating potential interethnic heterogeneity. A secondary analysis revealed 22 loci with evidence of age-specific effects (e.g., only in 20 to 29-year-olds). Age can be used to select samples with larger genetic effect sizes and more homogenous phenotypes, which may increase statistical power. Age-dependent effects identified through novel statistical approaches can provide insight into the biology and temporal regulation underlying BP associations.

[1]Division of Biostatistics, Washington University School of Medicine, St. Louis, MO 63110, USA; [2]Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA 98101, USA; [3]Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA 02215, USA; [4]Harvard Medical School, Boston, MA 02115, USA; [5]Center for Complex Disease Genomics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; [6]Cardiology, Department of Specialties of Internal Medicine, Geneva University Hospitals, Geneva 1211, Switzerland; [7]Research Center for Human Genetics, Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX 77030, USA; [8]Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute and Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, CA 90502, USA; [9]Framingham Heart Study, Framingham, MA 01702, USA; [10]Center for Population Studies, National Heart, Lung, and Blood Institute, Framingham, MA 01702, USA; [11]Department of Internal Medicine, Erasmus University Medical Center, 3015 GE Rotterdam, the Netherlands; [12]Icelandic Heart Association, 201 Kopavogur, Iceland; [13]Faculty of Medicine, University of Iceland, 101 Reykjavik, Iceland; [14]Department of Epidemiology, Erasmus University Medical Center, 3015 GE Rotterdam, the Netherlands; [15]Center for Statistical Genetics, University of Michigan, Ann Arbor, MI 48109, USA; [16]Centre for Genetic Origins of Health and Disease, University of Western Australia, Nedlands, WA 6009, Australia; [17]Genetic Epidemiology and Biostatistics Platform, Ontario Institute for Cancer Research, Toronto, ON M5G 0A3, Canada; [18]Samuel Lunenfeld Research Institute, Toronto, ON M5T 3L9, Canada; [19]Saw Swee Hock School of Public Health, National University of Singapore, Singapore 117597, Singapore; [20]Saw Swee Hock School of Public Health, National University Health System, Singapore 117597, Singapore; [21]Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore; [22]Department of Ophthalmology, National University Health System, Singapore 119228, Singapore; [23]Singapore Eye Research Institute, Singapore 168751, Singapore; [24]Centre for

Introduction

Age is a major predictor of cardiovascular health[1] but its impact on the genetic architecture of blood pressure (BP) has been largely unexplored. A Norwegian study of parent-offspring pairs, siblings, and twins reported that 60%–70% of the genetic variance of BP at ages 20 and 60 was attributable to genes active at both ages.[2] For the genes that are active across the age spectrum, we do not know whether the magnitude of the genetic effects are constant or vary with age.[3] Family and population studies suggest that age may modify the effects of some BP genes. Among relative pairs that shared 50% of their genes on average, the correlation of BP traits was higher in members of similar ages[2] and BPs of parents and offspring measured around the same age yielded correlations similar to that of sibpairs.[4] Variance components models that explicitly incorporated age-dependent genetic effects identified 26 loci that were missed by linkage analyses that assumed constant effects across ages.[5] Further strengthening the evidence for age-dependent effects, candidate gene studies have identified SNPs that interact with age to influence BP.[6–10]

No large-scale BP studies have assessed the pervasiveness of gene-age interactions by using common variants

Quantitative Medicine, Office of Clinical Sciences, Duke-NUS Graduate Medical School, Singapore 169857, Singapore; [25]Department of Medical Genetics, University of Lausanne, 1005 Lausanne, Switzerland; [26]Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland; [27]Department of Cardiology, University of Groningen, University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [28]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK; [29]Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford OX3 9DU, UK; [30]Clinical Pharmacology, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK; [31]Division of Human Genetics, Genome Institute of Singapore, Singapore 138672, Singapore; [32]Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore; [33]Department of Paediatrics, National University Health System, Singapore 119074, Singapore; [34]Cardiovascular Epidemiology and Genetics, IMIM (Hospital del Mar Medical Research Institute), 08003 Barcelona, Spain; [35]MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge, Cambridge CB2 0QQ, UK; [36]Department of Clinical Chemistry, Fimlab Laboratories, Tampere 30101, Finland; [37]Department of Clinical Chemistry, University of Tampere School of Medicine, Tampere 33101, Finland; [38]Department of Epidemiology, University of Groningen, University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [39]Centre for Molecular Epidemiology, National University of Singapore, Singapore 119260, Singapore; [40]Human Molecular Genetics Group, Institute of Molecular and Cell Biology, University of Tartu, Tartu 51010, Estonia; [41]Human Genetics Center, University of Texas Health Sciences Center, Houston, TX 77225, USA; [42]Human Genomics Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA; [43]Epidemiology and Public Health Network (CIBERESP), 08036 Barcelona, Spain; [44]Institute of Genetic Epidemiology, Helmholtz Zentrum München—German Research Center for Environmental Health, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany; [45]Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, NIH, Bethesda, MD 20892, USA; [46]U557 Institut National de la Santé et de la Recherche Médicale, U1125 Institut National de la Recherche Agronomique, Université Paris 13, 93000 Bobigny, France; [47]Department of Pulmonary Physiology and Sleep Medicine, Sir Charles Gairdner Hospital, Nedlands, WA 6009, Australia; [48]School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA 6009, Australia; [49]Cardiovascular Epidemiology and Human Genomics Branch, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892, USA; [50]Department of Clinical Physiology, Tampere University Hospital, Tampere 33521, Finland; [51]Department of Clinical Physiology, University of Tampere School of Medicine, Tampere 33521, Finland; [52]Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridge CB2 2SR, UK; [53]Department of Mathematics, Boston University, Boston, MA 02215, USA; [54]Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA; [55]Department of Internal Medicine, University of Groningen, University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [56]Division of Cardiovascular Sciences, National Heart, Lung, & Blood Institute, NIH, Bethesda, MD 20892, USA; [57]Department of Psychiatry/EMGO Institute/Neuroscience Campus, VU University Medical Centre, 1081 BT Amsterdam, the Netherlands; [58]Department of Psychiatry, Leiden University Medical Centre, 2333 ZD Leiden, the Netherlands; [59]Department of Psychiatry, University of Groningen, University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [60]Department of Epidemiology, University of Washington, Seattle, WA 98195, USA; [61]Department of Health Services, University of Washington, Seattle, WA 98195, USA; [62]Group Health Research Institute, Group Health Cooperative, Seattle, WA 98101, USA; [63]Medical Genetics Institute, Cedars-Sinai Medical Center, Pacific Theatres, Los Angeles, CA 90048, USA; [64]Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku 20521, Finland; [65]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku 20521, Finland; [66]Department of Biostatistics, University of Washington, Seattle, WA 98195, USA; [67]Istituto di Ricerca Genetica e Biomedica, CNR, Monserrato 09042, Italy; [68]Netherland Genomics Initiative, Netherlands Center for Healthy Aging, The Hague 2509, the Netherlands; [69]Department of Medicine, Johns Hopkins University, Baltimore, MD 21202, USA; [70]Department of Endocrinology, University of Groningen, University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [71]Divisions of Epidemiology and Cardiology, Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA; [72]Neuroscience and Behavioural Disorders (NBD) Program, Duke-NUS Graduate Medical School, Singapore 169857, Singapore; [73]Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, 17487 Greifswald, Germany; [74]Institute for Community Medicine, University of Greifswald, 17487 Greifswald, Germany; [75]Department of Ophthalmology, Duke University Medical Center, Durham, NC 27710, USA; [76]Division of Neuroscience, Duke-National University of Singapore, Singapore 169857, Singapore; [77]Institute of Social and Preventive Medicine, Lausanne University Hospital, 1010 Lausanne, Switzerland; [78]Interdisciplinary Center for Pathology of Emotions, University of Groningen, University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [79]Laboratory of Cardiovascular Science, National Institute on Aging, NIH, Bethesda, MD 21224, USA; [80]The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; [81]The Genetics of Obesity and Related Metabolic Traits Program, The Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; [82]The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; [83]U872 Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, Paris 75006, France; [84]Institute of Physiology, University of Greifswald, 17495 Karlsburg, Germany; [85]Department of Medicine, National University Health System and Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore; [86]Duke-National University of Singapore Graduate Medical School, Singapore 169857, Singapore; [87]Life Sciences Institute, National University of Singapore, Singapore 117456, Singapore; [88]Department of Statistics and Applied Probability, National University of Singapore, Singapore 117543, Singapore; [89]Genome Institute of Singapore, A*STAR, Singapore 138672, Singapore; [90]Department of Genetics, University of Groningen, University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [91]Durrer Center for Cardiogenetic Research, 3501 DG Utrecht, the Netherlands; [92]Boston University School of Medicine, Boston, MA 02118, USA; [93]Department of Medicine, Columbia University, New York, NY 10032, USA; [94]Netherland Genomics Initiative, Centre for Medical Systems Biology, 2300 RC Leiden, the Netherlands; [95]Departments of Psychiatry, Genetics, and Mathematics, Washington University School of Medicine, St. Louis, MO 63110, USA
[96]These authors contributed equally to this work
*Correspondence: jeannette@wubios.wustl.edu (J.S.), aravinda@jhmi.edu (A.C.)
http://dx.doi.org/10.1016/j.ajhg.2014.05.010.

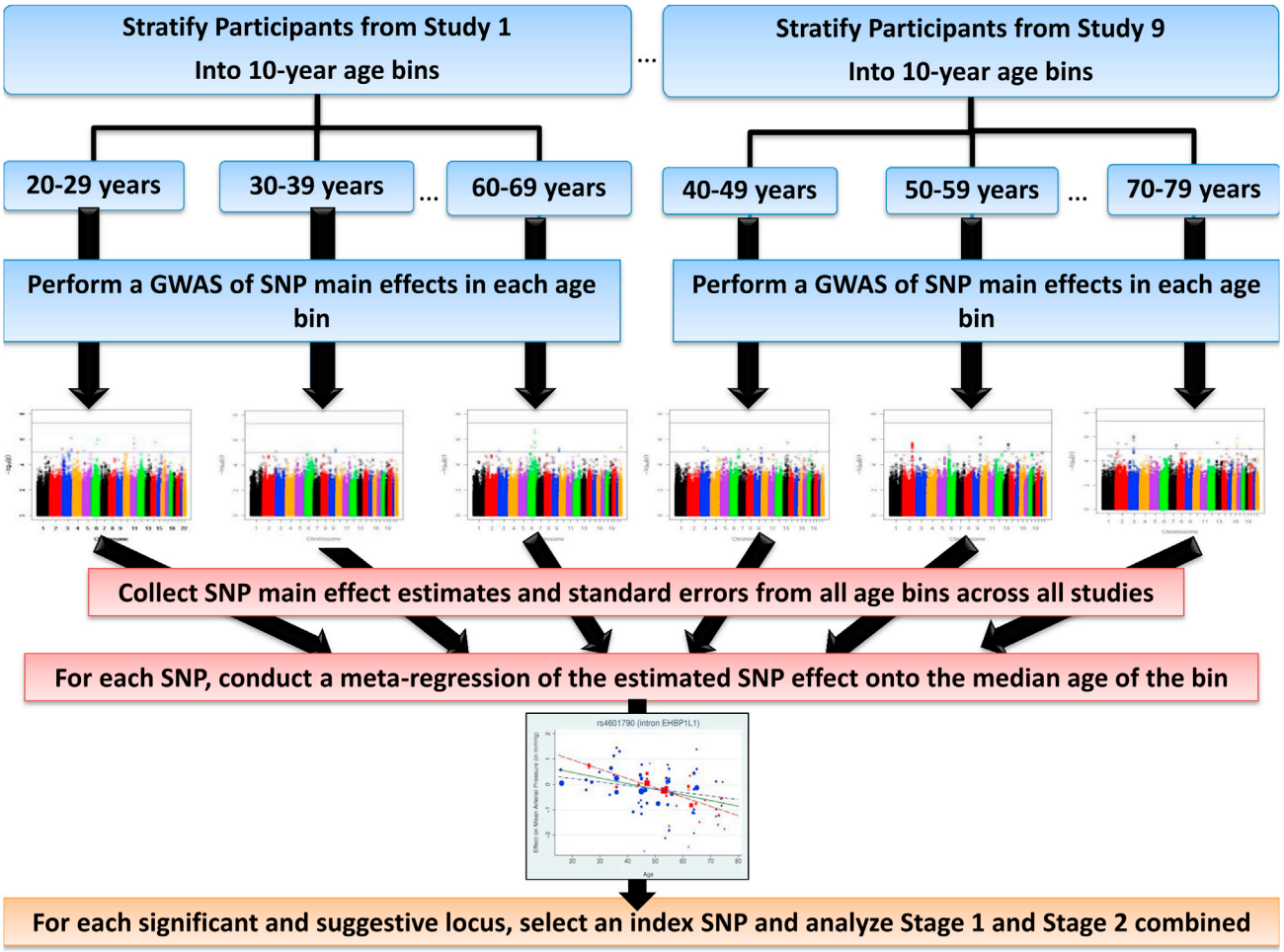


Figure 1. Study Design for the Primary Analysis

from genome-wide association studies (GWASs). Most aggregate studies have focused on the discovery of genetic main effects, relying on the meta-analysis of GWASs that included age as a continuous covariate in the study-specific analyses.[11–16] Not only do these studies fail to provide any knowledge about the change in genetic effects over time, but they use age adjustments that do not sufficiently control for the confounding by age[6–10] and they meta-analyze studies with substantially different age distributions (such as containing only the young or the elderly), which may obscure genetic effects that are age dependent. The primary aim of this investigation was to identify both known and novel BP loci whose magnitude of genetic effects differed by age. Identifying such gene-age interactions can provide insight into the biology and temporal regulation of known BP genes and facilitate the discovery of BP genes obscured in a main-effects-only analysis.

We employed age stratification and metaregression to identify BP loci whose magnitude of genetic effects differ by age (see Figure 1 for an overview of the design). This nonstandard approach was borne out of a previous analysis in which we failed to identify any loci when gene-age interaction analysis was performed within each study and the results meta-analyzed. Realizing that the null results could be due to study design issues relating to the way age was handled rather than a true lack of interactions, we developed a more computationally intensive alternative. We stratified participants from each study into 10-year age bins and conducted a GWAS of each BP trait (systolic BP [SBP], diastolic BP [DBP], mean arterial pressure [MAP], and pulse pressure [PP]) within each subgroup (representing a study and age bin). We then collected the SNP effect estimates (the coefficients from the GWAS that indicated the change in BP for each copy of the coded allele) and standard errors from all subgroups. We identified significant gene-age interactions through linear regression of the SNP effect estimates onto the median age of each subgroup; we referred to this as metaregression because the SNP effect estimates and the median ages were subgroup-level variables instead of measures on individuals and we weighted the subgroup results according to their precision (by the inverse variance of the SNP effects from the GWAS).

We also conducted a secondary "within-age bins" analysis to interrogate the 30%–40% of genetic variance in

BP that is generally attributed to age-specific genetic effects[2] and the differential influence of genetic mechanisms during different periods of life.[17] For the secondary analysis, we meta-analyzed the genetic effects across all studies within each age strata separately (e.g., a meta-analysis of 20- to 29-year-olds only). Overall, we show that explicit modeling of the age dependency of genetic effects can enhance our understanding of intraindividual variation in complex traits.

Subjects and Methods

Subjects

Participants from each study provided written informed consent and all studies received approval from their respective institutional review boards.

Stage 1 Samples

The stage 1 analysis (N = 55,796) included nine studies from the Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) Consortium: Age, Gene/Environment Susceptibility-Reykjavik (AGES; N = 3,128), Atherosclerosis Risk in Communities (ARIC; N = 9,306), Coronary Artery Risk Development in Young Adults (CARDIA; N = 1,713), Cardiovascular Health Study (CHS; N = 2,902), Framingham Heart Study (FHS; N = 7,520), Multi-Ethnic Study of Atherosclerosis (MESA; N = 2,339), Rotterdam Study I (RS I; N = 4,389), Rotterdam Study II (RS II; N = 1,912), and the Women's Genome Health Study (WGHS; N = 22,587). Participants aged <20 years or ≥80 years were excluded from the stage 1 samples except for the 17- to 20-year-olds included in the CARDIA Study; the latter was targeted to young adults, and therefore all subjects in this sample ranged from 17 to 32 years old. Detailed descriptions of the study designs and summary statistics are provided in the Supplemental Data and Tables S1 and S2.

Stage 2 Samples

Stage 2 included 15 studies and 43,445 participants of European ancestry, largely from the Global Blood Pressure Genetics Consortium (Global BPgen) and the ICBP (International Consortium for Blood Pressure). The stage 2 studies included the Busselton Health (BHS; N = 1,135), Cohorte Lausannoise (CoLaus; N = 4,943), European Prospective Investigation of Cancer-Norfolk (EPIC; N = 2,407), Fenland (N = 1,399), Kooperative Gesundheitsforschung in der Region Augsburg Third Survey (KORA S3; N = 1,594), LifeLines Cohort (N = 8,088), Myocardial Infarction Genetics Consortium (MIGen; N = 1,196), Netherlands Study of Depression and Anxiety (NESDA; N = 1,547), Prevention of Renal and Vascular End Stage Disease (PREVEND; N = 3,303), Precocious Coronary Artery Disease (PROCARDIS; N = 7,050), SardiNIA (N = 1,248), Study of Health In Pomerania (SHIP; N = 4,058), Supplementation en Vitamines et Mineraux Antioxydants (SUVIMAX; N = 1,673), Tracking Adolescent's Individual Lives Survey (TRAILS; N = 1,556), and the Young Finns (YFS; N = 2,248) studies. Detailed descriptions of the study designs and summary statistics are provided in the Supplemental Data and Tables S4 and S5. Individuals aged 20–80 years old were included in the analysis, along with the TRAILS clinical and population cohorts that included individuals <20 years old.

Singapore Samples

The Singapore samples included four studies of Asians comprised of 8,682 Chinese, Indian, and Malay individuals from Singapore. These studies were the Singapore Chinese Eye (N = 1,849), Singapore Indian Eye (N = 2,476), Singapore Malay Eye (N = 2,502), and the Singapore Prospective Study Program (N = 1,855). Detailed descriptions of the study designs and summary statistics are provided in the Supplemental Data and Tables S6 and S7.

Phenotypes

Blood pressure (BP) measurements and covariates were selected from a single visit that maximized the sample size or age range of the study. Each study conducted phenotype harmonization on systolic blood pressure (SBP) and diastolic blood pressure (DBP). For individuals on antihypertensive medications at the time of the chosen clinic visit, 10 and 5 mmHg were added to the measured SBP and DBP, respectively.[18] The addition of a constant to the measured BP in treated participants has been shown to increase statistical power and reduce shrinkage bias, compared to no medication adjustment or the exclusion of treated individuals.[19] Mean arterial pressure (MAP) and pulse pressure (PP) were calculated from the medication-adjusted SBP and DBP values as MAP = SBP/3 + 2DBP/3 and PP = SBP − DBP. Outliers, defined as those with BP values that were at least four standard deviations away from the mean of their subgroup (defined by study and age bin), were excluded from the analysis.

Genotypes

The genotyping platforms, SNP quality control filters, imputation software, and reference human genome used varied by study and are detailed in Tables S2, S5, and S7. Each study imputed the allele dosages for ~2.5 million SNP genotypes.

Association Analyses within Each Study-Age Bin Subgroup

Each stage 1 study stratified participants into six 10-year age bins (20–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, and 70–79 years) for a total of 28 subgroups (defined by study and age bin); the CARDIA study used one age bin from 17 to 32 years of age (the entire study sample). For age bins containing more than 250 individuals, a genome-wide association analysis (GWAS) of SNP main effects was conducted by regressing each BP trait (SBP, DBP, MAP, and PP) onto the allele dosage (the observed [genotyped data] or estimated [imputed data] number of copies of the coded allele in an individual) while adjusting for age, age-squared, body-mass-index, gender, and field center (if a multicenter study). The adjustment for both age and age-squared allowed age to have a nonlinear main effect on BP as suggested by multiple longitudinal studies.[20–22] The estimated SNP effect (the coefficient for the allele dosage) from the GWAS represented the BP change associated with each copy of the coded allele in that age bin. The GWAS analysis software used by each stage 1 study is detailed in Table S2. Genomic control was applied to the GWAS results from each stage 1 subgroup to control for population stratification (the genomic inflation factors, λ, ranged from 0.977 to 1.057; see Table S3).

The stage 2 and Singapore studies adopted a similar strategy: they stratified participants into 10-year age bins, combining adjacent age bins when necessary to achieve a sufficient sample size (two studies used an age bin for 20- to 39-year-olds, one study used an age bin for 60- to 79-year-olds, and another used age bins for 35- to 49-year-olds and 50- to 64-year-olds). Because many of the stage 2 and Singapore studies had smaller sample sizes than did the stage 1 studies, the association analysis was performed in all subgroups containing more than 124 individuals

(see Tables S5 and S7 for analysis software). After stage 1 analysis, the SNP with the smallest p value by the 2 df test, the "index" SNP, was chosen to represent each locus for each trait. The stage 2 and Singapore analyses were conducted only for these index SNPs, and therefore no genomic control was applied to their analyses. In all stages of this investigation, family-based studies maintained independence between bins and applied analysis methods to account for correlations between family members in the same bin.

Harmonization of Subgroup-Specific Association Results

The association results were harmonized to ensure that the beta coefficients from different subgroups represented the effect of the same allele on the BP trait. Autosomal SNPs were aligned to the positive strand of HapMap release 22 via NCBI Build 36. The LiftOver utility mapped SNP coordinates between NCBI builds. We supplemented the quality control performed by the individual studies by excluding (1) genotyped SNPs called in fewer than 90% of participants or with Hardy-Weinberg $p < 10^{-6}$ and (2) imputed SNPs with $r^2 < 0.3$ (ratio of the empirically observed variance of the allele dosage to the expected binomial variance). We further excluded SNPs with fewer than 40 copies of the minor allele in any stage 1 subgroup or fewer than 20 copies in the stage 2 or Singapore subgroups.

Aggregate Analyses

Metaregression to Reveal SNP-Age Interactions

For each BP trait (SBP, DBP, MAP, and PP), we collected the estimated SNP effects and standard errors (multiplied by the square root of the genomic inflation factor) from the stage 1 subgroup analyses. We performed a metaregression of the SNP association coefficients onto an intercept and the median age of the subgroup (using PROC MIXED in SAS 9.1, SAS Institute). We let α_i be the SNP main effect and age_i be the median age of included individuals from the GWAS of the subgroup indexed by i. We fit the regression $\alpha_i = \beta_0 + \beta_1 * age_i + e_i$, where the errors, e_i, were assumed to be independent and normally distributed with zero means and variances equal to that of the SNP coefficients from the subgroup-specific association analyses. The coefficient for the median age (β_1) represented the change in the SNP effect with each year of age (the gene-age interaction) and the intercept (β_0) represented the hypothetical SNP effect at age 0; the predicted SNP effect at a particular age was the addition of the intercept and the product of that age and the coefficient for age.

We performed a joint 2 degree of freedom (df) likelihood ratio test that there was no SNP main effect or SNP-age interaction ($\beta_0 = 0$ and $\beta_1 = 0$). We also performed a 1 df test of the SNP-age interaction ($\beta_1 = 0$), although this test was used to gauge whether interaction was driving the 2 df test and whether the 1 df interaction test could enhance gene discovery efforts. After metaregression, we applied genomic control to the joint 2 df tests (λ varied between 1.07 and 1.09) and 1 df interaction tests (λ varied between 1.02 and 1.03); Figure S1 contains the quantile-quantile plots for the raw p values.

We identified all suggestive ($5 \times 10^{-8} < p \leq 10^{-6}$) and significant ($p \leq 5 \times 10^{-8}$) results from the joint 2 df tests for each trait and divided them into distinct loci based on regional plots that extended up to one megabase in each direction from the most-significantly associated SNP ($r^2 < 0.4$ considered separate loci); we selected one index SNP (the most-significantly associated by the 2 df test) for each locus-trait combination and conducted separate metaregressions with the stage 2 subgroups. We then conducted a combined metaregression of all stage 1 and stage 2 subgroups for each index SNP-trait combination.

Main-Effects-Only Meta-analysis of Index SNPs

To determine whether the index SNP-trait associations would have been detected in a main-effects-only analysis, we conducted an inverse-variance weighted meta-analysis of SNP main effects by using the stage 1 and combined stages 1 and 2 subgroups (with SAS v.9.1). Genomic control was applied to the stage 1 meta-analysis results for each trait (λ varied between 1.10 and 1.17; see Figure S1) because the inflation factors were available. No genomic control adjustment was applied to the main-effects-only meta-analysis of stage 2 subgroups because we analyzed only the index SNPs.

Evaluating Significant Stage 1 and Combined Stages 1 and 2 Results in Singapore Subgroups

For each index SNP that achieved genome-wide significance in either the stage 1 or the combined stages 1 and 2 metaregression analyses, we conducted a joint 2 df test and a 1 df main-effects-only test using all Singapore subgroups. We performed both the main-effects-only and joint 2 df tests to evaluate potential differences in aging and interactions across populations. The age distributions in Singapore and stage 1 were similar (4.7% and 7.5% of participants were under 40 years of age, respectively, versus 21.9% of participants in stage 2), so we followed up significant SNPs from stage 1 even if they were not significant in the combined analysis with stage 2. Loci with main effects or linear gene-age interactions limited to those more than 40 years of age might be detected in the stage 1 and Singapore subgroups only. To maintain a 0.05 level of significance, a Bonferroni adjustment was applied for the two tests and the number of index SNPs chosen for that trait.

Secondary Within-Age Bins Meta-Analysis

We conducted an inverse-variance weighted meta-analysis of the SNP main effects separately within each age bin (i.e., meta-analysis using all subgroups in the 20–29 years age bin). We used the METAL software[23] to perform the genome-wide meta-analysis in each age bin that contained two or more stage 1 studies. The 30–39 years age bin contained only one study, so five meta-analyses were conducted for each BP trait. Genomic control was applied after meta-analysis (λ varied between 1.00 and 1.045; see Table S17). Significant and suggestive associations from each meta-analysis were separated into loci (regions that were suggestive/significant were narrow and spanned <110 kilobases each). The index SNP chosen to represent each locus-trait association was followed up in a combined meta-analysis of all stage 1 and stage 2 subgroups from the corresponding age bin, as well as the Singapore subgroups. Because some replication bins used different age ranges, the median of the replication bin determined age bin membership. The TRAILS cohort was included in replication analyses for the 20- to 29-year-olds.

Results

Table 1 displays the age distribution of the subjects in each stage 1 and stage 2 study. The narrow age ranges in CARDIA, CHS, and TRAILS demonstrate the utility of the age bin approach; these studies would have contributed little information to the meta-analysis if we incorporated gene-age interactions into these study-level analysis even

Table 1. Age Distribution of Each Stage 1 and Stage 2 Study

	Number of Individuals in Each Age Bin					
Study	**20–29**	**30–39**	**40–49**	**50–59**	**60–69**	**70–79**
Stage 1 Metaregression: 28 GWASs with N = 55,796						
AGES			1,260	1,603	265	
ARIC			2,392	4,772	2,142	
CARDIA	1,713					
CHS					1,230	1,672
FHS	533	1,926	2,608	1,916	537	
MESA			342	708	726	563
RS1				910	2,060	1,419
RS II				740	851	321
WGHS			7,219	10,386	4,271	711
Total	2,246	1,926	13,821	21,035	12,082	4,686
Stage 2 Metaregression: 59 GWAS with N = 43,445						
BHS		276	223	225	207	204
CoLaus		534	1,437	1,334	1,195	443
EPIC			442	775	819	371
Fenland		388	607	404		
KORA S3			191	984	419	
LifeLines	393	1,576	3,039	1,893	899	288
MIGen		124	527	391	154	
NESDA	340	361	424	422		
PREVEND		853	980	820	650	
PROCARDIS			649	2,399	3,362	640
SardiNIA	287		232	268	257	204
SHIP	550	729	726	760	733	560
SUVIMAX			819	854		
TRAILS CC	266					
TRAILS Pop	1,290					
YFS		1,562	686			
Total	3,126	6,403	10,982	11,529	8,695	2,710

Age bins that encompassed more than one decade were assigned the bin containing their median age.

though they provided information on undersampled age groups. For the stage 1 meta-analysis of gene-age interactions, we analyzed 28 GWASs (one for each study and age bin subgroup) per trait representing 55,796 individuals of European ancestry. We then followed up the significant ($p \leq 5 \times 10^{-8}$) and suggestive ($5 \times 10^{-8} < p \leq 10^{-6}$) loci in a combined analysis of the stage 1 subgroups with 59 stage 2 subgroups comprised of 43,445 participants of European ancestry. A Singapore sample, which included 19 subgroups containing 8,682 Chinese, Indian, and Malay individuals, was used to assess the interethnic generalizability of significant findings. An overview of the primary results is provided in Figure 2.

Metaregression of Blood Pressure onto Age: Identifying Linear Gene-Age Interactions

In the metaregression of stage 1 subgroups, 13 loci attained genome-wide significance and 17 loci exhibited suggestive evidence for at least one BP trait by a 2 df joint test of the SNP main effect and SNP-age interaction (see Tables S8, S9, S10, S11, and S12). Ten of these 30 loci were not reported in published GWAS results, including the Fer-1-like 5 (*FER1L5*) locus that achieved genome-wide significance. Eleven of the significant or suggestive loci demonstrated nominal ($p \leq 0.05$) evidence of age dependency through the 1 df test of SNP-age interaction. For each trait, we selected an index SNP (most significantly associated by the 2 df test) to represent each significant or suggestive locus so that the stage 2 analyses could be conducted. A total of 63 index SNP-trait combinations were followed up across the 30 loci. A total of 20, 17, 22, and 4 SNPs were followed up for SBP, DBP, MAP, and PP, respectively (Figure S2 contains the regional association plots for the stage 1 analyses, created with LocusZoom[24]).

As shown in Table 2, 20 loci harbored index SNPs that were significant in the combined metaregression of stage 1 and stage 2. The strongest statistical evidence for interaction was provided by the EH domain binding protein 1-like 1 (*EHBP1L1*) locus associated with MAP ($p = 2.9 \times 10^{-7}$ for the 1 df interaction test); this locus was discovered only through the inclusion of the age interaction (Figure S2 contains the regional plot for this locus). Of the 20 loci that achieved genome-wide significance, 9 exhibited at least nominal ($p \leq 0.05$) evidence of gene-age interactions (see Table 2). The index SNPs in *CASZ1* (MIM 609895), *EHBP1L1*, and *GOSR2* (MIM 604027) exhibited the largest modulation of BP effects by age (as shown by the magnitude of the interaction coefficients), with the coded alleles increasing their respective BP traits in young individuals but decreasing them in older individuals (see Figure 3). For these three loci, the estimated difference in SNP effects on the primary trait for 20-year-olds compared to 80-year-olds ranged from 1.17 mmHg to 1.58 mmHg. The age at which the variant changed direction of effect was ~27 years for *GOSR2*, 33 (SBP) to 36 (MAP) years for *CASZ1*, and 41 (MAP) to 42 (DBP) years for *EHBP1L1*.

As shown in Table S13, five loci (*EHBP1L1*, *CASZ1*, *MAP4* [MIM 157132]-*CDC25A* [MIM 116947], *CCDC71L-PIK3CG* [MIM 601232], *GOSR2*) would have been missed by the two-stage main-effects-only meta-analysis but were captured by the two-stage joint 2 df tests. Four of these five loci (*MAP4-CDC25A*, *CCDC71L-PIK3CG*, *EHBP1L1*, *GOSR2*) lacked suggestive main effects in the stage 1 main-effects-only analysis and would not have been followed up in stage 2, and the *CASZ1* locus would have been followed up but failed to achieve genome-wide significance in the combined stage 1 and stage 2 main-effects-only analysis.

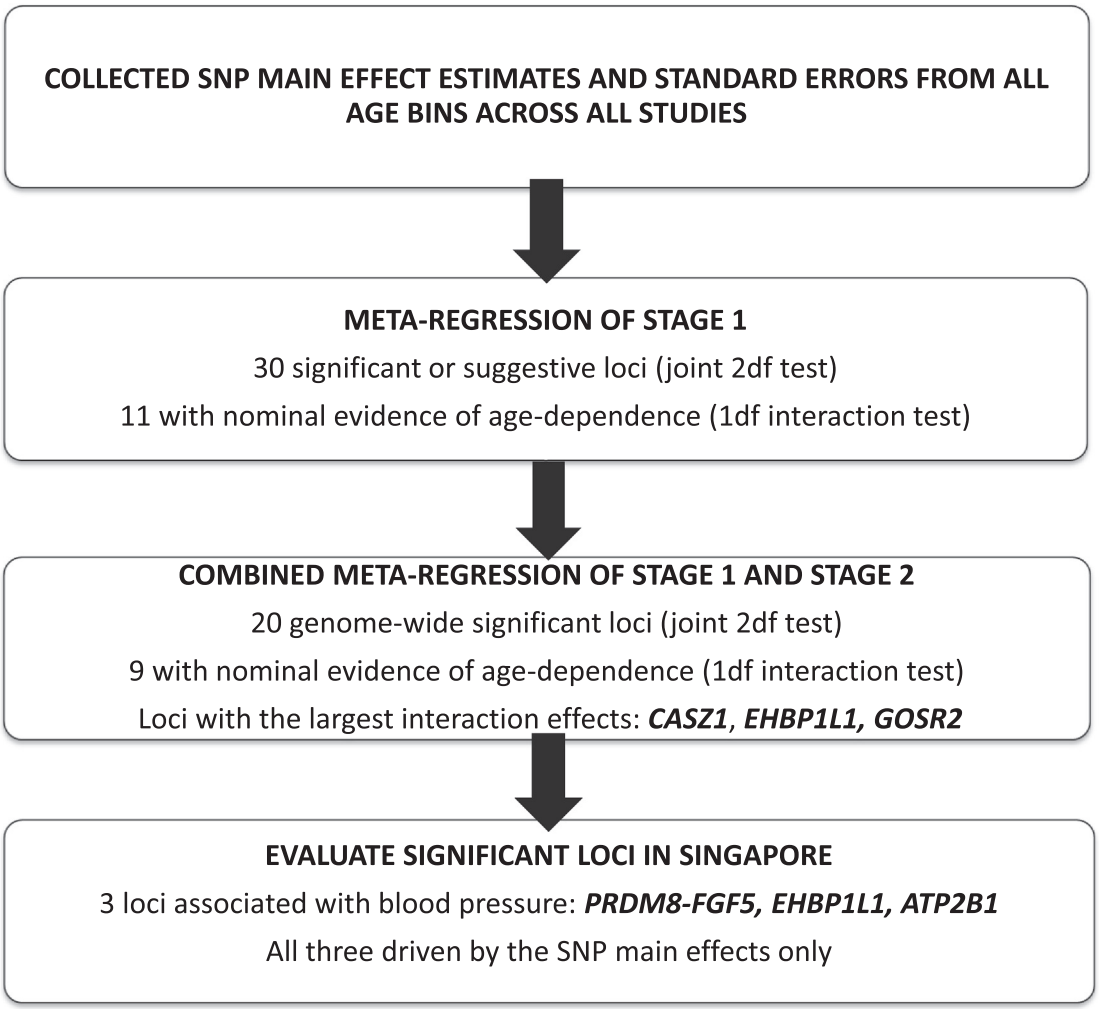


Figure 2. Overview of Results from Each Stage of the Primary Analysis

In summary, the joint analysis of SNP main effects and SNP-age interactions by metaregression identified 20 genome-wide significant loci, 9 of which exhibited nominal gene-age interactions. Five loci, including the *EHBP1L1* locus with the strongest statistical evidence of interaction, were missed when SNP-age interactions were excluded from the model.

Generalizability of Genome-wide Significant Associations to Singapore Subgroups

We examined the interethnic generalizability of the 47 index SNP-trait associations (from 22 loci) that achieved genome-wide significance in the metaregression of stage 1 subgroups only (*AGT* [MIM 106150] and *FER1L5* loci) or in the metaregression of the combined stage 1 and stage 2 subgroups (the 20 loci in Table 2). We evaluated 13, 15, 17, and 2 SNPs for SBP, DBP, MAP, and PP, respectively; however, two SNPs were not available in the Singapore subgroups. Because of the potential differences in aging and interactions across ethnic populations, we performed the SNP main-effects-only test and the joint 2 df test in the meta-analysis of Chinese, Indian, and Malay subgroups from Singapore. After a Bonferroni correction for the planned number of SNPs tested per trait and the two tests performed, 2, 1, 2, and 0 SNPs met the significance threshold for SBP ($p \leq 1.92 \times 10^{-3}$), DBP ($p \leq 1.67 \times 10^{-3}$), MAP ($p \leq 1.47 \times 10^{-3}$), and PP ($p \leq 0.0125$), respectively, corresponding to three loci replicating an association for at least one BP trait. The Singapore subgroups confirmed an association between *EHBP1L1* and DBP (main effects only $p = 8.3 \times 10^{-4}$), as well as the associations between SBP and MAP with the *PRDM8-FGF5* (MIM 165190) and *ATP2B1* (MIM 108731) loci.

As shown in Table S13, the index SNPs in *CASZ1*, *CCDC71L-PIK3CG*, *EHBP1L1*, and *GOSR2*, which were identified through the joint 2 df test in the primary analysis, had stronger evidence of main effects ($p < 0.05$) than interactions (tested with the joint 2 df test) in Singapore subgroups. The missense variant in *FER1L5* that was significantly associated with DBP in the stage 1 analysis demonstrated nominal ($p = 0.03$) evidence of a main effect in the same direction in the meta-analysis of Singapore subgroups. In addition, an intronic variant (rs11741255) in *C5orf56* that demonstrated suggestive

Table 2. Significant Findings from the Combined Metaregression of Stage 1 and Stage 2 Subgroups

SNP ID	Chr	Position (in basepairs)	Genomic Location	Primary Trait	Other Trait(s)	N_S	N	Ref Freq	Ref All	p Value Main-Effects-Only Model	Metaregression Model with SNP Main Effect and Age Interaction (2 df)					
											β_0	se(β_0)	β_1	se(β_1)	p Value of Interaction	p Value of 2 df Test
At Least Nominal Evidence ($p < 0.05$) of Interactions																
rs880315	1	10,719,453	intron *CASZ1*	SBP	MAP	56	74,498	0.64	T	2.35×10^{-7}	0.861	0.353	−0.026	0.007	1.52×10^{-4}	1.21×10^{-9}*
rs6797587	3	48,172,618	near 3′ *CDC25A*	MAP	DBP[a]	87	99,189	0.68	G	4.69×10^{-11}*	0.748	0.202	−0.008	0.004	0.04	4.36×10^{-11}*
rs11099098	4	81,388,936	intergenic (*PRDM8-FGF5*)	SBP	MAP	81	96,217	0.29	T	2.85×10^{-13}*	−0.199	0.335	0.016	0.007	0.02	1.56×10^{-13}*
rs198846	6	26,215,442	downstream *HIST1H1T*	DBP	MAP[a]	87	99,207	0.84	G	1.78×10^{-13}*	0.088	0.250	−0.011	0.005	0.03	1.48×10^{-13}*
rs12705390	7	106,198,013	intergenic (*CCDC71L-PIK3CG*)	PP		87	99,094	0.78	G	1.08×10^{-12}*	0.281	0.264	−0.014	0.005	0.006	2.42×10^{-13}*
rs7070797	10	63,221,779	intergenic (*C10orf107-ARID5B*)	MAP	SBP, DBP[a]	87	99,189	0.84	G	6.31×10^{-19}*	0.014	0.282	0.012	0.006	0.02	5.62×10^{-19}*
rs4601790	11	65,110,482	intron *EHBP1L1*	MAP	DBP	87	99,188	0.27	G	0.001	0.909	0.220	−0.022	0.004	2.90×10^{-7}	9.93×10^{-9}*
rs11072518	15	73,021,663	upstream *COX5A*	MAP	SBP, DBP	87	99,189	0.36	T	3.91×10^{-21}*	0.973	0.192	−0.010	0.004	0.006	1.11×10^{-21}*
rs17608766	17	42,368,270	intron or UTR 3′ of *GOSR2*	PP		86	97,437	0.84	T	5.62×10^{-9}*	0.524	0.322	−0.019	0.006	0.003	4.49×10^{-10}*
Joint Test Driven by Main Effects Only																
rs7537765	1	11,809,890	intron *CLCN6*	MAP	SBP[a], DBP	87	99,181	0.16	G	1.66×10^{-19}*	−0.957	0.249	0.008	0.005	0.12	5.58×10^{-19}*
rs6707357	2	164,722,539	intergenic (*FIGN-GRB14*)	SBP		87	99,177	0.45	T	1.49×10^{-11}*	−0.634	0.268	0.004	0.005	0.50	1.03×10^{-10}*
rs7733138	5	157,807,971	intergenic (nearest gene *EBF1*)	MAP	DBP[a]	87	99,189	0.39	T	6.01×10^{-13}*	0.006	0.194	−0.007	0.004	0.05	8.75×10^{-13}*
rs4841569	8	11,489,586	intergenic (*BLK-GATA4*)	SBP	MAP	82	97,928	0.57	G	5.56×10^{-10}*	0.140	0.311	0.008	0.006	0.21	2.03×10^{-9}*
rs1813353	10	18,747,454	intron *CACNB2*	MAP	SBP, DBP	87	99,189	0.68	T	1.29×10^{-17}*	0.564	0.202	−0.003	0.004	0.52	1.13×10^{-16}*
rs11191454	10	104,649,994	intron *AS3MT*	SBP		84	97,234	0.08	G	6.50×10^{-12}*	−0.320	0.484	−0.011	0.010	0.25	2.98×10^{-11}*
rs1801253	10	115,795,046	missense *ADRB1*	MAP	SBP[a], DBP	82	97,928	0.27	G	7.71×10^{-14}*	−0.336	0.215	−0.002	0.004	0.71	6.86×10^{-13}*
rs381815	11	16,858,844	intron *PLEKHA7*	MAP		87	99,189	0.25	T	3.31×10^{-9}*	0.028	0.217	0.006	0.004	0.16	9.26×10^{-9}*
rs2681472	12	88,533,090	intron *ATP2B1*	SBP	DBP[a], MAP	87	99,177	0.17	G	4.59×10^{-23}*	−0.483	0.348	−0.008	0.007	0.24	2.84×10^{-22}*
rs3184504	12	110,368,991	missense *SH2B3*	MAP	SBP, DBP[a]	87	99,187	0.48	T	1.17×10^{-21}*	0.325	0.186	0.003	0.004	0.47	1.09×10^{-20}*
rs260014	20	57,192,854	upstream *ZNF831*	MAP	SBP[a], DBP	82	97,941	0.85	T	1.50×10^{-11}*	−0.302	0.284	−0.004	0.006	0.49	1.02×10^{-10}*

Abbreviations are as follows: Chr., chromosome; N_S, number of study and age bin subgroups included in the analysis; N, number of participants represented by the analysis; Ref Freq, frequency of the coded allele; Ref All, the coded allele; se, standard error; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure. The primary trait had the minimum p value for the joint 2 df test of the index SNP in that locus. The other traits column indicates nonprimary traits significantly associated with SNPs in this locus.

[a]The index SNP for this trait differed from the index SNP for the primary trait (see Tables S8, S9, S10, S11, and S12). The p value main effect test was derived from the model containing only the SNP main effect (i.e., test that the intercept is zero). For the model containing the SNP main effect and age interaction, β_0 is the theoretical SNP effect on blood pressure (in mmHg) at birth (age = 0) and β_1 is the change in the SNP effect on blood pressure (in mmHg) per 1 year increase in age; the estimated SNP effect at a particular age was the addition of the intercept and the product of that age and the coefficient for age. Asterisks (*) indicate values that achieve genome-wide significance.

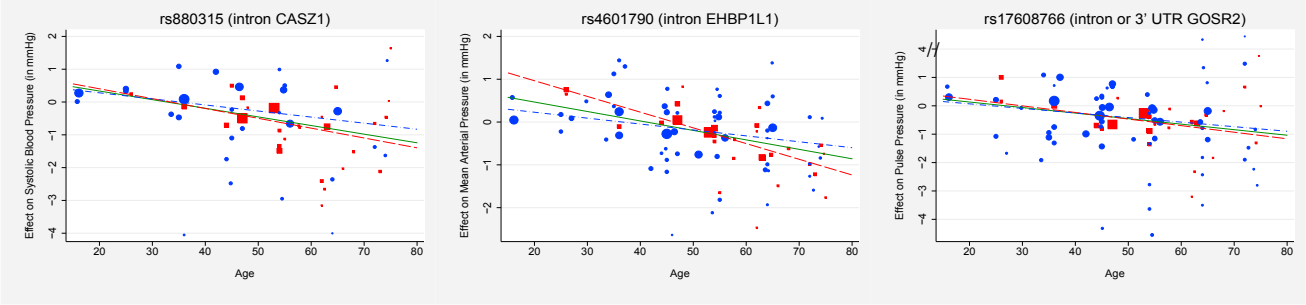


Figure 3. CASZ1, EHBP1L1, GOSR2, the Three Loci Exhibiting the Largest SNP-Age Interactions during the Combined Metaregression of Stage 1 and Stage 2 Subgroups
The figures display the SNP effect as a function of age. Study- and age bin-specific genetic effects from stage 1 and stage 2 are represented by red squares and blue circles, respectively, with the symbol size proportional to the inverse variance of the SNP main effect. The corresponding stage 1, stage 2, and combined consortia metaregressions are represented by red long-dashed, blue dashed-dotted, and green solid lines, respectively. The coded alleles of all three index SNPs are associated with increased blood pressure in the young but reduced blood pressure in the elderly.

evidence in the stage 1 analysis but that appeared to be driven by main effects achieved nominal ($p = 0.04$) evidence of a main effect in the same direction in the meta-analysis of the Singapore subgroups. The *C5orf56* index SNP, only 6.1 kb away from interferon regulatory factor 1 (*IRF1* [MIM 147575]), had a much larger effect size (2.28 mmHg versus 0.35 mmHg) and smaller minor allele frequency (0.02 versus 0.40) in Singapore subgroups than in stage 1 subgroups. Variants near or in *C5orf56* have been associated with biomarkers and diseases of inflammation such as for fibrinogen,[25,26] C-reactive protein,[27] and Crohn disease.[28,29]

In summary, the Singapore samples confirm associations between BP and the *EHBP1L1*, *PRDM8-FGF5*, and *ATP2B1* loci. Many of the loci found through age interactions in the populations of European descent exhibited stronger evidence of main effects in Singapore samples, indicating potential interethnic heterogeneity in age interactions.

Using the One Degree-of-Freedom Test to Detect Interactions

In the primary analysis, we used the 1 df interaction test to determine whether the associations identified by the joint 2 df test were driven by SNP main effects alone. To gauge the role of the 1 df interaction test in finding interaction loci, we repeated the two-stage metaregression analyses with the 1 df interaction test instead of the joint 2 df test. Only three loci exhibited significant (*RAB31* [MIM 605694]) or suggestive (*EHBP1L1* and *PGBD4-KATNBL1*) associations by the 1 df interaction test in the stage 1 analysis (see Tables S14, S15, and S16); the coded allele of SNP rs7233332 in an *RAB31* intron was associated ($p = 2.95 \times 10^{-8}$) with a decrease in PP for individuals aged <49.5 years and an increase in PP thereafter. However, this significant association failed to replicate in the stage 2 or Singapore subgroups. None of these suggestive or significant loci achieved genome-wide significance for the 1 df interaction test using the combined stage 1 and stage 2 analysis (see Table S16). Thus, the 1 df interaction test failed to produce any novel or known replicated loci, underscoring the importance of the joint 2 df test for identifying gene-age interactions.

The Secondary Analysis: Exploring Age-Specific Genetic Effects

As a secondary analysis, we explored age-specific genetic effects by meta-analyzing the GWAS results (SNP main effects) within each age bin separately (e.g., 20- to 29-year-old subgroup only). The second age bin (30- to 39-year-olds) contained only one study in stage 1; therefore, five age-bin-specific meta-analyses were conducted per trait. A total of 22 distinct loci (31 SNP-trait combinations) were significantly or suggestively associated with BP traits in the stage 1 analyses, yielding, respectively, 9, 6, 12, and 4 loci for SBP, DBP, MAP, and PP (see Tables S18, S19, S20, S21, and S22). Each locus was significant or suggestive in only one age bin; it is unlikely that a single 10-year age bin will isolate the age-dependent effect, and therefore the lack of a supportive pattern in adjacent bins may indicate a false positive or may be due to statistical issues (such as the lack of data for a meta-analysis in the adjacent 30- to 39-year age bin, differences in sample sizes between bins, or differences in study composition between age bins [such as the CARDIA study, which is present only in the youngest age bin]). In total, we identified six loci in the 20–29 years age bin ($N \approx 2{,}200$), five loci in the 40–49 years age bin ($N \approx 13{,}800$), six loci in the 50–59 years age bins ($N \approx 21{,}000$), four loci in the 60–69 years age bin ($N \approx 12{,}100$), and one locus in the 70–79 years age bin ($N = 3{,}014$). Thirteen of these loci (see Table 3), including all six in the youngest age bin and the lone finding in the oldest age bin, lacked strong evidence in the literature and failed to achieve even suggestive associations in the main-effects-only meta-analyses and SNP-age metaregressions using all age bins. This demonstrates the importance and promise of meta-analysis across cohorts within age bins. In the 20- to 29-year-olds, a SNP (rs16833934) in a locus near microRNA 1263 (*MIR1263*)

Table 3. Selected Significant and Suggestive Findings from the Within-Age-Bin Meta-analysis of Stage 1 Subgroups

Age Bin	rs Number (NCBI 36)	Chr.	Position	Genomic Location	Ref Allele	Trait	No. Studies	N	All Freq	β (in mmHg)	se (β)	λ	p Value	Direction of Effects
20–29	rs16833934	3	165,219,944	intergenic near *MIR1263*	G	DBP	2	2,242	0.26	−1.63	0.29	1.00	1.39×10^{-8}*	- -
						MAP	2	2,241	0.26	−1.33	0.27	1.02	7.12×10^{-7}	- -
	rs12195230	6	97,606,768	intron *KLHL32*	G	SBP	2	2,246	0.75	1.79	0.35	1.01	4.60×10^{-7}	++
	rs12195036	6	166,371,687	near *LINC00602*	T	MAP	2	2,241	0.95	−3.11	0.61	1.02	5.04×10^{-7}	- -
	rs2702888	8	6,752,442	*DEFB1-DEFA6*	G	PP	2	2,242	0.65	−1.36	0.27	1.00	3.87×10^{-7}	- -
	rs2196122	11	4,842,124	*OR51H1P-OR51H2P*	G	SBP	2	2,246	0.84	1.91	0.38	1.01	7.20×10^{-7}	++
	rs10143078	14	69,951,242	intron *SYNJ2BP*	C	SBP	2	2,246	0.04	−4.06	0.81	1.01	5.93×10^{-7}	- -
40–49	rs825937	2	4,785,902	near *LINC01249*	C	PP	5	13,810	0.83	0.83	0.16	1.01	2.32×10^{-7}	+++++
	rs11816631	10	99,552,562	*SFRP5-GOLGA7B*	G	PP	3	9,946	0.06	1.99	0.39	1.01	2.95×10^{-7}	+++
50–59	rs3118867	9	89,451,515	intron *DAPK1*	G	DBP	7	21,033	0.47	−0.49	0.09	1.05	3.87×10^{-7}	- - - - - - -
						MAP	7	21,035	0.47	−0.55	0.11	1.04	4.12×10^{-7}	- - - - - - -
60–69	rs4638749	2	108,250,474	downstream *SULT1C3*	G	DBP	8	12,082	0.76	−0.81	0.16	1.03	6.13×10^{-7}	+- - - - - - -
	rs4841895	9	136,563,863	*RXRA-COL5A1*	G	MAP	8	12,082	0.65	0.81	0.16	1.02	7.62×10^{-7}	+++++++++
	rs747685	17	721,801	intron *NXN*	T	MAP	8	12,082	0.86	1.50	0.30	1.02	6.32×10^{-7}	+++++++++
	rs747687	17	722,084	intron *NXN*	G	DBP	8	12,082	0.86	1.37	0.26	1.03	1.50×10^{-7}	+++++++++
70–79	rs603788	10	78,881,268	intron *KCNMA1*	G	DBP	4	3,014	0.50	1.73	0.35	1.00	9.37×10^{-7}	++++
						MAP	4	3,014	0.50	2.08	0.42	1.01	8.84×10^{-7}	++++

Abbreviations are as follows: Chr, chromosome; Ref Allele, coded allele; N, number of participants meta-analyzed in the age bin; All Freq, coded allele frequency; β, effect of each copy of the coded allele on blood pressure; se(β), standard error of the β; λ, genomic inflation factor in that age bin. Age bins 1 through 6 comprised individuals 20 to 29 years old and subsequently in 10 year increments. Asterisk (*) indicates value achieves the $p \leq 5 \times 10^{-8}$ threshold for significance.

was significantly ($p = 1.39 \times 10^{-8}$) associated with a 1.63 mmHg reduction of DBP per copy of the G allele.

Although none of the index SNPs from the 13 loci listed in Table 3 were significant in the combined stage 1 and stage 2 analysis (see Table S22), the significant association near the microRNA in the young is biological plausibile because microRNAs can change gene expression during aging.[30] Four known loci (*FIGN* [MIM 605295]-*GRB14* [MIM 601524], *PRDM8-FGF5*, *AS3MT* [MIM 611806], *POC1B* [MIM 614784]-*ATP2B1*) achieved genome-wide significance during the stage 1 or combined stage 1 and stage 2 within-age bins analysis; these loci had decent stage 1 ($N \approx 14{,}000$ to 21,000) and stage 2 ($N \approx 11{,}000$) sample sizes, were associated with BP in the 40–49 or 50–59 years age strata, and were implicated in the main effects meta-analyses with all age bins.

In summary, age-specific genetic effects can influence BP and designing studies to leverage age specificity, particularly in the young, may enhance gene-discovery efforts.

Discussion

Identifying gene-environment interactions that influence common complex traits and diseases is an arduous task. Linkage and candidate gene studies indicate the presence of environment-dependent genetic effects, yet few have been identified through published genome-wide interaction studies.[31–45] The complex genetic and environmental architecture underlying blood pressure is no exception. Even though previous epidemiological studies suggest age-dependent effects, we identified 20 loci for BP in the analysis of 99,241 participants of European descent ($N = 55{,}796$ in stage 1 and $N = 43{,}445$ in stage 2), 9 of which exhibited nominal evidence of gene-age interactions. Index SNPs in *CASZ1*, *EHBP1L1*, and *GOSR2* exhibited the largest gene-age interactions, with the coded alleles increasing BP traits in the young and decreasing them in the old. The effect of each of these SNPs on a BP trait may change by as much as 1.58 mmHg over 60 years.

The *EHBP1L1* locus demonstrated the most compelling evidence for gene-age interactions: it exhibited no appreciable main effects and its discovery depended on the inclusion of gene-age interactions. A missense variant (rs6591182) in *EHBP1L1* was suggestively associated with lobular inflammation in women with nonalcoholic fatty liver disease;[46] this variant was 4,150 basepairs from our index SNP but was in low linkage disequilibrium (r^2 = 0.315). Our index SNP (rs4601790) was associated with the expression of the small ubiquitin-like modifier-1 (*SUMO1* [MIM 601912]; $p = 4 \times 10^{-5}$) in HapMap CEU samples,[47] which causes posttranslational modifications in proteins influencing apoptosis, gene transcription, and protein stability. SUMO-1 negatively regulates reactive oxygen species production from NADPH oxidases in human vascular smooth muscle cells;[48] the overproduction of reactive oxygen species has been implicated in cardiovascular and age-related disease.[48] Other potential BP effectors near the *EHBP1L1* index SNP include potassium channel subfamily K member 7 (*KCNK7* [MIM 603940]), mitogen-activated protein kinase 11 (*MAP3K11* [MIM 600050] is a positive regulator of JNK signaling pathway), and microRNA 4690 (*MIR4690*).

Several biological phenomena could contribute to gene-age interactions. For example, intracellular levels of cyclic adenosine monophosphate (cAMP) may connect aging and the effect of the known *CASZ1* locus on BP. Basal levels of cAMP may vary by age[49] and changes in intracellular cAMP may alter CASZ1b and CASZ1a mRNA levels.[50] These *CASZ1* isoforms encode zinc finger transcription factors involved in cell survival and tumor suppression.[50] After tetracycline induction of *CASZ1* in neuroblastoma cell lines, 125 genes experienced expression level changes $\geq$1.5-fold, including the potential BP effectors tyrosine hydroxylase (MIM 191290; catalyzes the rate-limiting step in the synthesis of catecholamines), dopamine beta-hydroxylase (MIM 609312), angiotensin II receptor type 1 (MIM 106165), and endothelin receptor type A (MIM 131243).[51] Thus, the dynamic nature of gene expression and posttranslational protein modification could contribute to gene-age interactions.[8,9,52] A lifetime of behavioral and environmental exposures can trigger epigenetic mechanisms, such as DNA methylation, histone modification, and microRNA expression, causing changes in gene expression during aging.[30] Increased generation of reactive oxygen species and oxidative damage with age may mediate the accumulation of posttranslational modifications to proteins, thereby causing aging and age-related diseases like hypertension.[53] Changes in the cardiovascular environment, such as the increased vascular stiffness that often accompanies aging, may result in enhanced or muted genetic effects on BP. This is clinically relevant because different treatment strategies might be warranted at different ages if the mechanisms of BP regulation vary across the age spectrum.

We gleaned several important lessons from this investigation. First, the two loci that were significantly associated with PP (*CCDC71L-PIK3CG* and *GOSR2*) lacked corroboration from any other trait, indicating that the PP association might be independent of SBP and DBP. Second, careful sample selection might balance the need for massive sample sizes. The <30-year-old age bin yielded six significant or suggestive loci using $\approx$2,240 individuals from two studies; these loci were not detected in the meta-regression or main effects meta-analysis using all age bins. Young-onset hypertension is postulated to have a stronger genetic basis than older-onset hypertension[54] because the latter may be modulated by the accumulation of behavioral and lifetime exposures. We can reduce the sample size by recruiting individuals at the age when the genetic effect is the strongest[55] or by analyzing longitudinal (repeated-measures) data. The latter increases the probability that participants are examined at the age of largest genetic effect for the largest number of variants while providing stronger evidence of causation[6] and further insight into the landscape of hypertension genetics over an individual's lifespan. An alternate explanation for the discovery of significant loci in the <30-year-olds, which also supports careful sample selection, is a more accurate phenotype due to less confounding by antihypertensive medications; frequent use of antihypertensives may have masked putative associations in the older age groups. The third lesson we learned was that genetic replication may depend on the age distribution of the replication sample if gene-age interactions are present. The utilization of the main-effects and interaction tests may help remedy the nonreplication of genetic findings across samples and ethnicities.[5] Our *EHBP1L1* locus, identified only through gene-age interactions using individuals of European ancestry, replicated using main effects only in Singapore subgroups. In addition, three of the four known loci discovered only through gene-age interactions using cohorts of European ancestry had stronger evidence of main effects in Singapore subgroups, perhaps due to the limited age range of the latter (three of the four Singapore studies contained only individuals over 40 years old). Gene-age interactions coupled with different age distributions might contribute to the observed interethnic heterogeneity of BP loci.

The age bin approach we used has some advantages compared to the standard practice (meta-analysis of study-specific GWASs that adjust for age only through simple covariate adjustments). We adjusted for body mass index (BMI), gender, age, age-squared, and field center in the GWAS conducted within each age bin; the possibility of these coefficients varying across age bins contrasts with traditional unstratified GWAS where the same adjustment is applied for each covariate across all age bins. Because the effect of BMI, gender, and the SNP may differ by age, adjustments applied within each bin might produce more accurate estimates of all the covariate effects, thus potentially amplifying the SNP effects. Furthermore, the metaregression of the age-bin results made it possible to include all studies for investigating gene-age interactions,

even those like CARDIA with a narrow age range (17- to 32-year-olds) that contribute little information otherwise (when gene-age interactions are incorporated into the study-specific analysis).

A drawback is that our age bin method required a larger computational and data management burden than the standard approach. The standard approach would have required nine GWASs per trait in the stage 1 analysis, whereas we analyzed 28 GWASs per trait. We also managed an additional 78 files per trait for the stage 2 and the Singapore analyses, bringing our total data management burden to 106 files per trait (in total, we analyzed 424 files for the 4 BP traits instead of 120 if we did not use the age bin approach, a 3.5-fold increase in the data management burden). Because many studies have unstratified GWAS results available for common traits like SBP and DBP, reanalysis using age bins may have deterred study participation. Our sample size was also slightly reduced compared to the standard approach. We omitted individuals in age bins with insufficient sample sizes (<250 for stage 1 samples and <124 for stage 2 samples) and, in order to maintain independence across age bins, included family members from only one age bin. This reduced the sample size and hence reduced the power; this, coupled with fitting an extra parameter to the model for the interaction, may explain why we missed some of the known BP-associated loci. Similarly, a few stage 2 studies combined adjacent age bins to achieve the threshold sample size for analysis. The estimated SNP effect at the median age in these wide and sparse age bins may have greater error, impacting our ability to detect gene-age interactions in the metaregression and secondary meta-analyses.

Our analysis was predicated on several assumptions. The within-age-bin meta-analyses indicated that our assumption of linear interactions and additive main effects may not be valid. We may need to expand the toolbox of methods and develop new statistical models to properly capture complex gene-age interactions.[52] We made the implicit assumption of a strong correlation between biological and chronological age;[56] this correlation may differ across individuals and populations, and age may be a very different construct due to disparities in environment and lifestyle. For example, an association identified in 30- to 40-year-olds in one population may manifest in 50- to 60-year-olds in another population. There may even be heterogenity of aging within a population; conditions such as metabolic syndrome may be associated with premature vascular stiffness and biological aging. We assumed that the same covariates were important in all age strata. Because BP levels are often modulated by various diseases in the elderly, different covariate adjustments may enhance our ability to explain the genetic variability in the older age groups. We decided a priori to use a fixed-effects metaregression model that ignored any heterogeneity in SNP effects not due to age. We ignored heterogeneity due to other population attributes and assumed that there was one true SNP effect at each age (the fixed effects model) rather than a distribution of true SNP effects at each age (the random effects model).

There were some additional limitations to our analysis. The method we used to infer the underlying BP in treated participants ignored the number, dose, and type of antihypertensive medications taken and might not accurately impute the blood pressure, particularly in resistant individuals or those on multiple medications. There were also differences in genotyping and reference panels for imputation across the studies and we restricted analysis to the index SNPs in the Singapore studies. Given that the allele frequencies and linkage disequilibrium patterns may differ across populations, this might hinder our ability to find these gene-age interactions in Singapore samples. Although BP physiology may be different in the female and male lifecourse due to hormonal regulation and menopause, we ignored sex-specific gene-age interactions.[6] Although important, stratifying by age bin and sex would have resulted in GWASs of inadequate sample sizes for many of the studies included in this investigation and lower statistical power. Two of the studies, CARDIA (stage 1) and TRAILS (stage 2), were designed to study young adults and adolescents, respectively, and contributed individuals under age 20; the inclusion of these young participants did not drive the significance of loci identified by the 2 df test because these were still significant for at least one trait when both studies were omitted from the analysis. Lastly, and importantly, this study was designed as a two-staged discovery; all promising gene-age interactions observed require replication in additional large independent samples with a diverse range of ages.

We report nine BP-associated loci whose effects might be age dependent, including the *EHBP1L1* locus, which exhibited the strongest statistical evidence of interaction and was discovered only through the inclusion of gene-age interactions. Our results highlight the context-dependent nature of genetic effects and demonstrate that modeling age-dependent effects can enhance our understanding of the temporal regulation of known genes and identify additional genes influencing intraindividual variation in complex traits like BP.

Supplemental Data

Supplemental Data include acknowledgments, study descriptions, 22 tables, and 2 figures and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.05.010.

Consortia

The members of the LifeLines Cohort Study are Behrooz Z. Alizadeh, Rudolf A. de Boer, H. Marike Boezen, Marcel Bruinenberg, Lude Franke, Pim van der Harst, Hans L. Hillege, Melanie M. van der Klauw, Gerjan Navis, Johan Ormel, Dirkje S. Postma, Judith G.M. Rosmalen, Joris P. Slaets, Harold Snieder, Ronald P. Stolk, Bruce H.R. Wolffenbuttel, and Cisca Wijmenga.

Acknowledgments

B.M.P. served on a data and safety monitoring board for a clinical trial (Zoll LifeCor) and a steering committee for the Yale Open Data Access Project funded by Medtronic. P.M.R. received a grant from Amgen (>$10,000) for genotyping the WGHS. A.C. is on the Science Advisory Board of Affymetrix and Biogen Idec.

Received: February 22, 2014
Accepted: May 20, 2014
Published: June 19, 2014

Web Resources

The URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/
HUGO Gene Nomenclature Committee, http://www.genenames.org/
International HapMap Project, http://hapmap.ncbi.nlm.nih.gov/
Lift Genome Annotations, http://genome.ucsc.edu/cgi-bin/hgLiftOver
LocusZoom, http://csg.sph.umich.edu/locuszoom/
METAL, http://www.sph.umich.edu/csg/abecasis/metal/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
SCAN: SNP and CNV Annotation Database, http://www.scandb.org/newinterface/about.html
SNAP Pairwise LD, http://www.broadinstitute.org/mpg/snap/ldsearchpw.php
UCSC Genome Browser, http://genome.ucsc.edu

References

1. North, B.J., and Sinclair, D.A. (2012). The intersection between aging and cardiovascular disease. Circ. Res. *110*, 1097–1108.
2. Tambs, K., Eaves, L.J., Moum, T., Holmen, J., Neale, M.C., Naess, S., and Lund-Larsen, P.G. (1993). Age-specific genetic effects for blood pressure. Hypertension *22*, 789–795.
3. Wang, X., and Snieder, H. (2011). Familial aggregation of blood pressure. In Clinical Hypertension and Vascular DIseases: Pediatric Hypertension, J.T. Flynn, ed. (New York: Springer Science+Business Media LLC), pp. 241–258.
4. Havlik, R.J., Garrison, R.J., Feinleib, M., Kannel, W.B., Castelli, W.P., and McNamara, P.M. (1979). Blood pressure aggregation in families. Am. J. Epidemiol. *110*, 304–312.
5. Shi, G., Gu, C.C., Kraja, A.T., Arnett, D.K., Myers, R.H., Pankow, J.S., Hunt, S.C., and Rao, D.C. (2009). Genetic effect on blood pressure is modulated by age: the Hypertension Genetic Epidemiology Network Study. Hypertension *53*, 35–41.
6. Takeuchi, F., Isono, M., Katsuya, T., Yamamoto, K., Yokota, M., Sugiyama, T., Nabika, T., Fujioka, A., Ohnaka, K., Asano, H., et al. (2010). Blood pressure and hypertension are associated with 7 loci in the Japanese population. Circulation *121*, 2302–2309.
7. Bao, X., Mills, P.J., Rana, B.K., Dimsdale, J.E., Schork, N.J., Smith, D.W., Rao, F., Milic, M., O'Connor, D.T., and Ziegler, M.G. (2005). Interactive effects of common beta2-adrenoceptor haplotypes and age on susceptibility to hypertension and receptor function. Hypertension *46*, 301–307.
8. Strazzullo, P., Iacone, R., Siani, A., Cappuccio, F.P., Russo, O., Barba, G., Barbato, A., D'Elia, L., Trevisan, M., and Farinaro, E. (2001). Relationship of the Trp64Arg polymorphism of the beta3-adrenoceptor gene to central adiposity and high blood pressure: interaction with age. Cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study. J. Hypertens. *19*, 399–406.
9. Jin, H.S., Sober, S., Hong, K.W., Org, E., Kim, B.Y., Laan, M., Oh, B., and Jeong, S.Y. (2011). Age-dependent association of the polymorphisms in the mitochondria-shaping gene, OPA1, with blood pressure and hypertension in Korean population. Am. J. Hypertens. *24*, 1127–1135.
10. Bhatnagar, V., Liu, L., Nievergelt, C.M., Richard, E., Brophy, V.H., Pandey, B., Lipkowitz, M.S., and O'Connor, D.T. (2012). Paraoxonase 1 (PON1) C/T-108 association with longitudinal mean arterial blood pressure. Am. J. Hypertens. *25*, 1188–1194.
11. Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M.D., Bochud, M., Coin, L., Najjar, S.S., Zhao, J.H., Heath, S.C., Eyheramendy, S., et al.; Wellcome Trust Case Control Consortium (2009). Genome-wide association study identifies eight loci associated with blood pressure. Nat. Genet. *41*, 666–676.
12. Levy, D., Ehret, G.B., Rice, K., Verwoert, G.C., Launer, L.J., Dehghan, A., Glazer, N.L., Morrison, A.C., Johnson, A.D., Aspelund, T., et al. (2009). Genome-wide association study of blood pressure and hypertension. Nat. Genet. *41*, 677–687.
13. Kato, N., Takeuchi, F., Tabara, Y., Kelly, T.N., Go, M.J., Sim, X., Tay, W.T., Chen, C.H., Zhang, Y., Yamamoto, K., et al. (2011). Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. Nat. Genet. *43*, 531–538.
14. Wain, L.V., Verwoert, G.C., O'Reilly, P.F., Shi, G., Johnson, T., Johnson, A.D., Bochud, M., Rice, K.M., Henneman, P., Smith, A.V., et al.; LifeLines Cohort Study; EchoGen consortium; AortaGen Consortium; CHARGE Consortium Heart Failure Working Group; KidneyGen consortium; CKDGen consortium; Cardiogenics consortium; CardioGram (2011). Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat. Genet. *43*, 1005–1011.
15. Ehret, G.B., Munroe, P.B., Rice, K.M., Bochud, M., Johnson, A.D., Chasman, D.I., Smith, A.V., Tobin, M.D., Verwoert, G.C., Hwang, S.J., et al.; International Consortium for Blood Pressure Genome-Wide Association Studies; CARDIoGRAM consortium; CKDGen Consortium; KidneyGen Consortium; EchoGen consortium; CHARGE-HF consortium (2011). Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature *478*, 103–109.
16. Fox, E.R., Young, J.H., Li, Y., Dreisbach, A.W., Keating, B.J., Musani, S.K., Liu, K., Morrison, A.C., Ganesh, S., Kutlar, A., et al.; International Consortium for Blood Pressure Genome-wide Association Studies (ICBP-GWAS) (2011). Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Hum. Mol. Genet. *20*, 2273–2284.
17. Flynn, J.T., Ingelfinger, J.R., and Portman, R.J. (2011). Pediatric Hypertension (New York: Humana Press).
18. Cui, J.S., Hopper, J.L., and Harrap, S.B. (2003). Antihypertensive treatments obscure familial contributions to blood pressure variation. Hypertension *41*, 207–210.
19. Tobin, M.D., Sheehan, N.A., Scurrah, K.J., and Burton, P.R. (2005). Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. Stat. Med. *24*, 2911–2935.

20. Franklin, S.S., Gustin, W., 4th, Wong, N.D., Larson, M.G., Weber, M.A., Kannel, W.B., and Levy, D. (1997). Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. Circulation *96*, 308–315.
21. Landahl, S., Bengtsson, C., Sigurdsson, J.A., Svanborg, A., and Svärdsudd, K. (1986). Age-related changes in blood pressure. Hypertension *8*, 1044–1049.
22. Pearson, J.D., Morrell, C.H., Brant, L.J., Landis, P.K., and Fleg, J.L. (1997). Age-associated changes in blood pressure in a longitudinal study of healthy men and women. J. Gerontol. A Biol. Sci. Med. Sci. *52*, M177–M183.
23. Willer, C.J., Li, Y., and Abecasis, G.R. (2010). METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics *26*, 2190–2191.
24. Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Boehnke, M., Abecasis, G.R., and Willer, C.J. (2010). LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics *26*, 2336–2337.
25. Dehghan, A., Yang, Q., Peters, A., Basu, S., Bis, J.C., Rudnicka, A.R., Kavousi, M., Chen, M.H., Baumert, J., Lowe, G.D., et al.; Wellcome Trust Case Control Consortium (2009). Association of novel genetic Loci with circulating fibrinogen levels: a genome-wide association study in 6 population-based cohorts. Circ Cardiovasc Genet *2*, 125–133.
26. Danik, J.S., Paré, G., Chasman, D.I., Zee, R.Y., Kwiatkowski, D.J., Parker, A., Miletich, J.P., and Ridker, P.M. (2009). Novel loci, including those related to Crohn disease, psoriasis, and inflammation, identified in a genome-wide association study of fibrinogen in 17 686 women: the Women's Genome Health Study. Circ Cardiovasc Genet *2*, 134–141.
27. Dehghan, A., Dupuis, J., Barbalic, M., Bis, J.C., Eiriksdottir, G., Lu, C., Pellikka, N., Wallaschofski, H., Kettunen, J., Henneman, P., et al. (2011). Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels. Circulation *123*, 731–738.
28. Franke, A., McGovern, D.P., Barrett, J.C., Wang, K., Radford-Smith, G.L., Ahmad, T., Lees, C.W., Balschun, T., Lee, J., Roberts, R., et al. (2010). Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat. Genet. *42*, 1118–1125.
29. Barrett, J.C., Hansoul, S., Nicolae, D.L., Cho, J.H., Duerr, R.H., Rioux, J.D., Brant, S.R., Silverberg, M.S., Taylor, K.D., Barmada, M.M., et al.; NIDDK IBD Genetics Consortium; Belgian-French IBD Consortium; Wellcome Trust Case Control Consortium (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat. Genet. *40*, 955–962.
30. Berdasco, M., and Esteller, M. (2012). Hot topics in epigenetic mechanisms of aging: 2011. Aging Cell *11*, 181–186.
31. Basson, J., Sung, Y.J., Schwander, K., Kume, R., Simino, J., de las Fuentes, L., and Rao, D. (2014). Gene-education interactions identify novel blood pressure loci in the Framingham Heart Study. Am. J. Hypertens. *27*, 431–444.
32. Simino, J., Sung, Y.J., Kume, R., Schwander, K., and Rao, D.C. (2013). Gene-alcohol interactions identify several novel blood pressure loci including a promising locus near SLC16A9. Front. Genet. *4*, 277.
33. Figueroa, J.D., Han, S.S., Garcia-Closas, M., Baris, D., Jacobs, E.J., Kogevinas, M., Schwenn, M., Malats, N., Johnson, A., Purdue, M.P., et al. (2014). Genome-wide interaction study of smoking and bladder cancer risk. Carcinogenesis. Published online March 24, 2014. http://dx.doi.org/10.1093/carcin/bgu064.
34. Zhang, R., Chu, M., Zhao, Y., Wu, C., Guo, H., Shi, Y., Dai, J., Wei, Y., Jin, G., Ma, H., et al. (2014). A genome-wide gene-environment interaction analysis for tobacco smoke and lung cancer susceptibility. Carcinogenesis. Published online March 22, 2014. http://dx.doi.org/10.1093/carcin/bgu076.
35. Wu, T., Schwender, H., Ruczinski, I., Murray, J.C., Marazita, M.L., Munger, R.G., Hetmanski, J.B., Parker, M.M., Wang, P., Murray, T., et al. (2014). Evidence of gene-environment interaction for two genes on chromosome 4 and environmental tobacco smoke in controlling the risk of nonsyndromic cleft palate. PLoS ONE *9*, e88088.
36. Liao, S.Y., Lin, X., and Christiani, D.C. (2013). Gene-environment interaction effects on lung function- a genome-wide association study within the Framingham heart study. Environ. Health *12*, 101.
37. Boardman, J.D., Domingue, B.W., Blalock, C.L., Haberstick, B.C., Harris, K.M., and McQueen, M.B. (2013). Is the gene-environment interaction paradigm relevant to genome-wide studies? The case of education and body mass index. Demography *51*, 119–139.
38. Wang, L., Rundek, T., Beecham, A., Hudson, B., Blanton, S.H., Zhao, H., Sacco, R.L., and Dong, C. (2014). Genome-wide interaction study identifies RCBTB1 as a modifier for smoking effect on carotid intima-media thickness. Arterioscler. Thromb. Vasc. Biol. *34*, 219–225.
39. Tang, H., Wei, P., Duell, E.J., Risch, H.A., Olson, S.H., Bueno-de-Mesquita, H.B., Gallinger, S., Holly, E.A., Petersen, G.M., Bracci, P.M., et al. (2014). Genes-environment interactions in obesity- and diabetes-associated pancreatic cancer: a GWAS data analysis. Cancer Epidemiol. Biomarkers Prev. *23*, 98–106.
40. Surakka, I., Isaacs, A., Karssen, L.C., Laurila, P.P., Middelberg, R.P., Tikkanen, E., Ried, J.S., Lamina, C., Mangino, M., Igl, W., et al.; ENGAGE Consortium (2011). A genome-wide screen for interactions reveals a new locus on 4p15 modifying the effect of waist-to-hip ratio on total cholesterol. PLoS Genet. *7*, e1002333.
41. Velez Edwards, D.R., Naj, A.C., Monda, K., North, K.E., Neuhouser, M., Magvanjav, O., Kusimo, I., Vitolins, M.Z., Manson, J.E., O'Sullivan, M.J., et al. (2013). Gene-environment interactions and obesity traits among postmenopausal African-American and Hispanic women in the Women's Health Initiative SHARe Study. Hum. Genet. *132*, 323–336.
42. Wu, C., Kraft, P., Zhai, K., Chang, J., Wang, Z., Li, Y., Hu, Z., He, Z., Jia, W., Abnet, C.C., et al. (2012). Genome-wide association analyses of esophageal squamous cell carcinoma in Chinese identify multiple susceptibility loci and gene-environment interactions. Nat. Genet. *44*, 1090–1097.
43. Siegert, S., Hampe, J., Schafmayer, C., von Schönfels, W., Egberts, J.H., Försti, A., Chen, B., Lascorz, J., Hemminki, K., Franke, A., et al. (2013). Genome-wide investigation of gene-environment interactions in colorectal cancer. Hum. Genet. *132*, 219–231.
44. Gauderman, W.J., Zhang, P., Morrison, J.L., and Lewinger, J.P. (2013). Finding novel genes by testing G × E interactions in a genome-wide association study. Genet. Epidemiol. *37*, 603–613.
45. Sohns, M., Viktorova, E., Amos, C.I., Brennan, P., Fehringer, G., Gaborieau, V., Han, Y., Heinrich, J., Chang-Claude, J., Hung, R.J., et al. (2013). Empirical hierarchical bayes approach to gene-environment interactions: development and application to genome-wide association studies of lung cancer in TRICL. Genet. Epidemiol. *37*, 551–559.

46. Chalasani, N., Guo, X., Loomba, R., Goodarzi, M.O., Haritunians, T., Kwon, S., Cui, J., Taylor, K.D., Wilson, L., Cummings, O.W., et al.; Nonalcoholic Steatohepatitis Clinical Research Network (2010). Genome-wide association study identifies variants associated with histologic features of nonalcoholic fatty liver disease. Gastroenterology *139*, 1567–1576, e1–e6.
47. Gamazon, E.R., Zhang, W., Konkashbaev, A., Duan, S., Kistner, E.O., Nicolae, D.L., Dolan, M.E., and Cox, N.J. (2010). SCAN: SNP and copy number annotation. Bioinformatics *26*, 259–262.
48. Pandey, D., Chen, F., Patel, A., Wang, C.Y., Dimitropoulou, C., Patel, V.S., Rudic, R.D., Stepp, D.W., and Fulton, D.J. (2011). SUMO1 negatively regulates reactive oxygen species production from NADPH oxidases. Arterioscler. Thromb. Vasc. Biol. *31*, 1634–1642.
49. Birkenfeld, A., and Ben-Zvi, A. (1984). Age associated changes in intracellular cyclic adenosine monophosphate. Clin. Exp. Immunol. *55*, 651–654.
50. Liu, Z., Naranjo, A., and Thiele, C.J. (2011). CASZ1b, the short isoform of CASZ1 gene, coexpresses with CASZ1a during neurogenesis and suppresses neuroblastoma cell growth. PLoS ONE *6*, e18557.
51. Liu, Z., Yang, X., Li, Z., McMahon, C., Sizer, C., Barenboim-Stapleton, L., Bliskovsky, V., Mock, B., Ried, T., London, W.B., et al. (2011). CASZ1, a candidate tumor-suppressor gene, suppresses neuroblastoma tumor growth through reprogramming gene expression. Cell Death Differ. *18*, 1174–1183.
52. Bookman, E.B., McAllister, K., Gillanders, E., Wanke, K., Balshaw, D., Rutter, J., Reedy, J., Shaughnessy, D., Agurs-Collins, T., Paltoo, D., et al.; for the NIH G × E Interplay Workshop participants (2011). Gene-environment interplay in common complex diseases: forging an integrative model-recommendations from an NIH workshop. Genet. Epidemiol. *35*, 217–225.
53. Oien, D.B., Osterhaus, G.L., Latif, S.A., Pinkston, J.W., Fulks, J., Johnson, M., Fowler, S.C., and Moskovitz, J. (2008). MsrA knockout mouse exhibits abnormal behavior and brain dopamine levels. Free Radic. Biol. Med. *45*, 193–200.
54. Yang, H.C., Liang, Y.J., Wu, Y.L., Chung, C.M., Chiang, K.M., Ho, H.Y., Ting, C.T., Lin, T.H., Sheu, S.H., Tsai, W.C., et al. (2009). Genome-wide association study of young-onset hypertension in the Han Chinese population of Taiwan. PLoS ONE *4*, e5459.
55. Lasky-Su, J., Lyon, H.N., Emilsson, V., Heid, I.M., Molony, C., Raby, B.A., Lazarus, R., Klanderman, B., Soto-Quiros, M.E., Avila, L., et al. (2008). On the replication of genetic associations: timing can be everything!. Am. J. Hum. Genet. *82*, 849–858.
56. Izzo, J.L., Jr. (2001). Aging and systolic hypertension: cluster patterns and problem-solving strategies to answer the genetic riddle. Hypertension *37*, 1067–1068.

Note Added in Proof

Since this manuscript was submitted, a gene-centric meta-analysis of more than 150,000 individuals of European descent identified a BP-associated SNP near (55 kb away; $r^2 = 0.47$) our finding in *EHBP1L1*. The variant identified by Tragante et al. exhibited main effects, whereas our SNP exhibited only gene-age interactions.

Tragante, V., Barnes, M.R., Ganesh, S.K., Lanktree, M.B., Guo, W., Franceschini, N., Smith, E.N., Johnson, T., Holmes, M.V., Padmanabhan, S., et al. (2014). Gene-centric meta-analysis in 87,736 individuals of European ancestry identifies multiple blood-pressure-related loci. Am. J. Hum. Genet. *94*, 349–360.

The American Journal of Human Genetics, Volume *95*
Supplemental Data

Gene-Age Interactions in Blood Pressure Regulation: A Large-Scale Investigation with the CHARGE, Global BPgen, and ICBP Consortia

Jeannette Simino, Gang Shi, Joshua C. Bis, Daniel I. Chasman, Georg B. Ehret, Xiangjun Gu, Xiuqing Guo, Shih-Jen Hwang, Eric Sijbrands, Albert V. Smith, Germaine Verwoert, Jennifer L. Bragg-Gresham, Gemma Cadby, Peng Chen, Ching-Yu Cheng, Tanguy Corre, Rudolf A. de Boer, Anuj Goel, Toby Johnson, Chiea Chuen Khor, LifeLines Cohort Study, Carla Lluís-Ganella, Jian'an Luan, Leo-Pekka Lyytikäinen, Ilja M. Nolte, Xueling Sim, Siim Sõber, Peter J. van der Most, Niek Verweij, Jing Hua Zhao, Najaf Amin, Eric Boerwinkle, Claude Bouchard, Abbas Dehghan, Gudny Eiriksdottir, Roberto Elosua, Oscar H. Franco, Christian Gieger, Tamara B. Harris, Serge Hercberg, Albert Hofman, Alan L. James, Andrew D. Johnson, Mika Kähönen, Kay-Tee Khaw, Zoltan Kutalik, Martin G. Larson, Lenore J. Launer, Guo Li, Jianjun Liu, Kiang Liu, Alanna C. Morrison, Gerjan Navis, Rick Twee-Hee Ong, George J. Papanicolau, Brenda W. Penninx, Bruce M. Psaty, Leslie J. Raffel, Olli T. Raitakari, Kenneth Rice, Fernando Rivadeneira, Lynda M. Rose, Serena Sanna, Robert A. Scott, David S. Siscovick, Ronald P. Stolk, Andre G. Uitterlinden, Dhananjay Vaidya, Melanie M. van der Klauw, Ramachandran S. Vasan, Eranga Nishanthie Vithana, Uwe Völker, Henry Völzke, Hugh Watkins, Terri Young, Tin Aung, Murielle Bochud, Martin Farrall, Catharina A. Hartman, Maris Laan, Edward Lakatta, Terho Lehtimäki, Ruth J.F. Loos, Gavin Lucas, Pierre Meneton, Lyle J. Palmer, Rainer Rettig, Harold Snieder, E. Shyong Tai, Yik Ying Teo, Pim van der Harst, Nicholas J. Wareham, Cisca Wijmenga, Tien Yin Wong, Myriam Fornage, Vilmundur Gudnason, Daniel Levy, Walter Palmas, Paul M. Ridker, Jerome I. Rotter, Cornelia van Duijn, Jacqueline C.M. Witteman, Aravinda Chakravarti, and Dabeeru C. Rao

Table of Contents for the Supplemental Material

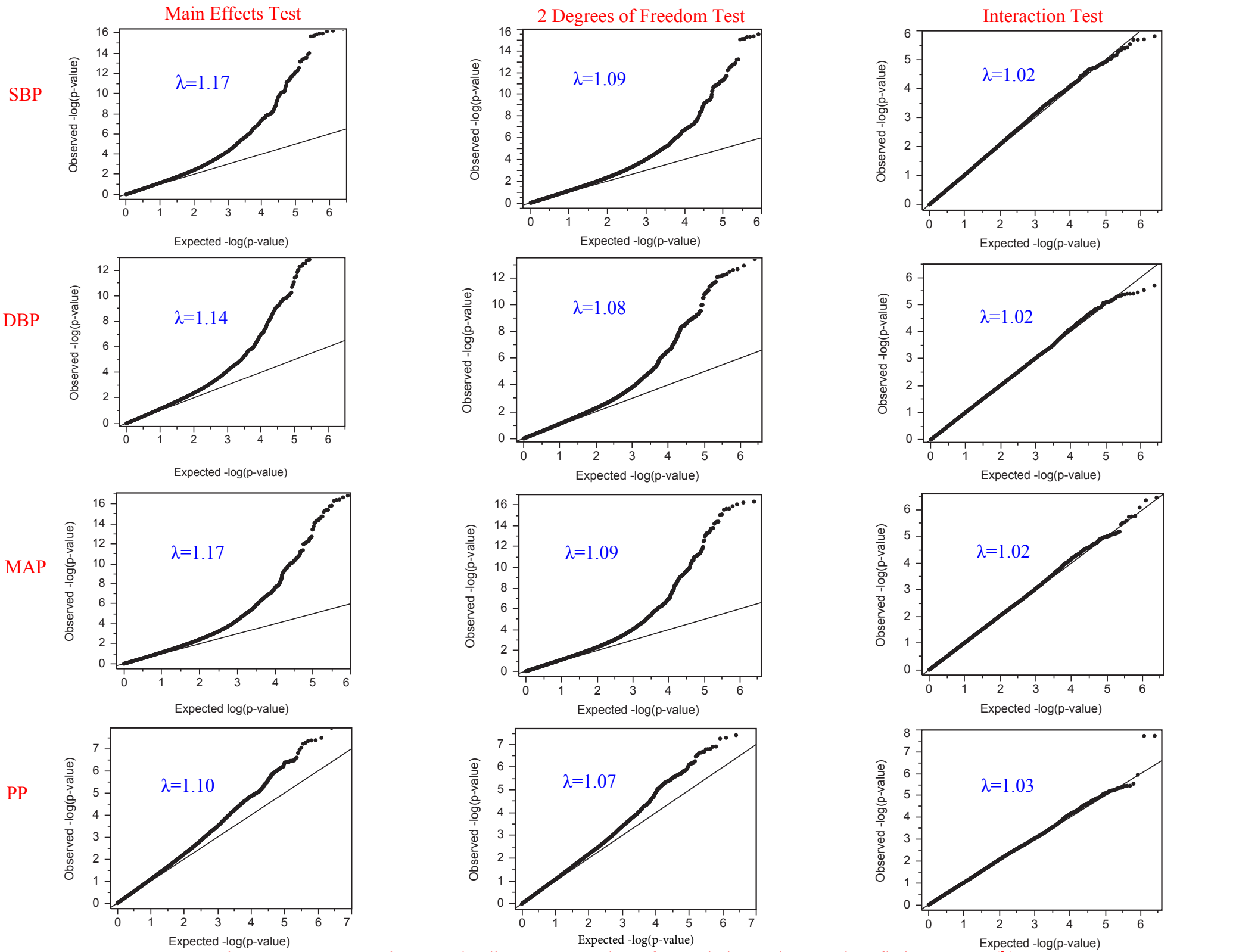


Figure S1: Pre-Genomic Control Adjustment:QQ Plots of Association and Genomic Inflation Factors (λ)

Figure S2: Regional Plots for the Joint 2 DF Test

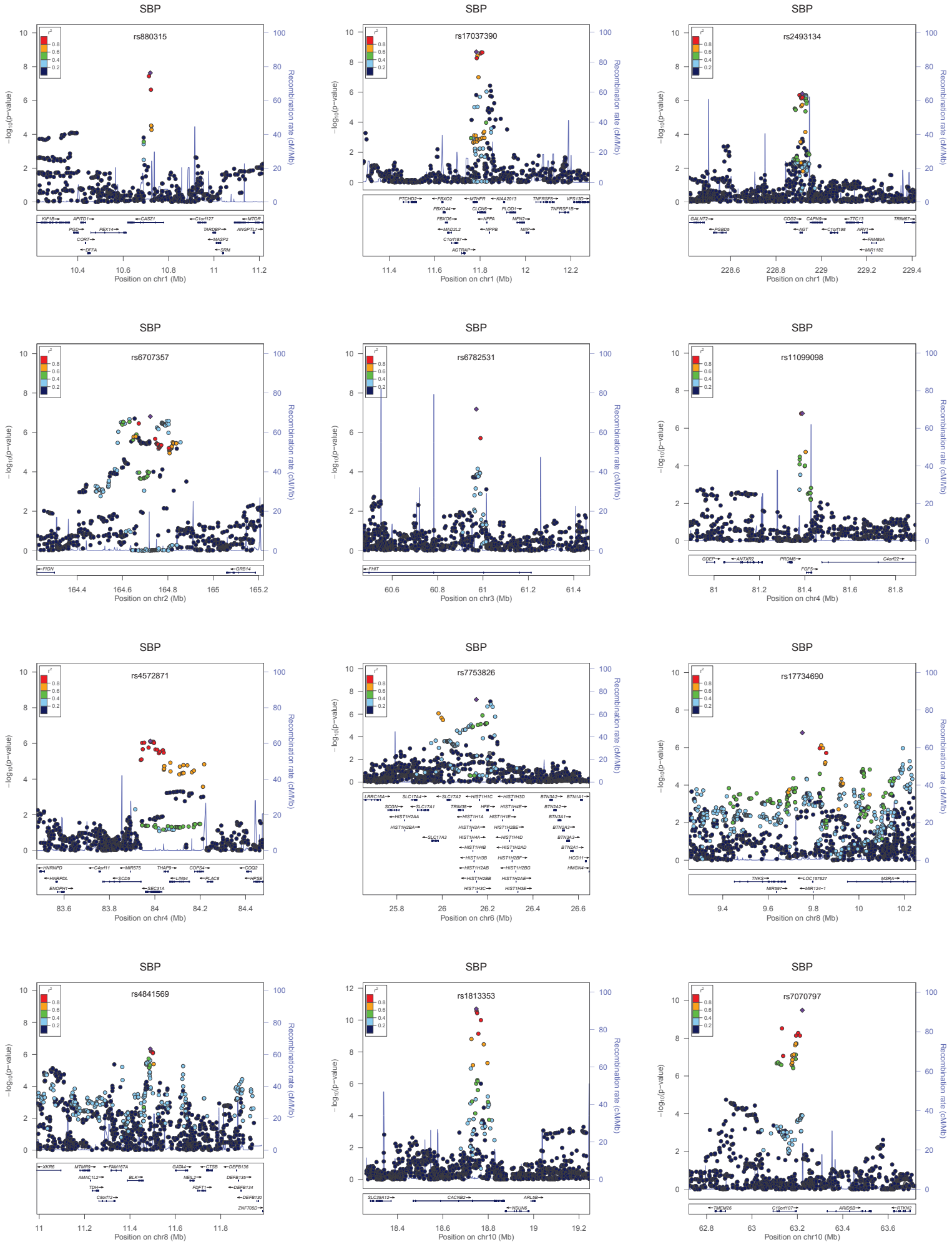


Figure S2: Regional Plots for the Joint 2 DF Te

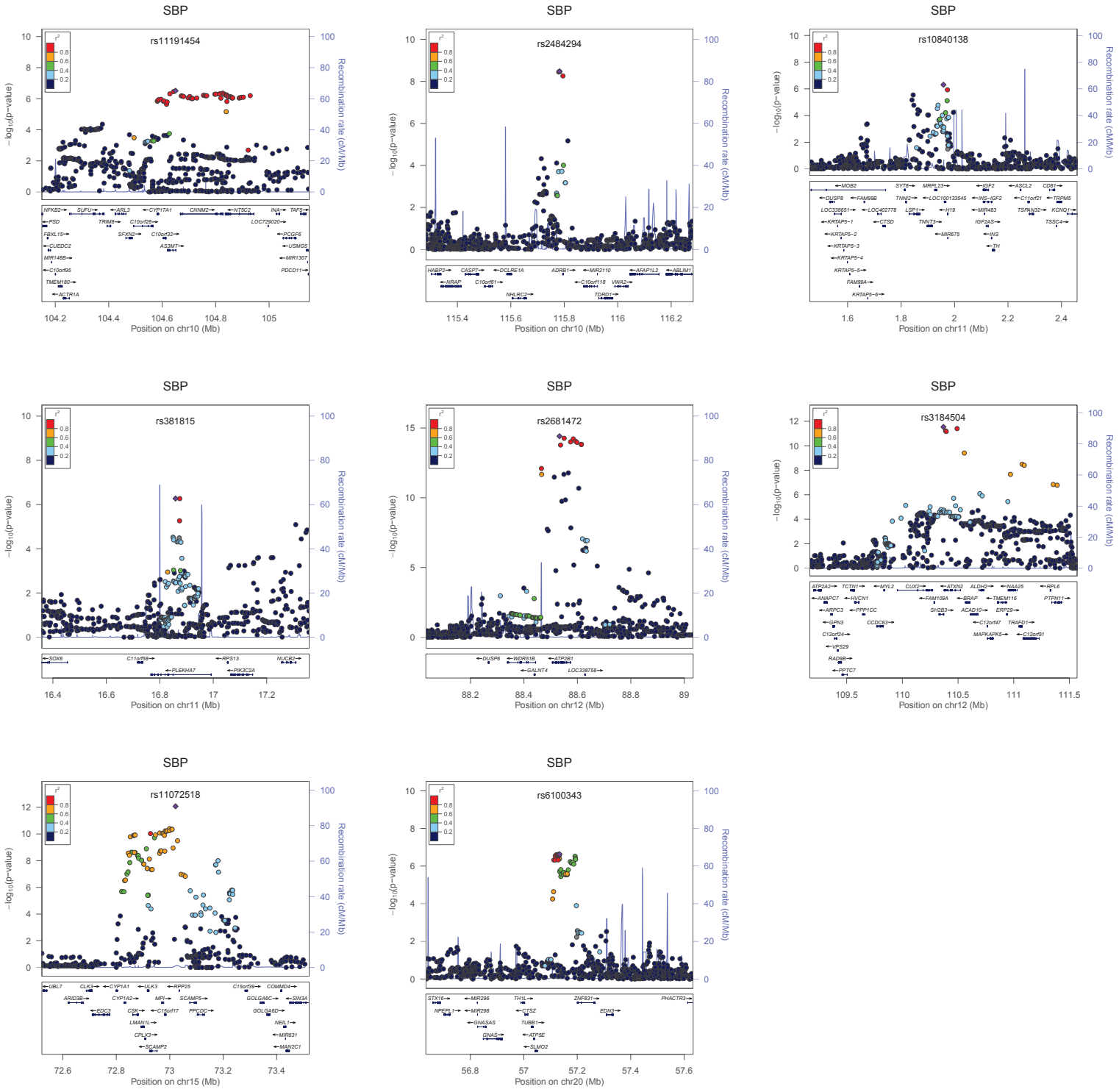


Figure S2: Regional Plots for the Joint 2 DF Te

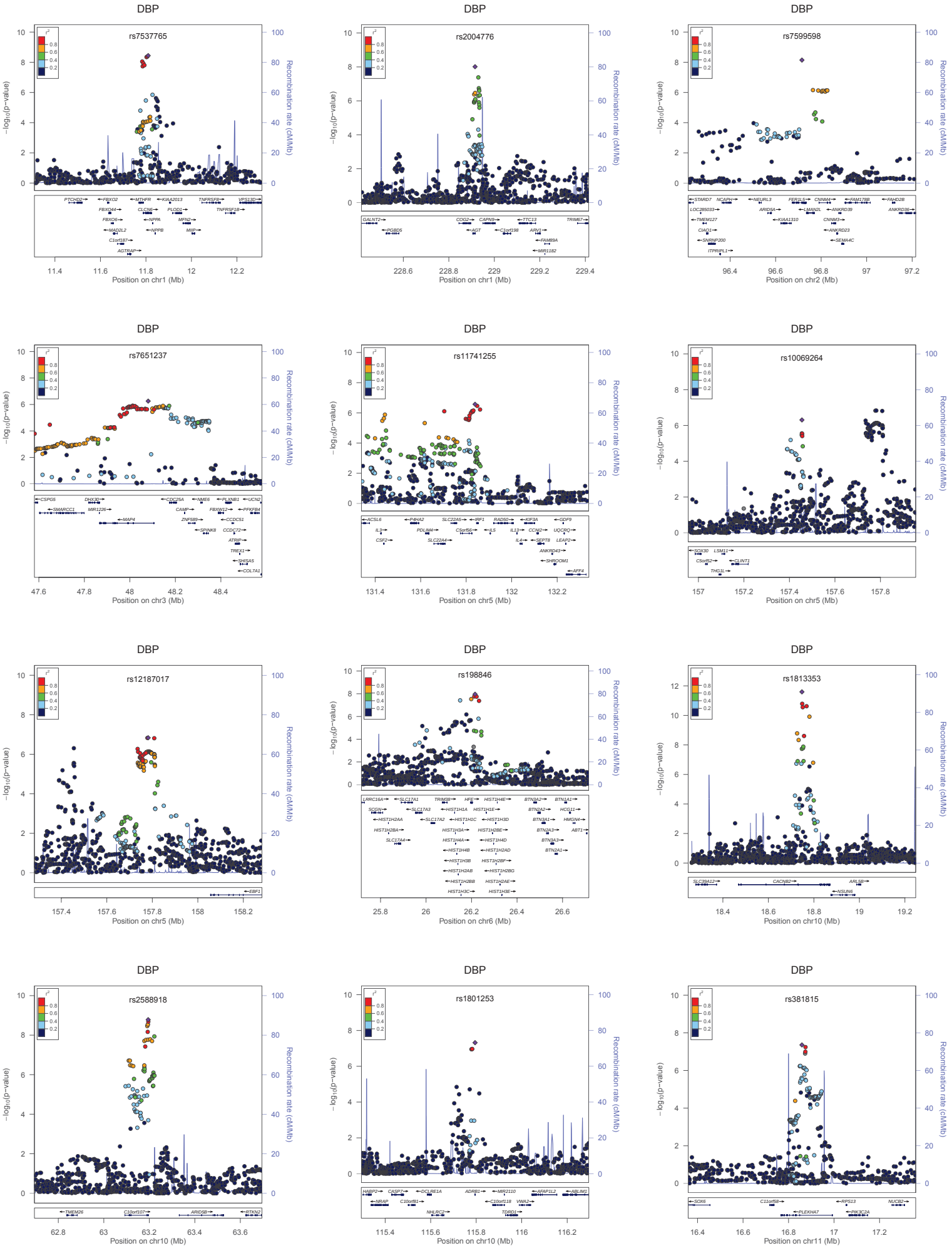
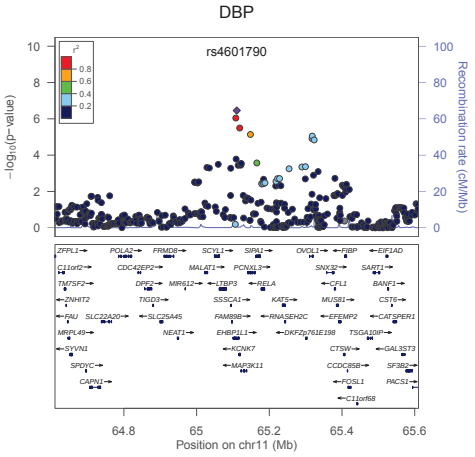
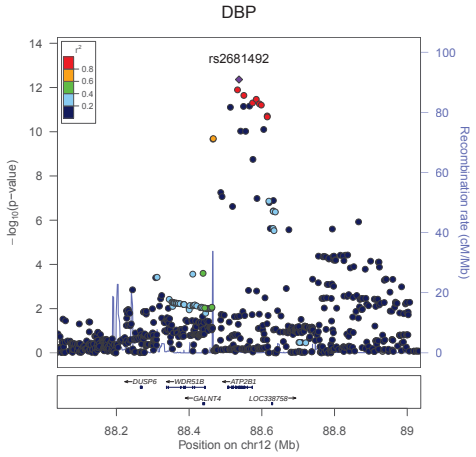
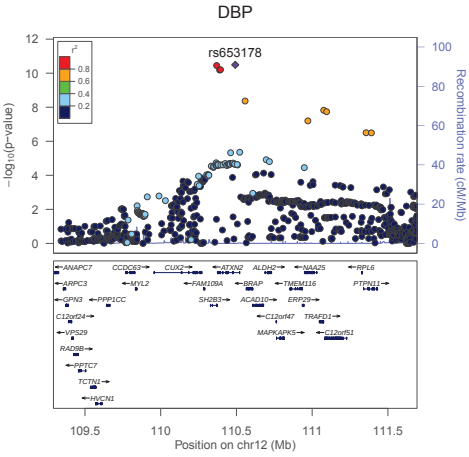
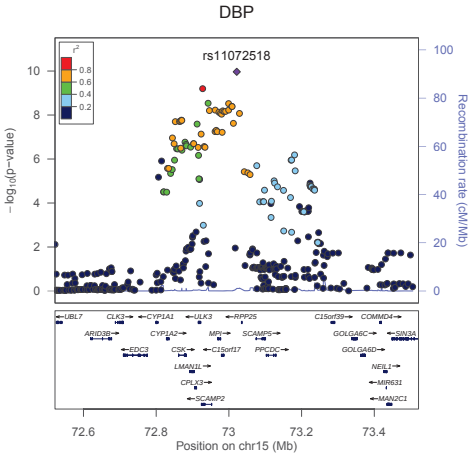


Figure S2: Regional Plots for the Joint 2 DF Te









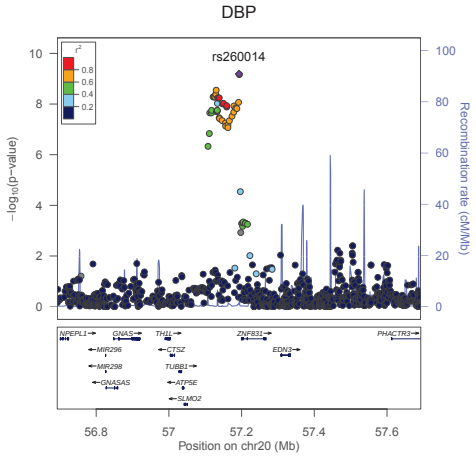


Figure S2: Regional Plots for the Joint 2 DF Te

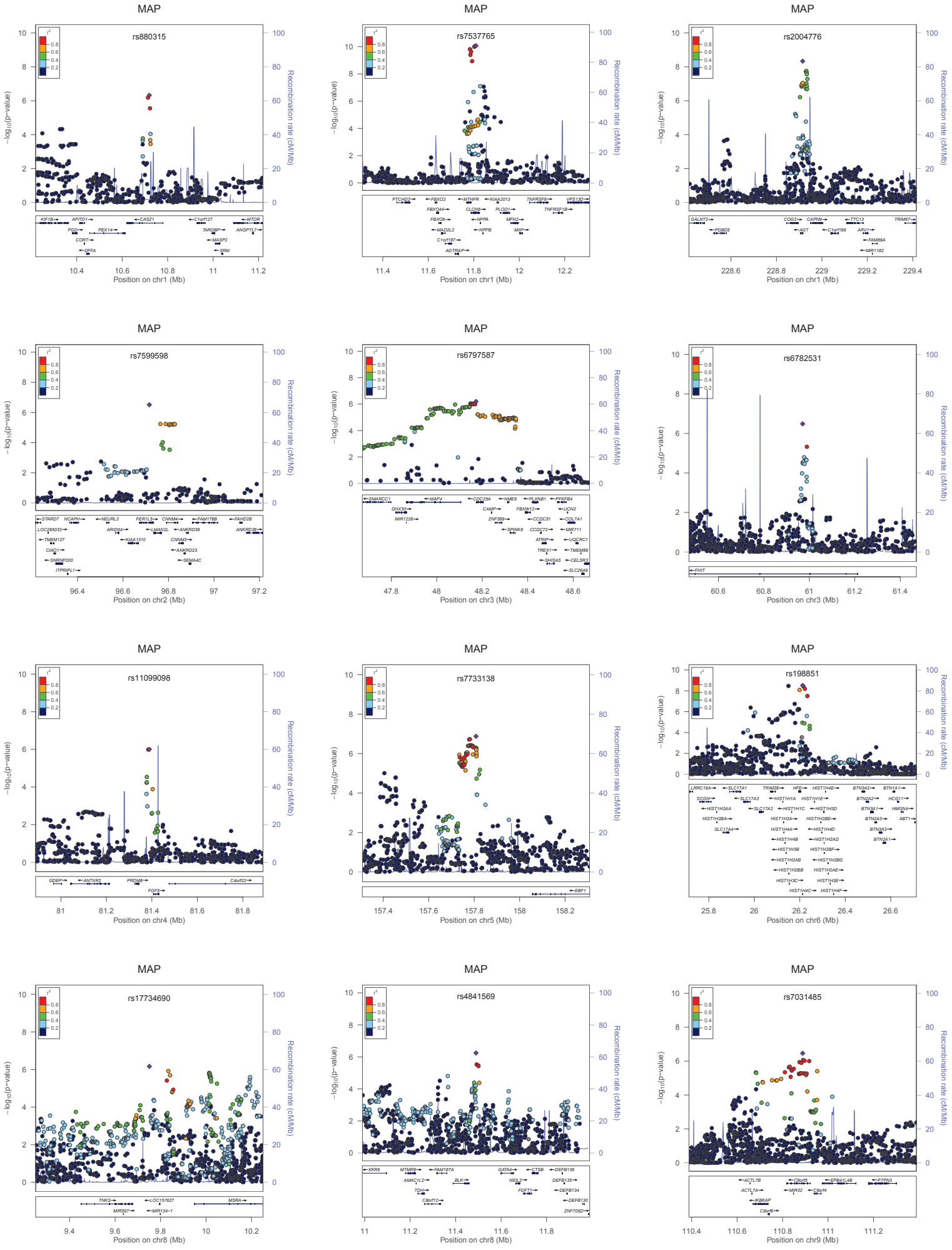
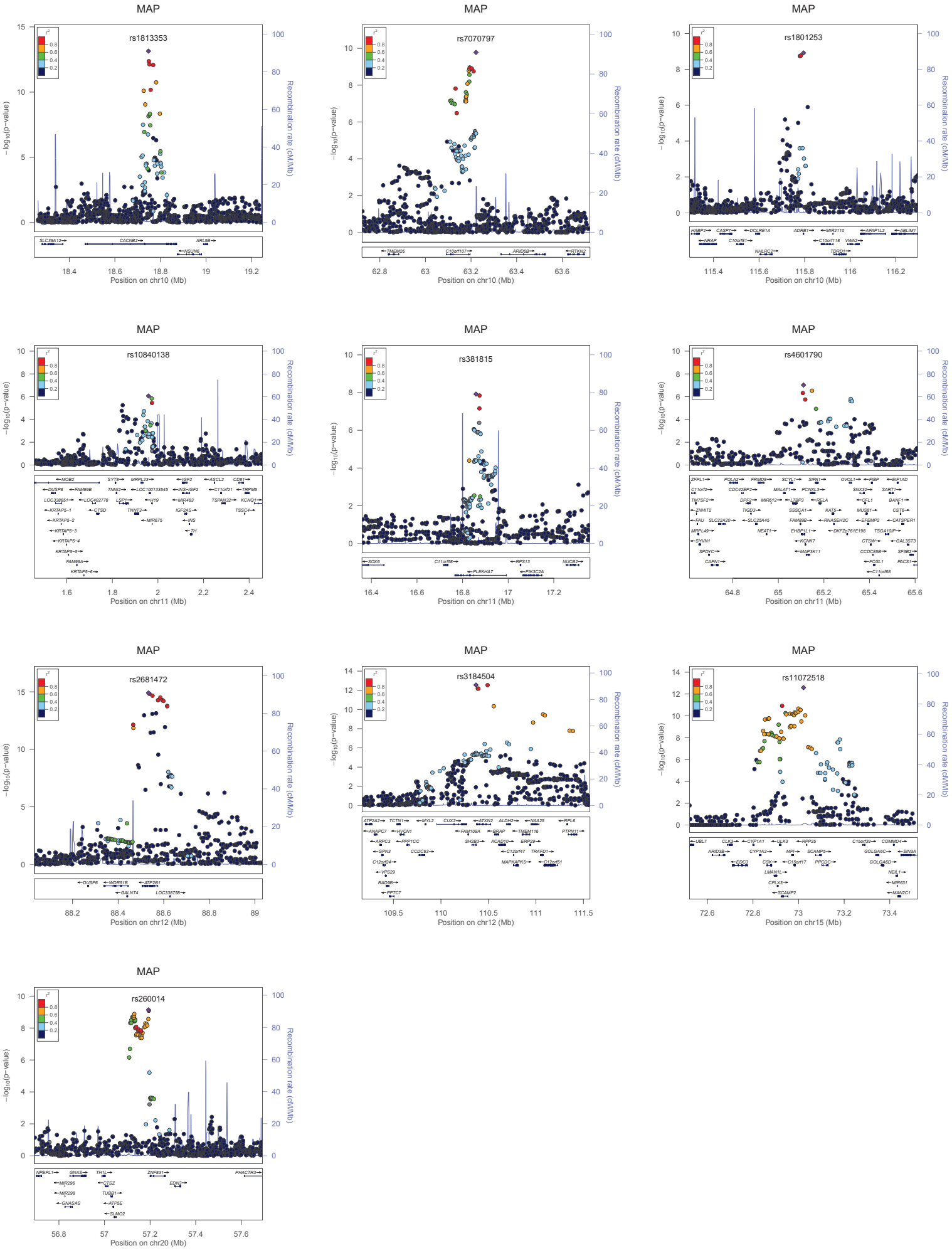


Figure S2: Regional Plots for the Joint 2 DF Test



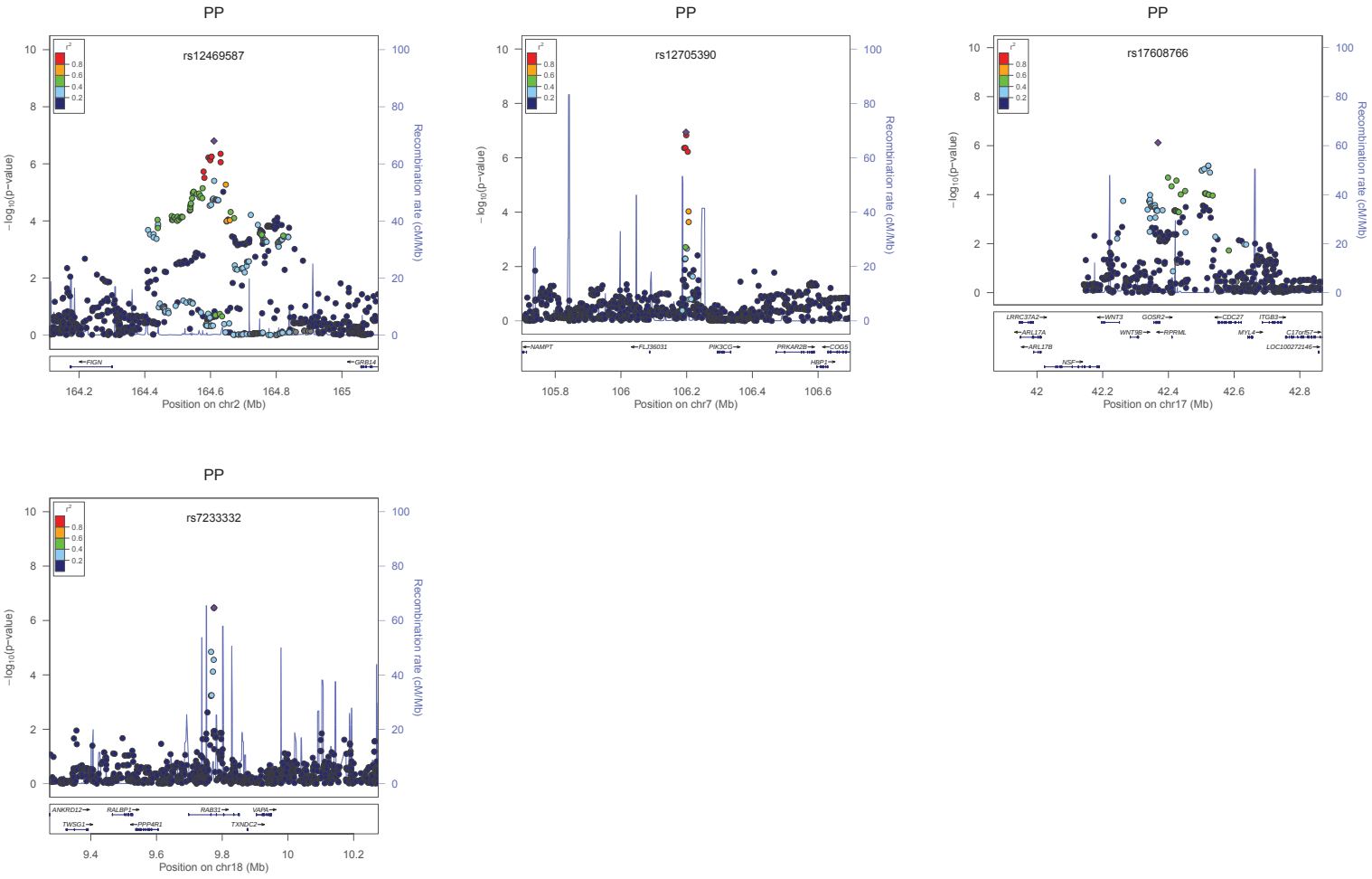


Figure S2: Regional Associations Plots for Suggestive and Significant Loci Identified by the Meta-Regression of Stage 1 Studies (Joint 2df tests of SNP Main Effects and SNP-age Interactions). Plots are presented by trait in ascending chromosome and basepair position.

STAGE 1 (CHARGE) STUDIES

Study Descriptions

The Age Gene/Environment Susceptibility-Reykjavik (AGES) Study originally comprised a random sample of 30,795 men and women born in 1907-1935 and living in Reykjavik in 1967. A total of 19,381 people attended, resulting in a 71% recruitment rate. The study sample was divided into six groups by birth year and birth date within month. One group was designated for longitudinal follow up and was examined in all stages; another was designated as a control group and was not included in examinations until 1991. Other groups were invited to participate in specific stages of the study. Between 2002 and 2006, the AGES-Reykjavik study re-examined 5,764 survivors of the original cohort who had participated before in the Reykjavik Study [1]. The midlife data blood pressure measurement was taken from stage 3 of the Reykjavik Study (1974-1979), if available. Half of the cohort attended during this period. Otherwise an observation was selected closest in time to the stage 3 visit. The supine blood pressure was measured twice by a nurse using a mercury sphygmomanometer after 5 minutes rest following World Health Organization recommendations[2]. Individuals with previous MI were excluded from the analyses (N=12).

The Atherosclerosis Risk In Communities (ARIC) Study is a population-based prospective cohort study of cardiovascular disease sponsored by the National Heart, Lung, and Blood Institute (NHLBI). ARIC included 15,792 individuals aged 45-64 years at baseline (1987-89), chosen by probability sampling from four US communities[3]. Cohort members completed four clinic examinations each spread over about three years, conducted approximately three years apart between 1987 and 1998. A detailed study protocol is available on the ARIC study website (http://www2.cscc.unc.edu/aric/). Blood pressure was measured using a standardized Hawksley random-zero mercury column sphygmomanometer with participants in a sitting position after a resting period of 5 minutes. The size of the cuff was chosen according to the arm circumference. Three sequential recordings for systolic and diastolic blood pressure were obtained; the mean of the last two measurements was used in this analysis, discarding the first reading. Blood pressure lowering medication use was recorded from the medication history. For this study the sample was restricted to individuals of European descent by self-report and principal component analysis using genome-wide genotypes.

The Coronary Artery Risk Development in Young Adults (CARDIA) Study is a prospective multicenter study with 5,115 adults Caucasian and African American participants of the age group 18-30 years, recruited from four centers at the baseline examination in 1985-1986. The recruitment was done from the total community in Birmingham, AL, from selected census tracts in Chicago, IL and Minneapolis, MN; and from the Kaiser Permanente health plan membership in Oakland, CA. The details of the study design for the CARDIA study have been previously

published[4]. Eight examinations have been completed since initiation of the study, respectively in the years 0, 2, 5, 7, 10, 15, 20 and 25. Written informed consent was obtained from participants at each examination and all study protocols were approved by the institutional review boards of the participating institutions. Systolic and diastolic blood pressure was measured in triplicate on the right arm using a random-zero sphygmomanometer with the participant seated and following a 5-min. rest. The average of the second and third measurements was taken as the blood pressure value. Blood pressure medication use was obtained by questionnaire. Baseline data were used for this study. In addition, the sample was restricted to individuals of European descent by self-report and principal component analysis using genome-wide genotypes.

The Cardiovascular Health Study (CHS) is a population-based cohort study of risk factors for cardiovascular disease in adults 65 years of age or older conducted across four field centers. The original predominantly white cohort of 5,201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists and an additional 687 African-Americans were enrolled in 1992-93 for a total sample of 5,888. Details of the study design are summarized elsewhere[5]. A total of 1,908 persons were excluded from the study sample due to prevalent coronary heart disease (N=1,195), congestive heart failure (N=86), peripheral vascular disease (N=93), valvular heart disease (N=20), stroke (N=166), or transient ischemic attack (N=56). Participants with missing BMI (N=10) or BP measurements (N=8) were also excluded. Research staff with central training in blood pressure measurement assessed repeated right-arm seated systolic and diastolic blood pressure levels at baseline with a Hawksley random-zero sphygmomanometer. Means of the repeated blood pressure measurements from the baseline examination of subjects of European ancestry were used for the analyses.

The Framingham Heart Study (FHS) began in 1948 with the recruitment of an original cohort of 5,209 men and women (mean age 44 years; 55 percent women). In 1971 a second generation of study participants was enrolled; this cohort (mean age 37 years; 52% women) consisted of 5,124 children and spouses of children of the original cohort. A third generation cohort of 4,095 children of offspring cohort participants (mean age 40 years; 53 percent women) was enrolled beginning in 2002. Details of study designs for the three cohorts are summarized elsewhere [6-8]. At each clinic visit, a medical history was obtained with a focus on cardiovascular content, and participants underwent a physical examination including measurement of height and weight from which BMI was calculated. Systolic and diastolic blood pressures were measured twice by a physician on the left arm of the resting and seated participant using a mercury column sphygmomanometer. Blood pressures were recorded to the nearest even number. The means of two separate systolic and diastolic blood pressure readings at each clinic examination were used for statistical analyses. To maximize the number of participants with age ranged from 20 to 80, we applied blood pressure measurements for the original cohort at the tenth examination, the second generation at the third examination, and the third generation at the first examination for GWAS. Individuals who had a myocardial infarction or congestive heart failure were excluded from the analyses because those conditions may affect blood pressure levels. We excluded participants with extreme values of systolic blood

pressures (greater or less than mean± 4 standard deviations) for the GWAS.

The Multi-Ethnic Study of Atherosclerosis (MESA) investigation is a population-based study of 6,814 men and women age 45 to 85 years, without clinical cardiovascular disease, recruited from six United States communities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; northern Manhattan, NY; and St. Paul, MN). The main objective of MESA is to determine the characteristics of subclinical cardiovascular disease and its progression. Sampling and recruitment procedures have been previously described in detail [9]. Adults with symptoms or history of medical or surgical treatment for cardiovascular disease were excluded. During the recruitment process, potential participants were asked about their race/ethnicity. Self-reported ethnicity was used to classify participants into groups [10]. After a 5-minute rest BP was measured three times at 1 minute intervals using a Dinamap PRO 100 automated oscillometric device (Critikon, Tampa, FL) with the subject seated, and the average of the second and third BP measurements was used in the analysis. This analysis included only individuals of European descent.

The Rotterdam Study (RS-I) and Rotterdam Extension Study (RS-II) are prospective population-based cohort studies; the RS-I comprises 7,983 subjects aged 55 years or older. Participants completed an interview at home and at the research center, where participants were subsequently examined. Baseline data were collected between 1990 and 1993. In 1999, inhabitants who turned 55 years of age or moved into the study district since the start of the study were invited to participate in an extension of the RS (RS-II), 3,011 participated (67% response rate). The rationale and design of the RS have been described in detail elsewhere [11-13]. At the research center, two seated blood pressure measurements of the right brachial artery were obtained with a random zero sphygmomanometer. The mean of two consecutive measurements was used in association analyses. Participants who had a history myocardial infarction or congestive heart failure were excluded because of the impact of these conditions on blood pressure levels.

The Women's Genome Health Study (WGHS) is a prospective cohort of female North American health care professionals representing participants in the Women's Health Study (WHS) trial who provided a blood sample at baseline and consent for blood-based analyses [14]. Participants in the WHS were 45 years or older at enrollment and free of cardiovascular disease, cancer or other major chronic illness. The current data are derived from 23,294 WGHS participants for whom whole genome genotype information was available at the time of analysis and for whom self-reported European ancestry could be confirmed by multidimensional scaling analysis of 1,443 ancestry informative markers in PLINK v. 1.06. Baseline BP in the WGHS was ascertained by a self-reported questionnaire, an approach which has been validated in the WGHS demographic, namely female health care professionals[15-17]. Questionnaires recorded systolic blood pressure in 9 categories (<110, 110-119, 120-129, 130-

139, 140-149, 150-159, 160-169, 170-179, ≥180 mmHg), and diastolic blood pressure in 7 categories (<65, 65-74, 75-84, 85-89, 90-94, 95-104, ≥105 mmHg). We adjusted for antihypertensive medication use (SBP + 10 mmHg and DBP + 5 mmHg) when assigning WGHS participants to BP categories. The midpoint of each category was used for this analysis.

Table S1: Descriptive Statistics for the Stage 1 (CHARGE) Studies

Discovery Study	Age Bin	N	Median Age	Female (%)	HT (%)	BMI Mean (SD)	SBP Mean (SD)	DBP Mean (SD)	MAP Mean (SD)	PP Mean (SD)
AGES	[40,50)	1,260	45							
	[50,60)	1,603	54							
	[60,70)	265	62							
ARIC	[40,50)	2,392	47	56.9	17.2	26.8 (5.1)	112.8 (14.4)	71.3 (9.9)	85.1 (10.7)	41.5 (9.3)
	[50,60)	4,772	54	52.6	24.9	27.0 (4.8)	118.0 (16.1)	71.9 (9.8)	87.2 (10.9)	46.1 (11.7)
	[60,70)	2,142	62	48.8	35.3	27.0 (4.5)	125.0 (17.6)	71.1 (10.0)	89.1 (11.2)	53.9 (14.2)
CARDIA†	[17,32)	1,713	26	52.4	2.1	23.7 (3.8)	109.3 (10.9)	68.3 (9.0)	82.0 (8.6)	41.0 (9.0)
CHS	[60,70)	1,230	68	66.0	47.0	26.8 (4.8)	134.6 (20.6)	74.8 (12.4)	94.7 (13.7)	59.8 (15.7)
	[70,80)	1,672	73	59.5	55.0	26.1 (4.3)	139.8 (22.6)	73.8 (12.8)	95.8 (14.2)	66.1 (18.5)
FHS	[20,30)	533	26	52.2	0.9	25.3 (5.0)	112.9 (11.7)	71.6 (9.2)	85.4 (9.2)	41.4 (9.1)
	[30,40)	1,926	36	54.2	3.6	26.0 (5.1)	114.0 (12.5)	74.8 (9.6)	87.9 (10.0)	39.2 (7.8)
	[40,50)	2,608	44	53.6	9.2	26.8 (5.3)	119.6 (15.0)	77.9 (9.8)	91.8 (10.9)	41.8 (9.4)
	[50,60)	1,916	54	54.6	16.8	26.8 (4.8)	128.9 (18.0)	80.6 (10.1)	96.7 (12.0)	48.3(12.3)
	[60,70)	537	62	54.6	27.9	26.7 (4.3)	138.3 (20.1)	81.5 (10.1)	100.4(12.3)	56.9(14.9)
MESA	[40,50)	342	48	53.2	14.9	27.5 (5.6)	112.9 (15.8)	70.2 (10.3)	84.5 (11.6)	42.9 (9.7)
	[50,60)	708	55	52.8	28.3	28.2 (5.4)	119.9 (18.8)	71.9 (10.4)	87.9 (12.3)	47.8 (12.8)
	[60,70)	726	66	50.3	44.2	28.0 (5.0)	130.3 (21.0)	72.8 (10.3)	92.0 (12.5)	57.7 (16.5)
	[70,80)	563	75	50.8	53.6	27.0 (4.3)	136.5 (22.4)	71.3 (10.3)	93.0 (13.0)	65.3 (17.4)
RS I	[50,60)	910	58	58.5	35.6	25.9 (3.4)	129.3 (20.4)	75.0 (11.0)	93.0 (14.0)	56.0 (15.0)
	[60,70)	2,060	65	57.7	51.8	26.4 (3.6)	137.3 (20.8)	75.0 (12.0)	97.0 (14.0)	64.0 (17.0)
	[70,80)	1,419	74	62.8	65.8	26.3 (3.7)	144.9 (21.3)	75.0 (12.0)	99.0 (14.0)	73.0 (18.0)
RS II	[50,60)	740	58	54.9	50.7	27.4 (4.7)	137.8 (19.1)	90.0 (11.0)	100.0 (13.0)	59.0 (14.0)
	[60,70)	851	63	53.8	58.4	27.3 (4.0)	142.0 (20.1)	81.0 (11.0)	102.0 (13.0)	64.0 (17.0)
	[70,80)	321	75	58.9	78.5	26.9 (3.6)	152.1 (21.7)	79.0 (11.0)	104.0 (14.0)	76.0 (18.0)
WGHS	[40,50)	7,219	47	100.0	15.5	25.9 (5.3)	120.5 (12.9)	75.4 (9.5)	90.5 (10.0)	45.1 (8.5)
	[50,60)	10,386	53	100.0	24.2	26.0 (4.9)	124.6 (15.0)	77.5 (9.7)	93.2 (10.8)	46.9 (9.6)
	[60,70)	4,271	63	100.0	37.2	25.8 (4.6)	131.0 (16.5)	79.6 (9.6)	96.7 (11.0)	51.3 (11.6)
	[70,80)	711	72	100.0	45.7	24.9 (3.9)	135.0 (16.4)	79.6 (9.1)	98.1 (10.4)	55.4 (12.9)

† Except for N and the median age, the descriptive statistics for CARDIA were derived on those 20 to 30 years old.

Table S2: Genotyping, Imputation, and Analysis Software Information for the Stage 1 (CHARGE) Studies

Discovery Study	Genotyping Platform	NCBI human genome reference used	Imputation Procedure	Pre-Imputation QC filter information	Pre-Association Filters (sample or SNP)	Association Analysis Software
AGES	Illumina 370 CNV	NCBI36/HapMap22	MACH			R & ProbABEL
ARIC	Affymetrix 6.0		MACH			
CARDIA	Affymetrix 6.0	Build 36	BEAGLE with reference HapMap2, release 22	Sample: Call rate ≥ 98%; duplicate samples, gender mismatch; outlier in PCA. SNP: MAF ≥ 2%; SNP call rate ≥ 95%; HWE ≥ 10^{-4}	Imputation quality R^2>0.3; MAF≥ 1%	ProbABEL
CHS	Illumina 370 CNV	Build 36, release 22	BIMBAM, single imputation of posterior mean genotype (dosage); CEPH Build 36 reference haplotypes; Build 36 positions.	Samples were excluded from analysis for: sex mismatch, discordance with prior genotyping, or call rate < 95%. SNPS: the following exclusions were applied to identify a final set of 306,655 autosomal SNPs: call rate < 97%, HWE P < 10^{-5}, > 2 duplicate errors or Mendelian inconsistencies (for reference CEPH trios), heterozygote frequency = 0, SNP not found in HapMap.	Variance of imputed SNP dosage < 0.01	R
FHS	Affymetrix 500k and MIPS 50K combined	36.2	MACH	Call-rate ≥97%, HWE p≤ 10^{-6}, Mishap p < 1e-9	Call-rate ≥97%, subject heterozygosity ≤ 5 SD from the mean	R statistics, LMEKIN. the linear mixed model for GWAS
MESA	Affymetrix 6.0	36.3	HapMap1+2 IMPUTE2	Not Applicable	HWE>=≥1E-6 MAF>=0.01	SNPTEST v 2.1.1
RS I & RS II	Illumina 550 K	36	MACH, Hapmap r22 (build 36)			ProbABEL
WGHS	Illumina Human-Hap300 Duo-plus BeadChip platform	36	Imputation used HapMap2 CEU r.22 reference panel with MaCH v. 1.0.16	HWE p-value < 10^{-6}	None	ProbABEL

Table S3: Genomic Inflation Factors of the GWAS Results from Each Stage 1 (CHARGE) Study and Age Bin

Study	Age Bin	SBP		DBP		MAP		PP	
		# SNPs after QC filtering	Genomic Inflation Factor	# SNPs after QC filtering	Genomic Inflation Factor	# SNPs after QC filtering	Genomic Inflation Factor	# SNPs after QC filtering	Genomic Inflation Factor
AGES	3	2,367,558	1.003	2,367,573	1.005	2,367,573	1.004	2,367,573	1.002
	4	2,391,184	1.055	2,391,199	1.044	2,391,199	1.052	2,391,199	1.049
	5	1,973,973	1.012	1,973,977	1.023	1,973,977	1.018	1,973,977	1.003
ARIC	3	2,458,877	1.020	2,458,877	1.011	2,458,877	1.010	2,458,877	1.023
	4	2,481,308	1.024	2,481,308	1.028	2,481,308	1.030	2,481,308	1.011
	5	2,453,141	1.022	2,453,141	1.010	2,453,141	1.026	2,453,141	1.018
CARDIA	1	2,272,313	1.017	2,272,244	1.001	2,272,244	1.001	2,272,244	1.014
CHS	5	2,186,656	1.024	2,186,085	1.021	2,186,085	1.021	2,186,085	1.023
	6	2,190,440	1.015	2,189,859	1.027	2,189,859	1.024	2,189,859	1.013
FHS	1	2,135,392	1.040	2,224,899	1.038	2,224,373	1.036	2,224,899	1.015
	2	2,333,148	1.035	2,405,991	1.017	2,406,012	1.025	2,406,012	1.019
	3	2,352,606	1.027	2,421,561	1.013	2,421,543	1.018	2,421,487	1.023
	4	2,377,100	1.020	2,405,583	1.007	2,405,662	1.014	2,405,428	1.022
	5	2,192,250	1.049	2,226,881	1.049	2,226,881	1.057	2,221,888	1.046
MESA	3	*2,210,077	1.008	2,190,470	1.010	2,190,470	1.011	2,190,470	1.012
	4	*2,428,339	0.997	2,401,091	1.010	2,401,091	1.007	2,401,091	0.977
	5	*2,431,813	1.012	2,403,807	0.993	2,403,807	1.001	2,403,807	1.008
	6	*2,376,153	1.013	2,351,180	0.997	2,351,180	1.004	2,351,180	1.011
RS I	4	2,361,278	1.010	2,361,278	1.005	2,361,278	1.009	2,361,278	1.004
	5	2,449,265	1.005	2,449,265	1.018	2,449,265	1.011	2,449,265	1.012
	6	2,418,155	1.008	2,418,155	1.000	2,418,155	1.001	2,418,155	0.997
RS II	4	2,322,498	1.000	2,322,498	1.004	2,322,498	1.000	2,322,498	1.000
	5	2,349,165	1.002	2,349,165	1.003	2,349,165	1.001	2,349,165	1.000
	6	2,077,844	1.004	2,077,844	1.014	2,077,844	1.012	2,077,844	0.998
WGHS	3	2,477,551	1.045	2,477,551	1.029	2,477,551	1.040	2,477,551	1.031
	4	2,481,199	1.047	2,481,199	1.038	2,481,199	1.051	2,481,159	1.019
	5	2,468,197	1.017	2,468,197	1.009	2,468,197	1.010	2,468,142	1.015

	6	2,308,858	1.006	2,308,858	1.013	2,308,858	1.010	2,308,858	1.001

* Some SNPs in the MESA datasets were exclusive to that cohort and did not match back to our HapMap legend file.
For SBP in MESA: We analyzed 2,117,061 SNPs in age bin 3; 2,327,960 in age bin 4; 2,331,257 in age bin 5; and 2,277,690 in age bin 6
For DBP, MAP, and PP in MESA: We analyzed 2,097,712 in age bin 3; 2,301,058 in age bin 4; 2,303,611 in age bin 5; 2,253,038 in age bin 6

STAGE 2 STUDIES

Study Descriptions

The Busselton Health Study (BHS) includes a series of seven cross sectional population health surveys of adult residents of the Shire of Busselton in the South-West of Western Australia, undertaken between 1966 and 1995. A cross-sectional community follow-up study in 1994-1995 included the collection of blood for DNA extraction for all survivors of previous surveys. A total of 4,554 individuals participated in this follow-up. BP was measured in the 1994-1995 follow-up study using a standard mercury sphygmomanometer (Baumanometer, New York) as described previously[18]. The participants were asked to refrain from caffeine for 12 hours and to not smoke prior to attending the survey. Three BP readings were recorded on the participant's survey chart to the nearest 2 mmHg and the average of the readings was used for the analyses.

The Cohorte Lausannoise (CoLaus) is a population-based study aimed at assessing the prevalence and molecular determinants of cardiovascular risk factors in the population of Lausanne, Switzerland[19]. Participants in the study (4,969) were randomly selected from the population register of Lausanne in 2003 (N=56,694, aged 35-75 years). All individuals were of European origin, defined as having both parents and grandparents born in a defined list of European countries. Blood pressure was measured using the Omron HEM-907 machine, in the seated position. Three measures were taken on the left arm; the mean of the last two measures was used in the analyses.

The European Prospective Investigation of Cancer (EPIC-Norfolk) is a population-based cohort study of Europid men and women aged 39-79 years recruited in Norfolk, UK between 1993 and 1997[20]. Blood pressure was measured using the Accutorr oscillometric BP machine; the mean of two readings was taken and used in the analysis. This analysis was performed based on the subcohort sample of the EPIC-Norfolk case-cohort design (N=2,417) of which 2,411 had information on blood pressure and 2,408 had passed quality control (QC).The study design and more detailed information is available from Loos et al.[21].

The Fenland Study is an ongoing population-based cohort study (started in 2005) designed to investigate the association between genetic and lifestyle environmental factors and the risk of obesity, insulin sensitivity, hyperglycemia, and related metabolic traits in men and women aged 30 to 55 years. Potential volunteers were recruited from general practice sampling frames in the Fenland, Ely, and Cambridge areas of the Cambridgeshire Primary Care Trust in the UK. Exclusion criteria for the study were: prevalent diabetes, pregnant and lactating women, inability to participate including terminal illness, psychotic illness, or inability to walk unaided. Currently,

the study comprises more than 3,000 participants; volunteers with complete anthropometric data were genotyped and included in the current analyses. All participants were measured at the MRC Epidemiology Unit Clinical Research Facilities in Ely, Wisbech and Cambridge. Blood pressure measurements were taken with an Accutorr automated sphygmomanometer using the average of three measurements made at one-minute intervals with the participant seated for 5 minutes prior to measurement. Of the 1,500 individuals that were genotyped 98 individuals were excluded as their genotyping data did not meet the quality control criteria applied. In total, 1,399 individuals were included in the genome-wide association analyses.

The Kooperative Gesundheitsforschung in der Region Augsburg Third Survey (KORA S3) is an epidemiological cohort recruited from the general population of Augsburg, Germany in 1994-1995[22; 23]. A subset of this survey (1,644 subjects), were genotyped using the Affymetrix 500K array (http://epi.helmholtz-muenchen.de/kora-gen/). In this study subjects with BMI<35 kg/m2 were included; diabetics were excluded. Blood pressure was measured using a random zero sphygmomanometer in the seated position at the first examination cycle. Three measurements were taken at least three minutes apart and the numbers entering the database were the mean of the last two measurements.

The LifeLines Cohort Study [24] is a multi-disciplinary prospective population-based cohort study using a unique three-generation design to examine 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. In addition, the LifeLines project comprises a number of cross-sectional sub-studies which investigate specific age-related conditions. These include investigations into metabolic and hormonal diseases, including obesity, cardiovascular and renal diseases, pulmonary diseases and allergy, cognitive function and depression, and musculoskeletal conditions. All survey participants are between 18 and 90 years old at the time of enrollment. Recruitment has been going on since the end of 2006, and over 130,000 participants had been included by April 2013. At the baseline examination, the participants in the study were asked to fill in a questionnaire (on paper or online) before the first visit. During the first and second visit, the first or second part of the questionnaire, respectively, are checked for completeness, a number of investigations are conducted, and blood and urine samples are taken. In the first visit to the LifeLines' study center, trained technicians measure subjects' systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse rate, every minute for a period of 10 minutes using a DINAMAP Monitor i.e. 10 measures for each of the indices.

The Myocardial Infarction Genetics Consortium (MIGen) cohort is composed of a subset of the controls of a case-control study aimed at identifying genetic variants associated with early-onset myocardial infarction. Most of the controls are selected from population

based cross-sectional or cohort studies and come from five different studies: Heart Attack Risk in Puget Sound (Seattle, USA), REGICOR (Girona, Spain), MGH Premature Coronary Artery Disease Study (Boston, USA), FINRISK (Finland); Malmö Diet and Cancer Study (Malmö, Sweden). There is a minimal overlap of samples between the resources (N=30). For the majority of studies, blood pressure was measured twice using calibrated sphygmomanometers, in the seated position after at least 5 minutes of rest; the mean of the two measurements was used in the analysis. The first two principal components from an identical by state (IBS) analysis were used to adjust for potential population stratification.

The Netherlands Study of Depression and Anxiety (NESDA)[25], is a multi-center study designed to examine the long-term course and consequences of depressive and anxiety disorders (http://www.nesda.nl). NESDA included both individuals with depressive and/or anxiety disorders and controls without psychiatric conditions. Inclusion criteria were age 18-65 years and self-reported western European ancestry while exclusion criteria were not being fluent in Dutch and having a primary diagnosis of another psychiatric condition (psychotic disorder, obsessive compulsive disorder, bipolar disorder, or severe substance use disorder). For all participants DNA was isolated from the baseline blood sample. Through funding from the fNIH GAIN program (www.fnih.gov/gain), whole genome scan analysis was conducted for 1859 NESDA (1702 depressed cases and 157 controls) participants. A hundred subjects were excluded because of various quality control issues [26]. Additional exclusions were made based on disease history, phenotype availability and medication use (e.g., all subjects using antidepressants other than SSRIs were excluded) [27]. Systolic blood pressure and diastolic blood pressure were measured twice using an OMRON IntelliSense Professional Digital Blood Pressure Monitor, HEM-907XL (Omron Healthcare, Inc., Bannockburn, Illinois) during supine rest on the right arm, and were averaged over the two measurements [27].

The Prevention of Renal and Vascular End Stage Disease (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 [28; 29]. 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). Details of the protocol have been described elsewhere (www.prevend.org). Blood pressure was measured in the supine position every minute for 10 and 8 minutes, respectively, with an automatic Dinamap XL Model 9300 series monitor (Critikon, Tampa, Florida). Systolic and diastolic blood pressures were calculated as the mean of the last two measurements at the two visits.

The Precocious Coronary Artery Disease study (PROCARDIS) (www.procardis.org) is a European consortium investigating the genetics of precocious coronary artery disease (CAD) in

German, Italian, Swedish, and British CAD patients and controls [30]. Country of origin was a covariate in all analyses. The CAD cases (N= 5,480) and controls (N= 1,570) were included in this study; the controls had no personal history of CAD, hypertension, or diabetes. Blood pressure was measured twice using various sphygmomanometers, in the seated position after at least 5 minutes of rest; the mean of the two measurements was used.

The SardiNIA study is a longitudinal study examining age-related quantitative traits in individuals from the Ogliastra region of Sardinia, Italy[31]. Genotype data was available for 4,305 related individuals (age >14 years). Blood pressure was measured using a mercury sphygmomanometer; the average of the second and third reading was used for the analyses. Due to the family-based nature of the SardiNIA study, this analysis allowed each family to appear in only one age bin; the selection of individuals maximized the sample size while trying to achieve 250 individuals in each age bin.

The Study of Health In Pomerania (SHIP) is a population-based survey in West Pomerania, the northeast area of Germany [32]. A sample from the adult population aged 20 to 79 years was drawn based on population registries of cities and towns in the region. SHIP finally comprised 4,308 participants (corresponding to a final response rate of 68.8%). Blood pressure was measured three times in a seated position using a digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan). The initial reading occurred after 5 minutes of rest, with 3 minutes between sequential measurements. The mean of the second and third measurements was used in the analyses.

The Supplementation en Vitamines et Mineraux Antioxydants study (SUVIMAX) is a longitudinal study performed on a national sample of healthy volunteers from France between 1996 and 2001. 1,673 individuals, aged 35-65 years at baseline were included in this analysis [33]. Blood pressure was measured using a mercury sphygmomanometer in the seated position; the average of three readings taken from the first examination (1996) was used in the analysis.

TRacking Adolescents' Individual Lives Survey (TRAILS) is a prospective cohort study of Dutch adolescents with bi- or triennial measurements from age 11 to at least age 25 and consists of a general population and a clinical cohort (for a cohort profile see [34]). In the population cohort, four assessment waves have been completed to date, which ran from March 2001 to July 2002 (T1), September 2003 to December 2004 (T2), September 2005 to August 2007 (T3), and October 2008 to September 2010 (T4). Data for the present study were collected during the third assessment wave. At T1, 2230 (pre)adolescents were enrolled in the study (response rate 76.0%, mean age 11.09, SD 0.55, 50.8% girls [35], of whom 81.4% (N = 1816, mean age 16.27, SD 0.73, 52.3% girls) participated at T3. The TRAILS Clinical Cohort runs in parallel with the TRAILS

general population cohort. The clinical cohort consists of 543 children of initially 10–12 years of age (mean age 10.89 years) who have been referred to one child psychiatric outpatient clinic in the Northern Netherlands at any point in their life. Systolic and diastolic blood pressure were measured in duplicate with a Dinamap Critikon 1846SX (Critikon Inc, Tampa, FL), from which we calculated means. Blood samples were obtained after at least 8 hours of fasting. Genome-wide genotyping was done with the Illumina Cyto SNP12 v2 array. This data was imputed using IMPUTE2 and association analysis was performed with SNPTEST v2.2.0.

Cardiovascular Risk in Young Finns Study (YFS) was set up to determine the contribution of childhood lifestyle, biological, and psychological measures to the risk of cardiovascular diseases in adulthood. In 1980, over 3,500 children and adolescents from all over Finland participated in the baseline study. Thereafter these subjects were followed up with several examinations including comprehensive risk factor assessments. The 27-year follow-up was performed in 2007 and the blood pressure measurements at this time point were used for this study. Blood pressure was measured by nursing staff three times using a random-zero sphygmomanometer and the average of the three measurements was taken. Individuals were excluded if BMI, systolic or diastolic blood pressure measurements or genotype data were missing.

Table S4: Descriptive Statistics for the Stage 2 Studies

Stage 2 Study	Age Bin	N	Median Age	Female (%)	HT (%)	BMI Mean (SD)	SBP Mean (SD)	DBP Mean (SD)	MAP Mean (SD)	PP Mean (SD)
BHS	[20,40)	276	34	60.1	2.9	25.0 (3.7)	115.6 (11.8)	71.6 (9.4)	86.3 (8.9)	44.0 (10.4)
	[40,50)	223	45	58.7	6.7	25.9 (4.1)	117.8 (14.4)	75.6 (10.4)	89.7 (11.2)	42.2 (8.8)
	[50,60)	225	55	51.1	14.7	26.7 (4.5)	125.9 (15.7)	79.1 (10.1)	94.7 (11.2)	46.7 (10.7)
	[60,70)	207	65	61.4	36.2	26.8 (4.0)	135.3 (17.8)	79.0 (9.4)	97.8 (10.8)	56.3 (14.8)
	[70,80)	204	74	55.4	50.0	26.2 (3.9)	145.3 (18.9)	77.8 (10.7)	100.3 (11.8)	67.5 (16.0)
CoLaus	[30,40)	534	37	50.4	2.4	24.9 (4.1)	118.5 (12.4)	75.6 (9.6)	95.7 (12.1)	49.0 (12.2)
	[40,50)	1,437	45	49.8	6.4	25.0 (4.3)	122.1 (15.0)	78.0 (10.4)	92.5 (11.1)	43.7 (8.1)
	[50,60)	1,334	55	55.5	16.4	26.0 (4.8)	130.0 (17.8)	81.1 (11.0)	96.0 (12.2)	49.2 (12.3)
	[60,70)	1,195	64	54.8	32.3	26.8 (4.6)	139.9 (19.6)	81.4 (10.6)	96.2 (12.3)	49.4 (12.3)
	[70, 80)	443	73	56.3	38.7	26.7 (4.4)	145.5 (19.6)	78.1 (10.9)	95.9 (12.2)	49.2 (12.3)
EPIC	[40,50)	442	47	56.3	5.7	25.4 (4.0)	127.4 (14.1)	80.1 (10.6)	95.9 (11.4)	47.4 (6.9)
	[50,60)	775	55	56.3	10.1	26.1 (3.8)	132.5 (17.0)	81.9 (10.8)	98.8 (12.4)	50.5 (9.6)
	[60,70)	819	65	49.8	19.1	26.9 (3.7)	143.1 (18.4)	85.6 (11.1)	104.7 (13.0)	57.5 (11.1)
	[70, 80)	371	72	50.1	24.3	26.8 (3.9)	147.8 (18.5)	86.4 (11.3)	106.9 (13.0)	61.4 (11.5)
Fenland	[30,40)	388	36	50.0	8.0	26.3 (4.7)	117.7 (13.0)	72.5 (9.4)	87.6 (9.9)	45.2 (8.7)
	[40,50)	607	45	58.2	16.6	27.4 (5.0)	121.9 (15.0)	75.5 (10.2)	91.0 (11.3)	46.4 (8.7)
	[50,60)	404	54	59.4	31.9	27.4 (4.8)	127.8 (17.7)	77.4 (10.9)	94.2 (12.6)	50.4 (10.5)
KORA S3	[40,50)	191	46	49.7	32.5	26.5 (4.5)	126.9 (18.7)	82.5 (10.8)	97.3 (12.8)	44.4 (11.5)
	[50,60)	984	55	51.3	45.0	27.7 (4.1)	134.9 (19.3)	84.3 (11.1)	101.2 (13.1)	50.5 (12.6)
	[60,70)	419	64	47.7	60.9	28.4 (3.9)	144.4 (20.6)	83.4 (11.4)	103.8 (13.1)	61.0 (15.8)
LifeLines	[20,30)	393	27	58.5	10.7	24.3 (3.8)	124.2 (12.2)	70.5 (6.5)	88.4 (7.4)	53.7 (10.2)
	[30,40)	1,576	36	57.1	14.2	25.7 (4.6)	123.7 (12.8)	72.9 (7.9)	89.8 (9.1)	50.8 (10.2)
	[40,50)	3,039	45	58.6	24.7	26.1 (4.4)	126.9 (16.2)	75.8 (10.0)	92.7 (11.6)	50.9 (11.1)
	[50,60)	1,893	51	56.0	37.3	26.7 (4.2)	131.2 (17.1)	77.8 (10.0)	95.5 (11.9)	53.5 (11.9)
	[60,70)	899	64	55.1	58.6	27.3 (3.9)	138.3 (20.8)	78.2 (10.1)	97.8 (13.9)	60.1 (14.8)
	[70,80)	288	72	54.5	75.3	27.8 (3.9)	145.2 (20.8)	78.5 (9.2)	100.0 (14.2)	65.4 (17.1)
MIGen	[30,40)	124	36	23.4	16.1	25.8 (4.5)	119.1 (14.4)	74.9 (9.7)	89.6 (10.4)	44.1 (10.6)
	[40,50)	527	45	26.9	20.1	26.7 (4.5)	123.9 (15.6)	79.7 (10.7)	94.4 (11.5)	44.3 (10.5)
	[50,60)	391	54	62.1	30.2	27.8 (5.4)	132.2 (19.8)	82.7 (11.9)	99.2 (13.6)	49.5 (13.6)
	[60,80)	154	64	29.9	46.1	28.0 (4.1)	141.0 (21.8)	82.7 (11.5)	102.1 (13.9)	58.3 (15.4)

NESDA	[20,30)	340	25	76.5	0.9	23.6 (4.4)	126.5 (13.0)	74.9 (7.9)	92.1 (8.5)	51.6 (10.6)
	[30,40)	361	35	72.9	3.3	25.1 (5.1)	126.6 (13.6)	77.6 (9.1)	94.0 (9.7)	49.1 (9.4)
	[40,50)	424	44	64.9	9.7	26.0 (5.0)	133.4 (17.1)	82.2 (10.5)	99.3 (12.0)	51.1 (11.2)
	[50, 60)	422	54	61.6	27.0	26.7 (4.7)	144.1 (21.3)	86.4 (11.3)	105.6 (13.8)	57.6 (13.8)
PREVEND	[30,40)	853	34	55.2	8.3	24.5 (4.0)	119.4 (14.0)	64.4 (8.0)	85.4 (9.2)	50.9 (9.9)
	[40,50)	980	45	50.4	16.8	25.8 (4.3)	124.4 (17.2)	73.7 (9.9)	90.6 (11.9)	50.8 (10.4)
	[50,60)	820	54	48.6	32.1	27.0 (4.2)	133.2 (20.8)	78.6 (10.8)	96.7 (13.3)	54.5 (13.6)
	[60,70)	650	64	42.0	52.0	27.5 (4.0)	146.5 (22.4)	82.1 (10.7)	103.6 (13.8)	64.1 (15.4)
PROCARDIS	[40,50)	649	47	20.6	*	27.8 (4.9)	131.5 (16.7)	84.7 (10.4)	100.3 (11.6)	46.8 (11.7)
	[50,60)	2,399	56	24.3	*	28.3 (4.6)	137.6 (18.4)	85.3 (10.6)	102.8 (12.2)	52.2 (13.4)
	[60,70)	3,362	65	29.3	*	27.9 (4.3)	144.3 (20.8)	83.5 (10.8)	103.8 (12.7)	60.9 (16.5)
	[70,80)	640	72	30.8	*	27.9 (4.3)	148.5 (22.1)	81.8 (11.8)	104.0 (13.7)	66.7 (17.4)
SardiNIA	[20,40)	287	30	59.2	0.7	23.2 (3.6)	117.0 (12.0)	72.0 (8.0)	87.0 (9.0)	45.0 (10.0)
	[40,50)	232	45	63.3	3.9	25.8 (4.3)	126.0 (16.0)	81.0 (10.0)	96.0 (11.0)	45.0 (12.0)
	[50,60)	268	54	49.6	21.6	28.0 (4.0)	138.0 (19.0)	86.0 (11.0)	103.0 (13.0)	53.0 (13.0)
	[60,70)	257	64	49.0	30.0	28.9 (4.3)	142.0 (17.0)	85.0 (10.0)	104.0 (11.0)	57.0 (13.0)
	[70,80)	204	74	51.0	46.1	28.5 (4.6)	151.0 (20.0)	85.0 (11.0)	107.0 (12.0)	66.0 (17.0)
SHIP	[20,30)	550	25	54.5	19.5	24.3 (4.3)	121.9 (14.4)	75.7 (8.4)	91.1 (9.6)	46.2 (10.4)
	[30,40)	729	35	53.4	24.8	25.7 (4.3)	125.9 (17.1)	81.3 (10.4)	96.2 (12.1)	44.5 (10.3)
	[40,50)	726	45	53.2	38.0	27.7 (4.8)	135.4 (20.8)	87.1 (11.9)	103.2 (14.4)	48.3 (11.7)
	[50,60)	760	55	52.5	44.7	28.4 (4.8)	142.5 (21.3)	88.8 (11.5)	106.7 (14.1)	53.8 (13.6)
	[60,70)	733	64	46.1	53.2	28.8 (4.5)	148.9 (21.3)	87.2 (10.7)	107.8 (13.2)	61.7 (15.6)
	[70,80)	560	74	44.5	58.0	28.5 (4.2)	154.6 (22.4)	85.0 (12.5)	108.2 (14.5)	69.6 (16.7)
SUVIMAX	[35, 50)	819	46	74.6	11.6	22.8 (3.1)	117.5 (11.1)	76.0 (7.8)	89.8 (8.4)	41.5 (6.8)
	[50,65)	854	55	47.1	26.1	24.3 (3.3)	124.4 (12.6)	80.0 (7.9)	94.8 (8.9)	44.3 (8.0)
TRAILS: clinical Cohort	<20	266	16	30.0	0.0	21.6 (3.6)	119.2 (12.6)	61.0 (6.6)	80.4 (7.3)	58.2 (11.4)
TRAILS: population cohort	<20	1,290	16	52.0	0.0	21.3 (3.2)	118.1 (12.4)	61.1 (6.9)	80.1 (7.4)	57.0 (11.1)
YFS	[30,40)	1,562	36	53.7	5.4	25.8 (4.8)	120.0 (13.9)	74.7 (11.3)	89.8 (11.5)	45.3 (8.9)
	[40,50)	686	42	54.2	13.3	26.4 (4.6)	124.3 (15.7)	78.6 (11.7)	93.8 (12.4)	45.7 (9.3)

* The PROCARDIS study was a case-control study of coronary artery disease; the % of cases in age bins 3 through 6 were 56.7%, 74.3%, 81.8%, and 90.6%, respectively.

Table S5: Genotyping, Imputation, and Association Analysis Information for the Stage 2 Studies

Stage 2 Study	Genotyping Platform	NCBI human genome reference used	Imputation Procedure	Pre-Imputation QC filter information	Pre-Association Filters (sample or SNP)	Association Analysis Software
BHS	Illumina 610K Chip	36	MACH v1.0.16.b with reference HapMap II CEU v22, Build 36	Hardy-weinberg eqilibrium(HWE) p-value: 1E-07, SNP callrate: 0.95, Sample callrate: 0.97, MAF:0.01, IBD>0.1875 removed, Ethnic outliers and gender mismatches removed		Mach2qtl V1.0.8
CoLaus	Affymetrix 500K	35v21	MACH	HWE p-values:1E-7, SNP Callrate: 0.9, MAF:0.01		In house Matlab script
EPIC	Affymetrix 500K	35	IMPUTE v0.3.1	HWE p-values:1E-6, SNP Callrate: 0.9, MAF:0.01		SNPTEST v1.1.5
Fenland	Affymetrix GeneChip Human Mapping 500K Array Set	36.2	IMPUTE v2.1.2	Sample callrate: 0.95; Heterozygosity between 27.3% and 28.8%; Duplicate Check, Relatedness Check, SNP callrate: 0.90, HWE p-value: 1E-06, MAF: 0.01		SNPTEST v1.1.5
KORA S3	Affymetrix 500k platform	36.1	MACH1 with HapMap release 22	MAF: 0.0008, HWE p-value: 1E-5, SNP callrate:0.9		ProbABEL 0.1-9e
LifeLines	Illumina Cyto SNP12 v2	HapMap II build 36, release 24	Beagle	SNP callrate: 0.95, MAF: 0.01, HWE p-value:1E-4, Sample callrate: 0.95, unrelatedness (pi-hat<0.4), gender match, caucasians	Analyze SNPs with MAF>0	PLINK
MIGen	Affymetrix 6.0 GeneChip	35	MACH 1.0	SNP call rate: 0.95, sample call rate 0.95; HWE p-value:1E-6; MAF: 0.01; SNPs with CHI-MISSING p < 1e-3		ProbABEL v0.0-6
NESDA	Perlegen 600k chip (N=1,747) and the Affymetrix 6.0 array (N=100)	36 release 23	IMPUTE v2	SNP call rate: 0.95, MAF: 0.01, not mapped, HWE p-value: 1E-6, strand ambiguities, high concordance between genotyping platforms or between positive controls, random genotypic failure, <5% Mendelian errors, samples callrate: 0.95, unrelatedness, gender match, caucasians	None	SNPTEST v2
PREVEND	Illumina CytoSNP12 v2	37	Beagle	Sample callrate 0.95, Relatedness >0.1, Ethnic outliers Z-score > 3 for first 5 PCA, MAF: 0.01, HWE p-value: 1E-03, SNP Callrate 0.9	plink filters on INFO <0.1, MAF <0.01	PLINK 1.07
PROCARDIS	Illumina Infinium BeadChips (1M and HumanHap	36	MACH with reference HapMap2 release 22	SNP callrate: 0.955, sample callrate: 0.955, HWE p-value: 1E-6, Non-European ancestry dropped, Duplicates dropped	Exclude SNPs with MAF<5% and MACH	Stata accounting for clustering in covariance due to sibships

Stage 2 Study	Genotyping Platform	NCBI human genome reference used	Imputation Procedure	Pre-Imputation QC filter information	Pre-Association Filters (sample or SNP)	Association Analysis Software
	610)				R^2 <0.3	
SardiNIA	Combination of Affymetrix 10K, 500K, 6.0 chip	36	MACH 1.0 with reference HapMap CEU v22	Sample callrate: 0.95, SNP callrate>0.90 for 10K and 500K chips, SNP callrate>0.95 for 6.0 chip, HWE p-value: 1E-06, MAF>0.05 for 10K and 500K chips, MAF>0.01 for Affymetrix 6.0 chip		MERLIN
SHIP	Affymetrix 6.0	36.1 36 (dbSNP 126)	IMPUTE v0.5.0 with reference HapMap II CEU v22, Build 36	Excluded arrays with call rates < 86%; Final, Duplicate samples (by IBD), gender mismatch		QUICKTEST v0.95
SUVIMAX	Illumina HumanHap 317	35 CEU release 21	IMPUTE v0.3.2	Samples call rate: 0.94, SNP call rate: 0.97, HWE p-value: 1E-7		QUICKTEST
TRAILS	Illumina Cyto SNP12 v2	Data were imputed using HapMap II build 36, release 22	IMPUTE v2	SNPs callrate: 0.95, MAF: 0.01, HWE p-value:1E-4, chr X >1%, heterozygous in men, samples callrate: 0.95, heterozygosity <4SD from mean, non-duplicates, gender match, caucasians	None	SNPTEST v2
YFS	Illumina 670k custom	36.3 dbSNP 126	MACH 1.0 with reference HapMap II CEU v22	HWE p-value: 1E-06, Sample callrate: 0.95, SNP callrate: 0.95, MAF: 0.01		ProbABEL v. 0.1-3

SINGAPORE STUDIES

Study Descriptions

The Singapore Chinese Eye Study (SCES) is a population-based, cross-sectional study of Chinese adults aged 40–80+ years residing in the South-Western part of Singapore, which is part of the Singapore Epidemiology of Eye Disease (SEED). Age stratified random sampling was used to select 6,350 eligible participants, of which 3,300 participated in the study (73% response rate). Detailed methodology has been published [36]**.** Two readings of blood pressure were taken from participants after 5 minutes of rest, seated, using an automated blood pressure monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany) by trained observers. One of two cuff sizes (regular, large) was chosen on the basis of the circumference of the participant's arm. A third reading was performed if the difference between two readings of either the systolic blood pressure was greater than 10mmHg or the diastolic blood pressure was greater than 5mmHg. The mean values of the closest two readings were calculated.

The Singapore Malay Eye Study (SiMES) is a population-based cross-sectional epidemiological study of 3,280 individuals from one of the three major ethnic groups residing in Singapore[37; 38]. SiMES is part of the Singapore Epidemiology of Eye Disease (SEED) study. In summary, 5600 individuals have been selected by an age-stratified sampling strategy. Among these 4168 individuals are eligible for this study. 3280 individuals finally participated in the study. All subjects were Malay and aged 40-80 years. Two readings of blood pressure were taken from participants after 5 minutes of rest, seated, using an automated blood pressure monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany) by trained observers. One of two cuff sizes (regular, large) was chosen on the basis of the circumference of the participant's arm. A third reading was performed if the difference between two readings of either the systolic blood pressure was greater than 10mmHg or the diastolic blood pressure was greater than 5mmHg. The mean values of the closest two readings were calculated.

The Singapore Indian Eye Study (SINDI) is a population-based, cross-sectional study of Asian Indian adults aged 40–80+ years residing in the South-Western part of Singapore, which is part of the Singapore Epidemiology of Eye Disease (SEED). Age stratified random sampling was used to select 6,350 eligible participants, of which 3,400 participated in the study (75.6% response rate). Detailed methodology has been published [36]. Two readings of blood pressure were taken from participants after 5 minutes of rest, seated, using an automated blood pressure monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany) by trained observers. One of two cuff sizes (regular, large) was chosen on the basis of the circumference of the participant's arm. A third reading was performed if the difference between two readings of either the systolic blood pressure was greater than 10mmHg or the diastolic blood pressure was greater than 5mmHg. The mean values of the closest two readings were calculated.

The Singapore Prospective Study Program (SP2) is a population-based study of diabetes and cardiovascular disease in Singapore that has been described previously [39]. Eligible subjects included 10,633 Chinese, Malay, and Indian subjects from four cross-sectional studies that were conducted in Singapore between 1984 and 1998.

Subjects were aged 18-69 at baseline and represented a random sample of the Singapore population. Subjects were re-visited between 2003 and 2007. Data from this re-visit were utilized for this study. Two readings of blood pressure were taken from participants after 5 min of rest, seated, using an automated blood pressure monitor (Dinamap Pro100V2; Criticon, Norderstedt, Germany) by trained observers. One of two cuff sizes (regular, large) was chosen on the basis of the circumference of the participant's arm. A third reading was performed if the difference between two readings of either the systolic blood pressure was greater than 10mmHg or the diastolic blood pressure was greater than 5mmHg. The mean values of the closest two readings were calculated.

Table S6: Descriptive Statistics for the Singapore Studies

Singapore Study	Age Bin	N	Median Age	Female (%)	HT (%)	BMI Mean (SD)	SBP Mean (SD)	DBP Mean (SD)	MAP Mean (SD)	PP Mean (SD)
SCES	[40,50)	470	47	49.8	14.7	23.8 (3.7)	130.1 (17.5)	79.4 (10.8)	96.3 (12.3)	50.7 (11.6)
	[50,60)	633	55	52.3	26.7	23.8 (3.4)	137.1 (18.6)	80.8 (10.1)	99.6 (12.0)	56.3 (13.4)
	[60,70)	497	63	46.9	46.7	23.7 (3.4)	146.9 (18.6)	81.7 (9.4)	103.4 (11.2)	65.2 (14.7)
	[70,80)	249	73	39.8	59.8	23.8 (3.5)	155.9 (19.3)	80.5 (8.1)	105.6 (10.3)	75.4 (16.6)
SiMES	[40,50)	590	45	52.2	41.4	26.4 (5.4)	134.9 (19.8)	79.7 (11.2)	98.1 (13.3)	55.2 (13.2)
	[50,60)	750	54	55.1	65.5	27.1 (4.9)	147.2 (22.8)	82.4 (11.6)	104.0 (14.2)	64.8 (16.6)
	[60,70)	599	65	50.1	84.0	26.7 (5.0)	156.9 (23.8)	81.7 (11.3)	106.7 (14.2)	75.3 (18.0)
	[70,80)	563	74	43.3	89.9	25.1 (5.0)	162.3 (23.8)	81.0 (11.5)	108.1 (14.5)	81.3 (17.4)
SINDI	[40,50)	632	46	51.7	23.3	26.2 (4.6)	127.9 (18.6)	78.6 (11.0)	95.1 (12.7)	49.3 (12.3)
	[50,60)	799	54	50.3	36.1	27.0 (4.8)	135.9 (19.3)	80.2 (10.7)	98.8 (12.6)	55.6 (13.7)
	[60,70)	723	63	46.2	48.6	25.8 (4.7)	146.1 (20.6)	79.9 (10.0)	102.0 (12.2)	66.2 (16.2)
	[70,80)	322	73	47.5	60.3	25.6 (5.0)	152.1 (19.9)	77.0 (8.6)	102.0 (11.0)	75.1 (16.6)
SP2 (Illumina 1M platform)	[30,40)	205	36	42.9	7.3	22.4 (3.5)	120.1 (14.1)	73.3 (9.4)	88.9 (10.4)	46.8 (8.7)
	[40,50)	405	45	39.8	11.9	22.9 (3.4)	126.1 (14.6)	78.0 (10.6)	94.0 (11.3)	48.0 (9.1)
	[50,60)	203	54	28.6	23.2	23.6 (3.6)	134.7 (18.2)	82.3 (11.3)	99.8 (12.8)	52.4 (12.0)
SP2 (Illumina 610 platform)	[30,40)	201	35	75.1	4.0	22.2 (4.7)	115.7 (13.1)	70.8 (8.2)	85.8 (9.2)	44.9 (8.7)
	[40,50)	366	45	80.6	11.2	22.5 (3.7)	122.4 (16.4)	73.8 (9.9)	90.0 (11.4)	48.6 (10.7)
	[50,60)	337	54	78.0	23.7	23.1 (3.7)	132.1 (18.3)	77.0 (10.9)	95.3 (12.3)	55.2 (13.2)
	[60,70)	138	65	73.2	37.7	23.4 (3.2)	150.0 (20.2)	78.4 (9.2)	102.3 (11.6)	71.6 (16.1)

Table S7: Genotyping, Imputation, and Association Analysis Information for the Singapore Studies

Singapore Study	Genotyping Platform	NCBI human genome reference used	Imputation Procedure	Pre-Imputation QC filter information	Pre-Association Filters (sample or SNP)	Association Analysis Software
SCES	Illumina610 Quad	Build 36/hg18	IMPUTE2.2, HapMap phase2 release22 JPT+CHB	Sample callrate: 0.95, SNP callrate>0.95, HWE p-value: 1E-06, MAF>0; Sample call rate >0.95, population outlier, cryptic relationship, excessive heterozygosity, gender mismatch.	None	SNPTEST v2.2
SiMES	Illumina610 Quad	Build 36/hg18	IMPUTE0.5, HapMap phase2 release22 JPT+CHB+CEU+YRI	Sample callrate: 0.95, SNP callrate>0.95, HWE p-value: 1E-06, MAF>0; Sample call rate >0.95, population outlier, cryptic relationship, excessive heterozygosity, gender mismatch.	None	SNPTEST v2.2 Covariates PC1 and PC2
SINDI	Illumina610 Quad	Build 36/hg18	IMPUTE0.5, HapMap phase2 release22 JPT+CHB+CEU+YRI	Sample callrate: 0.95, SNP callrate>0.95, HWE p-value: 1E-06, MAF>0; Sample call rate >0.95, population outlier, cryptic relationship, excessive heterozygosity, gender mismatch.	None	SNPTEST v2.2 Covariates PC1, PC2 and PC3
SP2	Illumina 1M and Illumina 610	Build 36/hg18	IMPUTE0.5, HapMap phase2 release22 JPT+CHB	Sample callrate: 0.95, SNP callrate>0.95, HWE p-value: 1E-06, MAF>0; Sample call rate >0.95, population outlier, cryptic relationship, excessive heterozygosity, gender mismatch.	None	SNPTEST v2.2

TITLES OF SUPPLEMENTAL TABLES 8-22

NOTE: Red text denotes a table with results from all stages of analysis.

Table S8: Joint Effects of the SNP and SNP-age Interaction on Systolic Blood Pressure from the Meta-regression Using Stage 1 Subgroups (CHARGE)

Table S9: Joint Effects of the SNP and SNP-age Interaction on Diastolic Blood Pressure from the Meta-regression Using Stage 1 Subgroups (CHARGE)

Table S10: Joint Effects of the SNP and SNP-age Interaction on Mean Arterial Pressure from the Meta-regression Using Stage 1 Subgroups (CHARGE)

Table S11: Joint Effects of the SNP and SNP-age Interaction on Pulse Pressure from the Meta-regression Using Stage 1 Subgroups (CHARGE)

Table S12: Combined Meta-regression of all Stage 1 (CHARGE) and Stage 2 (largely Global BPgen) Subgroups for Loci with Suggestive or Significant Evidence by the Joint 2 DF test; Replication in the Singapore Subgroups

Table S13: Five Loci Identified With the Joint 2DF Test That Would Have Been Missed By a Two-Stage Main Effects Only Analysis

Table S14: One Degree of Freedom Test of SNP-age Interactions on Mean Arterial Pressure in Stage 1 Subgroups (CHARGE)

Table S15: One Degree of Freedom Test of SNP-age Interactions on Pulse Pressure in Stage 1 Subgroups (CHARGE)

Table S16: Replication of Loci Chosen Through the 1DF Test of the SNP-age Interaction; Combined Meta-regression of All Stage 1 and Stage 2 Subgroups (Individuals of European Ancestry)

Table S17: Genomic Inflation Factors from the Inverse-variance Weighted Meta-Analysis Conducted Within Each Age Bin; the Secondary Analysis Using Stage 1 Subgroups (CHARGE)

Table S18: Within Age Bin Meta-analysis of the SNP Effect on Systolic Blood Pressure: Significant and Suggestive Associations in the Secondary Analysis of Stage 1 (CHARGE)

Table S19: Within Age Bin Meta-analysis of the SNP Effect on Diastolic Blood Pressure: Significant and Suggestive Associations in the Secondary Analysis of Stage 1 (CHARGE)

Table S20: Within Age Bin Meta-analysis of the SNP Effect on Mean Arterial Pressure: Significant and Suggestive Associations in the Secondary Analysis of Stage 1 (CHARGE)

Table S21: Within Age Bin Meta-analysis of the SNP Effect on Pulse Pressure: Significant and Suggestive Associations in the Secondary Analysis of Stage 1 (CHARGE)

Table S22: Combined Within-Age Bin Meta-analysis of Stage 1 and Stage 2 Subgroups; Replication of Secondary Within-Age Bin Analyses Using Singapore Subgroups.

ACKNOWLEDGMENTS

AGES has been funded by NIH contract N01-AG-12100, the NIA Intramural Research Program Z01-AG-007380, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament). The study is approved by the Icelandic National Bioethics Committee, VSN: 00-063.

ARIC is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, N01-HC-55022 and R01HL087641; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. The meta-analysis and meta-regression analyses were funded by grant R01 HL086694 from the National Heart, Lung, and Blood Institute.

BHS: The Busselton Health Study acknowledges the generous support for the 1994/5 follow-up study from Healthway, Western Australia and the numerous Busselton community volunteers who assisted with data collection and the study participants from the Shire of Busselton. The Busselton Health Study is supported by The Great Wine Estates of the Margaret River region of Western Australia. The study gratefully acknowledges the assistance of the Western Australian DNA Bank (NHMRC Enabling Facility) with DNA samples and the support provided by the Ark (NHMRC Enabling Facility) for this study.

CARDIA: The Coronary Artery Risk Development in Young Adults (CARDIA) study is funded by contracts N01-HC-95095, N01-HC-48047, N01-HC-48048, N01-HC-48049, N01-HC-48050, N01-HC-45134, N01-HC-05187, N01-HC-45205, and N01-HC-45204 from the National Heart, Lung and Blood Institute to the CARDIA investigators. A full list of principal CARDIA investigators and institutions can be found at http://www.cardia.dopm.uab.edu/study-information/participating-institutions. Genotyping and data analyses was funded as part of the Gene Environment Association Studies (GENEVA) under GEI through grant U01-HG004729 from the National Human Genome Research Institute to MF. Funding support for genotyping, which was performed at the Broad Institute of MIT and Harvard, was provided by U01-HG04424. Assistance with genotype cleaning, as well as with general study coordination, was provided by the GENEVA Coordinating Center (U01-HG004446). The authors thank the investigators and staff of the GENEVA coordinating center and the Broad Institute genotyping center, as well as the staff and participants of the CARDIA study for their important contributions.

CHS: This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants HL080295, HL087652, HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG023629 from the National Institute on Aging (NIA). A full list of CHS investigators and institutions can be found at http://www.chs-nhlbi.org. DNA handling and genotyping at Cedars-Sinai Medical Center was supported in part by the National Center for Research Resources, grant UL1RR033176, and is now at the National Center for

Advancing Translational Sciences, CTSI grant UL1TR000124; in addition to the National Institute of Diabetes and Digestive and Kidney Disease grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

CoLaus: The CoLaus authors thank Yolande Barreau, Mathieu Firmann, Vladimir Mayor, Anne-Lise Bastian, Binasa Ramic, Martine Moranville, Martine Baumer, Marcy Sagette, Jeanne Ecoffey, and Sylvie Mermoud for data collection. The CoLaus study received financial contributions from GlaxoSmithKline, the Faculty of Biology and Medicine of Lausanne, the Swiss National Science Foundation (33CSCO-122661, 3200BO-111361/2, 3100AO-116323/1,310000-112552), the Swiss School of Public Health Plus, the Giorgi-Cavaglieri Foundation, the European Framework Project 6 (EuroDia, AnEuploidy and Hypergenes projects.

EPIC-Norfolk: The EPIC Norfolk study is supported by programme grants from the Medical Research Council, and Cancer Research UK. We acknowledge the contribution of the staff and participants of the EPIC-Norfolk Study.

Fenland: The Fenland Study is funded by the Medical Research Council (MC_U106179471). We are grateful to all the volunteers for their time and help, and to the General Practitioners and practice staff for assistance with recruitment. We thank the Fenland Study Investigators, Fenland Study Co-ordination team and the Epidemiology Field, Data and Laboratory teams.

FHS: From the Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. This work was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195) and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278), and by grants from the National Institute of Neurological Disorders and Stroke (NS17950; PAW) and the National Institute of Aging, (AG08122, AG16495; PAW). Analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARe) project.

KORA S3: KORA S3 500K blood pressure project was supported by Estonian Ministry of Education and Science core grant no SF0180022s12 (to M.L). The KORA Augsburg studies have been financed by the Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany and supported by grants from the German Federal Ministry of Education and Research (BMBF). The KORA study group consists of H-E. Wichmann (speaker), A. Peters, C. Meisinger, T. Illig, R. Holle, J. John and co-workers who are responsible for the design and conduct of the KORA studies. Part of this work was financed by the German National Genome Research Network (NGFN-2 and NGFNPlus:01GS0823) and supported within the Munich Center of Health Sciences (MC Health) as part of LMUinnovativ.

LifeLines: The LifeLines Cohort Study, and generation and management of GWAS genotype data for the LifeLines Cohort Study is supported by the Netherlands Organization of Scientific Research NWO (grant 175.010.2007.006), the Economic Structure Enhancing Fund (FES) of the Dutch government, the Ministry of Economic Affairs, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the Northern Netherlands Collaboration of Provinces (SNN), the Province of Groningen, University Medical Center Groningen, the University of Groningen, Dutch Kidney Foundation and Dutch Diabetes Research Foundation.We thank Behrooz Alizadeh, Annemieke Boesjes, Marcel Bruinenberg, Noortje Festen, Pim van der Harst, Ilja Nolte, Lude Franke, Mitra Valimohammadi for their help in creating the GWAS database, and Rob Bieringa, Joost Keers, René Oostergo, Rosalie Visser, Judith Vonk for their work related to data-collection and validation. The authors are grateful to the study participants, the staff from the LifeLines Cohort Study and Medical Biobank Northern Netherlands, and the participating general practitioners and pharmacists.

MESA: MESA and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, and CTSA UL1-RR-024156.

MIGen: The Myocardial Infarction Genetics Consortium (MIGen) was funded by grant R01 HL087676 (NIH, USA). This work was supported by the European Regional Development Fund (ERDF-FEDER), the Spanish Ministry of Economy and Innovation through the Carlos III Health Institute [CIBER Epidemiología y Salud Pública, Red de Investigación Cardiovascular, Heracles Program RD12/0042, PI09/90506], the Catalan Research and Technology Innovation Interdepartmental Commission [SGR 1195] and Fundació La Marató de TV3 [81810]. GL was funded by the Juan de la Cierva Program, Ministerio de Educación (JCI-2009-04684). Study Concept and Design: David Altshuler, Sekar Kathiresan, Diego Ardissino, Pier M Mannucci, David S Siscovick, Christopher J O'Donnell, Leena Peltonen, Veikko Salomaa, Stephen M Schwartz, Olle Melander; Phenotype Data Acquisition/QC: Stephen M Schwartz Genotype Data Acquisition/QC: Benjamin F Voight, Shaun Purcell;

NESDA: The infrastructure for the NESDA study is funded through the Geestkracht programme of the Dutch Scientific Organization (ZON-MW, grant number 10-000-1002) and matching funds from participating universities and mental health care organizations. Genotyping in NESDA was funded by the Genetic Association Information Network (GAIN) of the Foundation for the US National Institutes of Health. Statistical analyses were carried out on the Genetic Cluster Computer (http://www.geneticcluster.org), which is financially supported by the Netherlands Scientific Organization (NWO 480-05-003) along with a supplement from the Dutch Brain Foundation.

PREVEND: PREVEND genetics is supported by the Dutch Kidney Foundation (Grant E033), the EU project grant GENECURE (FP-6 LSHM CT 2006 037697), the National Institutes of Health (grant LM010098), The Netherlands organisation for health research and development (NWO VENI grant 916.761.70 & ZonMw grant 90.700.441), and the Dutch Inter University Cardiology Institute Netherlands (ICIN).

PROCARDIS was supported by the European Community Sixth Framework Program (LSHM-CT-2007-037273), AstraZeneca, the British Heart Foundation, the Swedish Research Council, the Knut and Alice Wallenberg Foundation, the Swedish Heart-Lung Foundation, the Torsten and Ragnar Söderberg Foundation, the Strategic Cardiovascular Program of Karolinska Institutet and Stockholm County Council, the Foundation for Strategic Research and the Stockholm County Council (560283). MF and HW acknowledge support from the Oxford British Heart Foundation Centre of Research Excellence and a Wellcome Trust core award (075491/Z/04).

RS I and RS II: The GWA database of the Rotterdam Study was funded through the Netherlands Organisation of Scientific Research NWO (nr. 175.010.2005.011). The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University, Rotterdam; the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

SardiNIA: The work was supported by the National Institute on Aging, Intramural Research Program.

SCES, SiMES, SINDI and SP2: SCES, SiMES, SINDI, and SP2 acknowledge the support of the Yong Loo Lin School of Medicine, the National University Health System and the Life Sciences Institute from the National University of Singapore. The National Medical Research Council provided support through the

individual research grant, the clinician scientist award and Singapore translational investigator award schemes. The Biomedical research council in Singapore also provided support through the individual research grant scheme. We also acknowledge the support from the National Research Foundation of Singapore (NRF-RF-2010-05).

SHIP: SHIP was funded by grants from the German Federal Ministry of Education and Research (BMBF, Grants 01ZZ0403, 01ZZ0103, 01GI0883), the Ministry for Education, Research and Cultural Affairs as well as the Ministry of Social Affairs of the Federal State of Mecklenburg-West Pomerania. Genome-wide data have been supported by the Federal Ministry of Education and Research (grant no. 03ZIK012) and a joint grant from Siemens Healthcare, Erlangen, Germany and the Federal State of Mecklenburg- West Pomerania. The University of Greifswald is a member of the 'Center of Knowledge Interchange' program of the Siemens AG.

SUVIMAX: We thank the Commissariat à l'Energie Atomique, the Université Paris 13, the Conservatoire National des Arts et Métiers, the Institut National de la Recherche Agronomique and the Institut National de la Santé et de la Recherche Médicale for their financial support.

TRAILS (TRacking Adolescents' Individual Lives Survey) is a collaborative project involving various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen, and the Parnassia Bavo group, all in the Netherlands. TRAILS has been financially supported by grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior and Dependence grants 60-60600-98-018 and 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 457-03-018, GB-MaGW 452-04-314, and GB-MaGW 452-06-004; NWO large-sized investment grant 175.010.2003.005; NWO Longitudinal Survey and Panel Funding 481-08-013); the Sophia Foundation for Medical Research (projects 301 and 393), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), and the participating universities. We are grateful to all adolescents, their parents and teachers who participated in this research and to everyone who worked on this project and made it possible. Statistical analyses were carried out on the Genetic Cluster Computer (http://www.geneticcluster.org), which is financially supported by the Netherlands Scientific Organization (NWO 480-05-003) along with a supplement from the Dutch Brain Foundation.

WGHS: The WGHS is funded by the Donald W. Reynolds Foundation (Las Vegas, NV), the Fondation LeDucq (Paris, France), the National Heart, Lung and Blood Institute (NHLBI; HL043851) and the National Cancer Institute (NCI; CA047988). Funding for genotyping and collaborative scientific support was provided by Amgen.

YFS: The Young Finns Study has been financially supported by the Academy of Finland: grants 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi), the Social Insurance Institution of Finland, Kuopio, Tampere and Turku University Hospital Medical Funds (grant 9M048 and 9N035 for TeLeht), Juho Vainio Foundation, Paavo Nurmi Foundation, Finnish Foundation of Cardiovascular Research and Finnish Cultural Foundation, Tampere Tuberculosis Foundation and Emil Aaltonen Foundation (T.L). The expert technical assistance in the statistical analyses by Ville Aalto and Irina Lisinen is gratefully acknowledged.

LIFELINES BANNER AUTHORSHIP

LifeLines Cohort Study: Behrooz Z Alizadeh (1), Rudolf A de Boer (2), H Marike Boezen (1), Marcel Bruinenberg (3), Lude Franke (4), Pim van der Harst (2), Hans L Hillege (1,2), Melanie M van der Klauw (5), Gerjan Navis (6), Johan Ormel (7), Dirkje S Postma (8), Judith GM Rosmalen (7), Joris P Slaets (9), Harold Snieder (1), Ronald P Stolk (1), Bruce HR Wolffenbuttel (5), Cisca Wijmenga (4)

(1) University of Groningen, University Medical Center Groningen, Department of Epidemiology, Groningen, The Netherlands
(2) University of Groningen, University Medical Center Groningen, Department of Cardiology, Groningen, The Netherlands
(3) University of Groningen, University Medical Center Groningen, LifeLines Cohort Study, Groningen, The Netherlands
(4) University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, The Netherlands
(5) University of Groningen, University Medical Center Groningen, Department of Endocrinology, Groningen, The Netherlands
(6) University of Groningen, University Medical Center Groningen, Department of Internal Medicine, Division of Nephrology, Groningen, The Netherlands
(7) University of Groningen, University Medical Center Groningen, Interdisciplinary Center of Psychopathology of Emotion Regulation (ICPE), Department of Psychiatry, Groningen, The Netherlands
(8) University of Groningen, University Medical Center Groningen, Department of Pulmonology, Groningen, The Netherlands
(9) University of Groningen, University Medical Center Groningen, University Center for Geriatric Medicine, Groningen, The Netherlands

REFERENCES

1. Harris, T.B., Launer, L.J., Eiriksdottir, G., Kjartansson, O., Jonsson, P.V., Sigurdsson, G., Thorgeirsson, G., Aspelund, T., Garcia, M.E., Cotch, M.F., et al. (2007). Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. Am J Epidemiol 165, 1076-1087.
2. Rose, G.A., and Blackburn, H. (1966). Cardiovascular population studies: Methods. (World Health Organization, Geneva).
3. The ARIC investigators. (1989). The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. Am J Epidemiol 129, 687-702.
4. Friedman, G.D., Cutter, G.R., Donahue, R.P., Hughes, G.H., Hulley, S.B., Jacobs, D.R., Jr., Liu, K., and Savage, P.J. (1988). CARDIA: study design, recruitment, and some characteristics of the examined subjects. J Clin Epidemiol 41, 1105-1116.
5. Fried, L.P., Borhani, N.O., Enright, P., Furberg, C.D., Gardin, J.M., Kronmal, R.A., Kuller, L.H., Manolio, T.A., Mittelmark, M.B., Newman, A., et al. (1991). The Cardiovascular Health Study: design and rationale. Ann Epidemiol 1, 263-276.
6. Dawber, T.R., Meadors, G.F., and Moore, F.E., Jr. (1951). Epidemiological approaches to heart disease: the Framingham Study. Am J Public Health Nations Health 41, 279-281.
7. Feinleib, M., Kannel, W.B., Garrison, R.J., McNamara, P.M., and Castelli, W.P. (1975). The Framingham Offspring Study. Design and preliminary data. Prev Med 4, 518-525.
8. Splansky, G.L., Corey, D., Yang, Q., Atwood, L.D., Cupples, L.A., Benjamin, E.J., D'Agostino, R.B., Sr., Fox, C.S., Larson, M.G., Murabito, J.M., et al. (2007). The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. Am J Epidemiol 165, 1328-1335.
9. Kramer, H., Han, C., Post, W., Goff, D., Diez-Roux, A., Cooper, R., Jinagouda, S., and Shea, S. (2004). Racial/ethnic differences in hypertension and hypertension treatment and control in the multi-ethnic study of atherosclerosis (MESA). Am J Hypertens 17, 963-970.
10. Bild, D.E., Bluemke, D.A., Burke, G.L., Detrano, R., Diez Roux, A.V., Folsom, A.R., Greenland, P., Jacob, D.R., Jr., Kronmal, R., Liu, K., et al. (2002). Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol 156, 871-881.
11. Hofman, A., Breteler, M.M., van Duijn, C.M., Krestin, G.P., Pols, H.A., Stricker, B.H., Tiemeier, H., Uitterlinden, A.G., Vingerling, J.R., and Witteman, J.C. (2007). The Rotterdam Study: objectives and design update. Eur J Epidemiol 22, 819-829.
12. Hofman, A., Breteler, M.M., van Duijn, C.M., Janssen, H.L., Krestin, G.P., Kuipers, E.J., Stricker, B.H., Tiemeier, H., Uitterlinden, A.G., Vingerling, J.R., et al. (2009). The Rotterdam Study: 2010 objectives and design update. Eur J Epidemiol 24, 553-572.
13. Hofman, A., van Duijn, C.M., Franco, O.H., Ikram, M.A., Janssen, H.L., Klaver, C.C., Kuipers, E.J., Nijsten, T.E., Stricker, B.H., Tiemeier, H., et al. (2011). The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol 26, 657-686.
14. Ridker, P.M., Chasman, D.I., Zee, R.Y., Parker, A., Rose, L., Cook, N.R., and Buring, J.E. (2008). Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy american women. Clin Chem 54, 249-255.
15. Lewington, S., Clarke, R., Qizilbash, N., Peto, R., and Collins, R. (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 360, 1903-1913.

16. Colditz, G.A., Martin, P., Stampfer, M.J., Willett, W.C., Sampson, L., Rosner, B., Hennekens, C.H., and Speizer, F.E. (1986). Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. Am J Epidemiol 123, 894-900.
17. Conen, D., Ridker, P.M., Buring, J.E., and Glynn, R.J. (2007). Risk of cardiovascular events among women with high normal blood pressure or blood pressure progression: prospective cohort study. BMJ 335, 432.
18. Adams, C., Burke, V., and Beilin, L.J. (2002). Accuracy of blood pressure measurement and anthropometry among volunteer observers in a large community survey. J Clin Epidemiol 55, 338-344.
19. Firmann, M., Mayor, V., Vidal, P.M., Bochud, M., Pecoud, A., Hayoz, D., Paccaud, F., Preisig, M., Song, K.S., Yuan, X., et al. (2008). The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord 8, 6.
20. Day, N., Oakes, S., Luben, R., Khaw, K.T., Bingham, S., Welch, A., and Wareham, N. (1999). EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. Br J Cancer 80 Suppl 1, 95-103.
21. Loos, R.J., Lindgren, C.M., Li, S., Wheeler, E., Zhao, J.H., Prokopenko, I., Inouye, M., Freathy, R.M., Attwood, A.P., Beckmann, J.S., et al. (2008). Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet 40, 768-775.
22. Heid, I.M., Vollmert, C., Hinney, A., Doring, A., Geller, F., Lowel, H., Wichmann, H.E., Illig, T., Hebebrand, J., and Kronenberg, F. (2005). Association of the 103I MC4R allele with decreased body mass in 7937 participants of two population based surveys. J Med Genet 42, e21.
23. Wichmann, H.E., Gieger, C., and Illig, T. (2005). KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen 67 Suppl 1, S26-30.
24. Stolk, R.P., Rosmalen, J.G., Postma, D.S., de Boer, R.A., Navis, G., Slaets, J.P., Ormel, J., and Wolffenbuttel, B.H. (2008). Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. Eur J Epidemiol 23, 67-74.
25. Penninx, B.W., Beekman, A.T., Smit, J.H., Zitman, F.G., Nolen, W.A., Spinhoven, P., Cuijpers, P., De Jong, P.J., Van Marwijk, H.W., Assendelft, W.J., et al. (2008). The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res 17, 121-140.
26. Sullivan, P.F., de Geus, E.J., Willemsen, G., James, M.R., Smit, J.H., Zandbelt, T., Arolt, V., Baune, B.T., Blackwood, D., Cichon, S., et al. (2009). Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. Mol Psychiatry 14, 359-375.
27. Licht, C.M., de Geus, E.J., Seldenrijk, A., van Hout, H.P., Zitman, F.G., van Dyck, R., and Penninx, B.W. (2009). Depression is associated with decreased blood pressure, but antidepressant use increases the risk for hypertension. Hypertension 53, 631-638.
28. Pinto-Sietsma, S.J., Janssen, W.M., Hillege, H.L., Navis, G., De Zeeuw, D., and De Jong, P.E. (2000). Urinary albumin excretion is associated with renal functional abnormalities in a nondiabetic population. J Am Soc Nephrol 11, 1882-1888.
29. Hillege, H.L., Fidler, V., Diercks, G.F., van Gilst, W.H., de Zeeuw, D., van Veldhuisen, D.J., Gans, R.O., Janssen, W.M., Grobbee, D.E., and de Jong, P.E. (2002). Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. Circulation 106, 1777-1782.
30. Broadbent, H.M., Peden, J.F., Lorkowski, S., Goel, A., Ongen, H., Green, F., Clarke, R., Collins, R., Franzosi, M.G., Tognoni, G., et al. (2008). Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet 17, 806-814.
31. Pilia, G., Chen, W.M., Scuteri, A., Orru, M., Albai, G., Dei, M., Lai, S., Usala, G., Lai, M., Loi, P., et al. (2006). Heritability of cardiovascular and personality traits in 6,148 Sardinians. PLoS Genet 2, e132.
32. John, U., Greiner, B., Hensel, E., Ludemann, J., Piek, M., Sauer, S., Adam, C., Born, G., Alte, D., Greiser, E., et al. (2001). Study of Health In Pomerania (SHIP): a health examination survey in an east German region: objectives and design. Soz Praventivmed 46, 186-194.

33. Hercberg, S., Galan, P., Preziosi, P., Roussel, A.M., Arnaud, J., Richard, M.J., Malvy, D., Paul-Dauphin, A., Briancon, S., and Favier, A. (1998). Background and rationale behind the SU.VI.MAX Study, a prevention trial using nutritional doses of a combination of antioxidant vitamins and minerals to reduce cardiovascular diseases and cancers. SUpplementation en VItamines et Mineraux AntioXydants Study. Int J Vitam Nutr Res 68, 3-20.
34. Huisman, M., Oldehinkel, A.J., de Winter, A., Minderaa, R.B., de Bildt, A., Huizink, A.C., Verhulst, F.C., and Ormel, J. (2008). Cohort profile: the Dutch 'TRacking Adolescents' Individual Lives' Survey'; TRAILS. Int J Epidemiol 37, 1227-1235.
35. de Winter, A.F., Oldehinkel, A.J., Veenstra, R., Brunnekreef, J.A., Verhulst, F.C., and Ormel, J. (2005). Evaluation of non-response bias in mental health determinants and outcomes in a large sample of pre-adolescents. Eur J Epidemiol 20, 173-181.
36. Lavanya, R., Jeganathan, V.S., Zheng, Y., Raju, P., Cheung, N., Tai, E.S., Wang, J.J., Lamoureux, E., Mitchell, P., Young, T.L., et al. (2009). Methodology of the Singapore Indian Chinese Cohort (SICC) eye study: quantifying ethnic variations in the epidemiology of eye diseases in Asians. Ophthalmic Epidemiol 16, 325-336.
37. Foong, A.W., Saw, S.M., Loo, J.L., Shen, S., Loon, S.C., Rosman, M., Aung, T., Tan, D.T., Tai, E.S., and Wong, T.Y. (2007). Rationale and methodology for a population-based study of eye diseases in Malay people: The Singapore Malay eye study (SiMES). Ophthalmic Epidemiol 14, 25-35.
38. Lim, L.S., Saw, S.M., Jeganathan, V.S., Tay, W.T., Aung, T., Tong, L., Mitchell, P., and Wong, T.Y. (2010). Distribution and determinants of ocular biometric parameters in an Asian population: the Singapore Malay eye study. Invest Ophthalmol Vis Sci 51, 103-109.
39. Nang, E.E., Khoo, C.M., Tai, E.S., Lim, S.C., Tavintharan, S., Wong, T.Y., Heng, D., and Lee, J. (2009). Is there a clear threshold for fasting plasma glucose that differentiates between those with and without neuropathy and chronic kidney disease?: the Singapore Prospective Study Program. Am J Epidemiol 169, 1454-1462.