SUPPLEMENTAL MATERIAL

Supplemental Methods

Data S1 – Study information

Atherosclerosis Risk In Communities

The Atherosclerosis Risk in Communities (ARIC) Study is a prospective community-based study of cardiovascular disease and its risk factors. At baseline (1987-89), 15,792 men and women age 45-64 were recruited from 4 communities in the US (Washington County, Maryland; Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis suburbs, Minnesota). Participants were mostly white in the Minnesota and Washington County field centers, white and African American in Forsyth County, and exclusively African American in the Jackson field center. Cohort members completed nine clinic examinations, conducted approximately three years apart between 1987 and 1998, with a fifth visit conducted from 2011 – 2013. Clinic examinations included assessment of cardiovascular risk factors, self-reported medical family history, employment and educational status, diet, physical activity, comorbidity, clinical and laboratory measurements. The present analyses utilized ECG measurements from the baseline assessment.

British Genetics of Hypertension study

Participants of the BRIGHT Study were recruited from the Medical Research Council General Practice Framework and other primary care practices in the UK. Each case had a history of hypertension diagnosed prior to 60 years of age with confirmed blood pressure recordings corresponding to seated levels >150/100mmHg (1 reading) or mean of 3 readings >145/95 mmHg. BRIGHT is focused on recruitment of hypertensive individuals with BMI<30. Sample selection for GWAS was based on DNA availability and quantity.

The Cooperative Health Research in South Tyrol study

The Cooperative Health Research in South Tyrol (CHRIS) study is a population-based cohort of 13,393 adults recruited from the alpine Val Venosta/Vinschgau district in South Tyrol (Italy). Baseline recruitment was conducted at a single study centre from 2011 to 2018, collecting socio-demographic, health, lifestyle, and exposure data from questionnaires-based interviews. Health assessments were based also on clinical examinations and urine and blood sampling. The latter were also used biobanking, DNA extraction, and multi-omic characterization. The study was approved by the Ethics Committee of the Healthcare System of the Autonomous Province of Bolzano-South Tyrol (protocol no. 21/2011 19 Apr 2011). All CHRIS participants provided written informed consent.

Cardiovascular Health Study

The Cardiovascular Health Study (CHS) is a population-based cohort study of risk factors for coronary heart disease and stroke in adults ≥65 years conducted across four field centers. The original predominantly European ancestry cohort of 5,201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists; subsequently, an additional predominantly African-American cohort of 687 persons was enrolled for a total sample of 5,888. Blood samples were drawn from all participants at their baseline examination and DNA was subsequently extracted from available

samples. Genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai among CHS participants who consented to genetic testing and had DNA available using the Illumina 370CNV BeadChip system (for European ancestry participants, in 2007) or the Illumina HumanOmni1-Quad_v1 BeadChip system (for African-American participants, in 2010). Calcium was measured on previously collected and stored serum samples from the 1992-1993 clinic exam. Due to low sample sizes in in the exposed groups after calcium dichotomization, the African-American participants are not included in these analyses. CHS was approved by institutional review committees at each field center and individuals in the present analysis had available DNA and gave informed consent including consent to use of genetic information for the study of cardiovascular disease.

INGI-CARLANTINO

INGI-CAR consisted of about 1000 subjects who were drawn from Carlantino, an isolated village of southern Italy. Ethics approval was obtained from the Ethics Committee of the "IRCCS Burlo Garofolo" in Trieste. Written informed consent was obtained from every participant of the study. The study population had undergone clinical and instrumental evaluations between 1998 and 2005. For all subjects, anthropometrics variables (such as height, weight, etc) were taken and a structured questionnaire about lifestyle and medical history was filled out. In addition, blood pressure, body-mass index, biochemical analyses, ECG and cardiovascular evaluation were collected.

INGI-Friuli Venezia Giulia

The INGI-FVG cohort consisted of about 1700 subjects drawn from the project "Genetic Park of Friuli Venezia Giulia". This study examined six isolated villages in the North-East of Italy between 2008 and 2010. Ethics approval was obtained from the Ethics Committee of the "IRCCS Burlo Garofolo" in Trieste. Written informed consent was obtained from every participant of the study. The study population had undergone clinical and instrumental evaluations. For all subjects, anthropometrics variables (such as height, weight, etc) were taken and a structured questionnaire about lifestyle and medical history was filled out. In addition, blood pressure, body-mass index, biochemical analyses, ECG and cardiovascular evaluation were collected.

Inter99 study

The Inter99 study carried out in 1999-2001 included invitation of12934 persons aged 30-60 years drawn from an age- and sex-stratified random sample of the population (16). The baseline participation rate was 52.5%, and the study included 6784 persons. The Inter99 study was a population-based randomized controlled trial (CT00289237, ClinicalTrials.gov) and investigated the effects of lifestyle intervention on CVD. Here 5827 participants with information on lipids and exome chip were analysed. ECG information was obtained from the MUSE Cardiology Information System (GE Healthcare, Wauwatosa, Wisconsin) analysed by Marquette 12SL algorithm version 21.

Kooperative Gesundheitsforschung in der Region Augsburg (Survey 2000) – F3/S4

The KORA study is a series of independent population-based epidemiological surveys of participants living in the city of Augsburg, Southern Germany, or the two adjacent counties. All survey participants are residents of German nationality identified through the registration office and aged between 25 and 74 years at recruitment. KORA F3: The baseline survey KORA S3 was conducted in

the years 1994/95. 3,006 participants from KORA S3 were reexamined in a 10-year follow-up (KORA F3) in the years 2004/05. KORA S4: All survey participants are residents of German nationality identified through the registration office and aged between 25 and 74 years at recruitment. The baseline survey KORA S4 was conducted in the years 1999-2001.

Lifelines Cohort Study

Lifelines is a longitudinal population study & biobank, recruiting from three nothern provinces of the Netherlands. The Lifelines Biobank initiative has been made possible by funding from the Dutch Ministry of Health, Welfare and Sport, the Dutch Ministry of Economic Affairs, the University Medical Center Groningen (UMCG the Netherlands), University of Groningen and the Northern Provinces of the Netherlands. The generation and management of GWAS genotype data for the Lifelines Cohort Study is supported by the UMCG Genetics Lifelines Initiative (UGLI). UGLI is partly supported by a Spinoza Grant from NWO, awarded to Cisca Wijmenga. UMCG Genetics Lifelines Initiative (UGLI) group author LifeLines Cohort Study Raul Aguirre-Gamboa (1), Patrick Deelen (1), Lude Franke (1), Jan A Kuivenhoven (2), Esteban A Lopera Maya (1), Ilja M Nolte (3), Serena Sanna (1), Harold Snieder (3), Morris A Swertz (1), Peter M. Visscher (3,4), Judith M Vonk (3), Cisca Wijmenga (1): (1) Department of Genetics, University of Groningen, University Medical Center Groningen, The Netherlands (2) Department of Pediatrics, University of Groningen, University Medical Center Groningen, The Netherlands (3) Department of Epidemiology, University of Groningen, University Medical Center Groningen, The Netherlands (4) Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia.

Multi-Ethnic Study of Atherosclerosis

The Multi-Ethnic Study of Atherosclerosis (MESA) is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. The cohort is a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84. Approximately 38 percent of the recruited participants are white, 28 percent African-American, 22 percent Hispanic, and 12 percent Asian (predominantly of Chinese descent). Participants were recruited during 2000-2002 from 6 field centers across the U.S. (at Wake Forest University; Columbia University; Johns Hopkins University; the University of Minnesota; Northwestern University; and the University of California – Los Angeles). All underwent anthropomorphic measurement and extensive evaluation by questionnaires at baseline, followed by 7 subsequent examinations at intervals of approximately 2-4 years. Age and sex were self-reported.

Microisolates in South Tyrol

The MICROS study was a population-based survey on adult volunteer participants who reside in three isolated villages in South Tyrol, Italy. These villages were selected because they had a small number of founders with old settlement, as well as slow/null population expansion. Extensive data was collected in 2002-03 regarding genealogy, and clinical measurements as well as collection of blood and urine samples and DNA isolation. An extensive standardized questionnaire was administered by interviewers to collect data on family history of disease and lifestyle exposures such as smoking and alcohol consumption. A serum sample was collected, prepared and stored at -80°C for subsequent analysis. The study was approved by the Ethics Committee of the Autonomous Province of Bolzano, and all participants of MICROS gave written informed consent.

Netherlands Epidemiology of Obesity

The NEO was designed for extensive phenotyping to investigate pathways that lead to obesity-related diseases. The NEO study is a population-based, prospective cohort study that includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. At baseline, information on demography, lifestyle, and medical history have been collected by questionnaires. In addition, samples of 24-h urine, fasting and postprandial blood plasma and serum, and DNA were collected. Genotyping was performed using the Illumina HumanCoreExome chip, which was subsequently imputed to the 1000 genome reference panel. Participants underwent an extensive physical examination, including anthropometry, electrocardiography, spirometry, and measurement of the carotid artery intima-media thickness by ultrasonography. In random subsamples of participants, magnetic resonance imaging of abdominal fat, pulse wave velocity of the aorta, heart, and brain, magnetic resonance spectroscopy of the liver, indirect calorimetry, dual energy X-ray absorptiometry, or accelerometry measurements were performed. The collection of data started in September 2008 and completed at the end of September 2012. Participants are currently being followed for the incidence of obesity-related diseases and mortality.

Ogliastra Genetic Park

The OGP study is a large epidemiological survey carried out in ten villages of a secluded area of Sardinia. We recruited and phenotyped ~ 12,500 individuals (80% of resident population). Participants gave a blood sample, underwent ECG, blood pressure and anthropometric measurements and, collection of genealogical information dating back to the seventeenth century, medical and pharmacology history data and family history of many pathologies. Written informed consent was obtained from every participant in the study

Orkney Complex Disease Study

The Orkney Complex Disease Study (ORCADES) is a family-based, cross-sectional study that seeks to identify genetic factors influencing cardiovascular and other disease risk in the isolated archipelago of the Orkney Isles in northern Scotland (McQuillan et al., 2008). Genetic diversity in this population is decreased compared to Mainland Scotland, consistent with the high levels of endogamy historically. 2078 participants aged 16-100 years were recruited between 2005 and 2011, most having three or four grandparents from Orkney, the remainder with two Orcadian grandparents. Fasting blood samples were collected and many health-related phenotypes and environmental exposures were measured in each individual. All participants gave written informed consent and the study was approved by Research Ethics Committees in Orkney and Aberdeen (North of Scotland REC).

Rotterdam study

Rotterdam Study, a prospective population-based cohort study. Details regarding design, objectives, and methods of the Rotterdam Study have been described in detail. (Hofman et al, 2016) In short, the Rotterdam study started in 1989 with an initial cohort of 7,983 persons (out of 10,215 invitees; response rate 78%) 55 years of age or older living in the Ommoord district in the city of Rotterdam in the Netherlands. In 2000, 3,011 participants (out of 4,472 invitees, response rate 67%) who had become 55 years of age or moved into the study district were added to the cohort. Approximately every 4-5 years follow-up examinations are conducted. Examinations consist of a home interview and an extensive set of test at a research facility in the study district. By linking the general practitioners'

and municipality records to the study database, participants are continuously monitored for major morbidity and mortality.

Study of Health in Pomerania

The Study of Health In Pomerania is a prospective longitudinal population-based cohort study in Western Pomerania assessing the prevalence and incidence of common diseases and their risk factors. SHIP encompasses the two independent cohorts SHIP-START and SHIP-TREND. The detailed study design has been published previously. In brief, participants aged 20 to 79 with German citizenship and principal residency in the study area were recruited from a random sample of residents living in the three local cities (with 17,076 to 65,977 inhabitants), 12 towns (with 1,516 to 3,044 inhabitants) as well as 17 out of 97 (with less than 1,500 inhabitants) randomly selected smaller towns. Individuals were randomly selected in proportion to the population size of the community and stratified by age and sex. For SHIP-START, a total of 4,308 participants were recruited between 1997 and 2001. Between 2008 and 2012 a total of 4,420 participants were recruited in the SHIP-TREND cohort. Individuals were invited to the SHIP study centre for computer-assisted personal interviews and extensive physical examinations. Individuals of both cohorts were analysed separately.

UK Biobank

UK Biobank (UKB, www.ukbiobank.ac.uk) is a large longitudinal biobank study in the United Kingdom which was established to improve understanding of the genetic and environmental causes of common diseases including CVDs. In addition to self-reported disease outcomes and extensive health and life-style questionnaire data, UKB participants are being tracked through their NHS records and national registries (including cause of death and Hospital Episode Statistics). In 2017, UKB released the genotypes of 488,377 participants profiled with a custom SNP array. Genotyping QC was performed centrally by UKB, and genotypes imputed to Haplotype Reference Consortium (HRC) panel were released for 488,377 participants. For the UKB-12lead sub-cohort, ECG measures were calculated from a resting 12-lead and 3-lead ECG rhythm strips as previously described (Young et al, PMID 33537064).

Viking Health Study

The Viking Health Study - Shetland (VIKING) is a family-based, cross-sectional study that seeks to identify genetic factors influencing cardiovascular and other disease risk in the population isolate of the Shetland Isles in northern Scotland. Genetic diversity in this population is decreased compared to Mainland Scotland, consistent with the high levels of endogamy historically. 2105 participants were recruited between 2013 and 2015, most having at least three grandparents from Shetland. Fasting blood samples were collected and many health-related phenotypes and environmental exposures were measured in each individual. All participants gave informed consent and the study was approved by the South East Scotland Research Ethics Committee.

Data S2 – Study level acknowledgments and funding information

Atherosclerosis Risk In Communities

The Atherosclerosis Risk in Communities study has been funded in whole or in part with Federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services, under Contract nos. (75N92022D00001, 75N92022D00002, 75N92022D00003, 75N92022D00004, 75N92022D00005). Funding was also supported by R01HL087641 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. The authors thank the staff and participants of the ARIC study for their important contributions.

British Genetics of Hypertension study

The BRIGHT study is extremely grateful to all the patients who participated in the study and the BRIGHT nursing team. This work forms part of the research program of the National Institutes of Health Research (NIHR), Barts Biomedical Centre award at Queen Mary University of London, UK.

The Cooperative Health Research in South Tyrol study

Full acknowledgements for the CHRIS study are reported here: http://translational-medicine.biomedcentral.com/articles/10.1186/s12967-015-0704-9#Declarations. The CHRIS study was funded by the Department of Innovation, Research, and University of the Autonomous Province of Bolzano-South Tyrol, and supported by the European Regional Development Fund (FESR1157).

Cardiovascular Health Study

Cardiovascular Health Study: This CHS research was supported by NHLBI contracts HHSN268200960009C, HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, 75N92021D00006; and NHLBI grants 1R01HL084443, U01HL080295, R01HL085251, R01HL087652, R01HL105756, R01HL103612, R01HL120393, and U01HL130114 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR001881, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

INGI-CARLANTINO

We are very grateful to the municipal administrators for their collaboration on the project and for logistic support. We would like to thank all participants to this study. Italian Ministry of Health - RC 35/17; Italian Ministry of Education, University and Research, D70-RESRICGIROTTO to GG.

INGI-Friuli Venezia Giulia

We are very grateful to the municipal administrators for their collaboration on the project and for logistic support. We would like to thank all participants to this study. Italian Ministry of Health - RC 35/17; Italian Ministry of Education, University and Research, D70-RESRICGIROTTO to GG.

Inter99 study

The Inter99 was initiated by Torben Jørgensen (PI), Knut Borch-Johnsen (co-PI), Hans Ibsen and Troels F. Thomsen. The steering committee comprises the former two and Charlotta Pisinger. The study was financially supported by research grants from the Danish Research Council, the Danish Centre for Health Technology Assessment, Novo Nordisk Inc., Research Foundation of Copenhagen County, Ministry of Internal Affairs and Health, the Danish Heart Foundation, the Danish Pharmaceutical Association, the Augustinus Foundation, the Ib Henriksen Foundation, the Becket Foundation, and the Danish Diabetes Association.

Kooperative Gesundheitsforschung in der Region Augsburg (Survey 2000) – F3/S4

The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ.

Lifelines Cohort Study

The authors wish to acknowledge the services of the Lifelines Cohort Study, the contributing research centers delivering data to Lifelines, and all the study participants. The Lifelines Biobank initiative has been made possible by funding from the Dutch Ministry of Health, Welfare and Sport, the Dutch Ministry of Economic Affairs, the University Medical Center Groningen (UMCG the Netherlands), University of Groningen and the Northern Provinces of the Netherlands. The generation and management of GWAS genotype data for the Lifelines Cohort Study is supported by the UMCG Genetics Lifelines Initiative (UGLI). UGLI is partly supported by a Spinoza Grant from NWO, awarded to Cisca Wijmenga.

Multi-Ethnic Study of Atherosclerosis

MESA and the MESA SHARe projects 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 75N92020D00001, HHSN268201500003I, N01-HC-95159, 75N92020D00005, N01-HC-95160, 75N92020D00002, N01-HC-95161, 75N92020D00003, N01-HC-95162, 75N92020D00006, N01-HC-95163, 75N92020D00004, N01-HC-95164, 75N92020D00007, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-000040, UL1-TR-001079, and UL1-TR-001420, UL1TR001881, DK063491, and R01HL105756. Funding for SHARe genotyping was provided by NHLBI Contract N02-HL-64278. Genotyping was performed at Affymetrix (Santa Clara, California, USA) and the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) using the Affymetrix Genome-Wide Human SNP Array 6.0. MESA Family is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA

investigators. Support is provided by grants and contracts R01HL071051, R01HL071205, R01HL071250, R01HL071251, R01HL071258, R01HL071259, and by the National Center for Research Resources, Grant UL1RR033176The authors thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutes can be found at http://www.mesa-nhlbi.org.

Microisolates in South Tyrol

We owe a debt of gratitude to all participants, all primary care practitioners, and the personnel of the Hospital of Silandro (Department of Laboratory Medicine) for their participation and collaboration in the research project. The study was supported by the Ministry of Health and Department of Educational Assistance, University and Research of the Autonomous Province of Bolzano, the South Tyrolean Sparkasse Foundation, and the European Union framework program 6 EUROSPAN project (contract no. LSHG-CT-2006-018947).

Netherlands Epidemiology of Obesity

The authors of the NEO study thank all individuals who participated in the Netherlands Epidemiology in Obesity study, all participating general practitioners for inviting eligible participants and all research nurses for collection of the data. We thank the NEO study group, Pat van Beelen, Petra Noordijk and Ingeborg de Jonge for the coordination, lab and data management of the NEO study. We also thank Arie Maan for the analyses of the electrocardiograms. The genotyping in the NEO study was supported by the Centre National de Génotypage (Paris, France), headed by Jean-Francois Deleuze. The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area Vascular and Regenerative Medicine. We thank Pascal Arp, Mila Jhamai, Dr Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the GWAS database. The authors are very grateful to the participants and staff from the Rotterdam Study, the participating general practitioners and the pharmacists. 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 Netherlands Heart Foundation; the Ministry of Education, Culture and Science; the Ministry of Health Welfare and Sports; the European Commission; and the Municipality of Rotterdam. Support for genotyping was provided by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Consortium for Healthy Aging (NCHA) project nr. 050- 060-810. Jacqueline Witteman is supported by NWO grant (vici, 918-76-619). Abbas Dehghan is supported by NWO grant (veni, 916.12.154) and the EUR Fellowship. O.H. Franco works in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.) and Metagenics Inc. Nestlé Nutrition (Nestec Ltd.) and Metagenics Inc. had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review or approval of the manuscript. The GWA study was funded by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Consortium for Healthy Aging (NCHA) project nr. 050-060-810.

Ogliastra Genetic Park

The OGP expresses its gratitude to all the study participants for their contributions and to the municipal administrations for their economic and logistic support. The OGP study was supported by grant from the Italian Ministry of Education, University and Research (MIUR) n°: 5571/DSPAR/2002.

Orkney Complex Disease Study

DNA extractions were performed at the Wellcome Trust Clinical Research Facility in Edinburgh. We would like to acknowledge the invaluable contributions of the research nurses in Orkney, the administrative team in Edinburgh and the people of Orkney. The Orkney Complex Disease Study (ORCADES) was supported by the Chief Scientist Office of the Scottish Government (CZB/4/276, CZB/4/710), a Royal Society URF to J.F.W., the MRC Human Genetics Unit quinquennial programme "QTL in Health and Disease", Arthritis Research UK and the European Union framework program 6 EUROSPAN project (contract no. LSHG-CT-2006-018947).

Rotterdam study

We thank Pascal Arp, Mila Jhamai, Dr Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the GWAS database. The authors are very grateful to the participants and staff from the Rotterdam Study, the participating general practitioners and the pharmacists. 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 Netherlands Heart Foundation; the Ministry of Education, Culture and Science; the Ministry of Health Welfare and Sports; the European Commission; and the Municipality of Rotterdam. Support for genotyping was provided by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Consortium for Healthy Aging (NCHA) project nr. 050- 060-810. Jacqueline Witteman is supported by NWO grant (vici, 918-76-619). Abbas Dehghan is supported by NWO grant (veni, 916.12.154) and the EUR Fellowship. O.H. Franco works in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.) and Metagenics Inc. Nestlé Nutrition (Nestec Ltd.) and Metagenics Inc. had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review or approval of the manuscript. The GWA study was funded by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Consortium for Healthy Aging (NCHA) project nr. 050-060-810.

Study of Health in Pomerania

SHIP is supported by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung (BMBF); grants 01ZZ9603, 01ZZ0103, and 01ZZ0403) and the German Research Foundation (Deutsche Forschungsgemeinschaft (DFG); grant GR 1912/5-1). SHIP-START and SHIP-TREND are part of the Community Medicine Research net (CMR) of the University of Greifswald which is funded by the BMBF as well as the Ministry for Education, Science and Culture and the Ministry of Labor, Equal Opportunities, and Social Affairs of the Federal State of

Mecklenburg-West Pomerania. The CMR encompasses several research projects that share data from SHIP. Genome-wide data have been supported by a joint grant from Siemens Healthcare, Erlangen, Germany and the Federal State of Mecklenburg-West Pomerania.

UK Biobank

This research has been conducted using the UK Biobank Resource under Application Number 8256. This research used data assets made available by National Safe Haven as part of the Data and Connectivity National Core Study, led by Health Data Research UK in partnership with the Office for National Statistics and funded by UK Research and Innovation (grant ref MC_PC_20029).

Viking Health Study

DNA extractions and genotyping were performed at the Edinburgh Clinical Research Facility, University of Edinburgh. We would like to acknowledge the invaluable contributions of the research nurses in Shetland, the administrative team in Edinburgh and the people of Shetland. The Viking Health Study – Shetland (VIKING) was supported by the MRC Human Genetics Unit quinquennial programme grant “QTL in Health and Disease”.

Data S3 – UK Biobank sensitivity analysis including calcium as a categorical variable and interaction term in the model

To determine whether inclusion of calcium as a category variable and as an interaction term SNP*calcium in the model would yield different results compared with our approach using EasyStrata, we performed this analysis using the largest cohort included in the meta-analysis, UK Biobank (N=45,509). Individuals with a serum calcium concentration in the top 20% were coded as "1" and the bottom 80% as "0" for the SNP-high calcium interaction analysis. Individuals with a serum calcium concentration in the bottom 20% were coded as "1" and the top 80% as "0" for the SNP-low calcium interaction analysis. First, a genetic relatedness matrix was created using the R-packages SeqArray (v1.34) and Genetic Estimation and Inference in Structured samples (GENESIS, v2.42.2). Subsequently a global null model was fitted using the R package GMMAT (v.1.4.2), that only accounts for covariates and therefore not including any genetic main effect. Subsequently the Mixed Model Association Test for Gene-Environment (MAGEE, v1.4.1) R-package was used to performed variant-calcium interaction and joint tests for QT and JT intervals[57].

The 2-degree of freedom [df] joint P-values, interaction P-values and interaction betas calculated by MAGEE, were compared with the results for UK Biobank output by EasyStrata[30] following the same approach as for the meta-analysis in this study. SNP-strata specific joint and interaction P-values were calculated between exposed (top or bottom 20% of the calcium distribution) and unexposed (bottom or top 80%) strata from the corresponding genome-wide association studies performed in UK Biobank using BOLT-LMM[20].

Comparing the two methods (MAGEE versus EasyStrata), high correlations (Spearman's rank correlation coefficients [corr]) were observed for the 2df joint P-values (corr=0.91-0.92), interaction P-values (corr=0.89) and interaction betas (corr=0.96) across all interaction analyses. This was supported by visual inspection of correlation plots (Supplementary Figures 7A-D) The strong levels of concordance observed between the two different analyses within this single large cohort, indicate that it is unlikely that a full GWAS meta-analysis conducted in this traditional GxE way, would significantly affect our conclusions.

Table Legends – see Excel file.

Table S1: Summary information for all participating studies

Table S2: Per study (and their sub-studies) summary of genotyping and GWAS software information

Table S3: Per study summary statistics of ECG measures, Calcium and covariates

Table S4: List of established QT-prolonging medication used as exclusion criteria

Table S5: Previously reported variants from main effects GWAS for resting QT and JT

Table S6: Lead variants at loci from the two-stage SNP x calcium QT JT interaction GWAS meta-analyses

Table S7: Lead variants at loci for the European ancestry only SNP by calcium QT JT interaction GWAS meta-analyses

Table S8: Lead variants at loci for the single-stage all cohorts SNP by calcium QT JT interaction GWAS meta-analyses

Table S9: Lead variants at loci with an interaction P-value below suggestive significance (1x10-6)

Table S10: Lead variants at loci including only individuals where serum samples and ECG acquisition were performed on the same day

Table S11: Lead variants at loci from the QT and JT interaction analysis including only individuals at the extremes of the calcium distribution (top and bottom 20%)

Table S12: Lead variants at loci from the QT and JT interaction analysis in UKB after defining exposed as top or bottom 1% of the calcium distribution

Table S13: Comparison of lead variants at loci when using albumin-corrected calcium and uncorrected calcium in interaction analyses

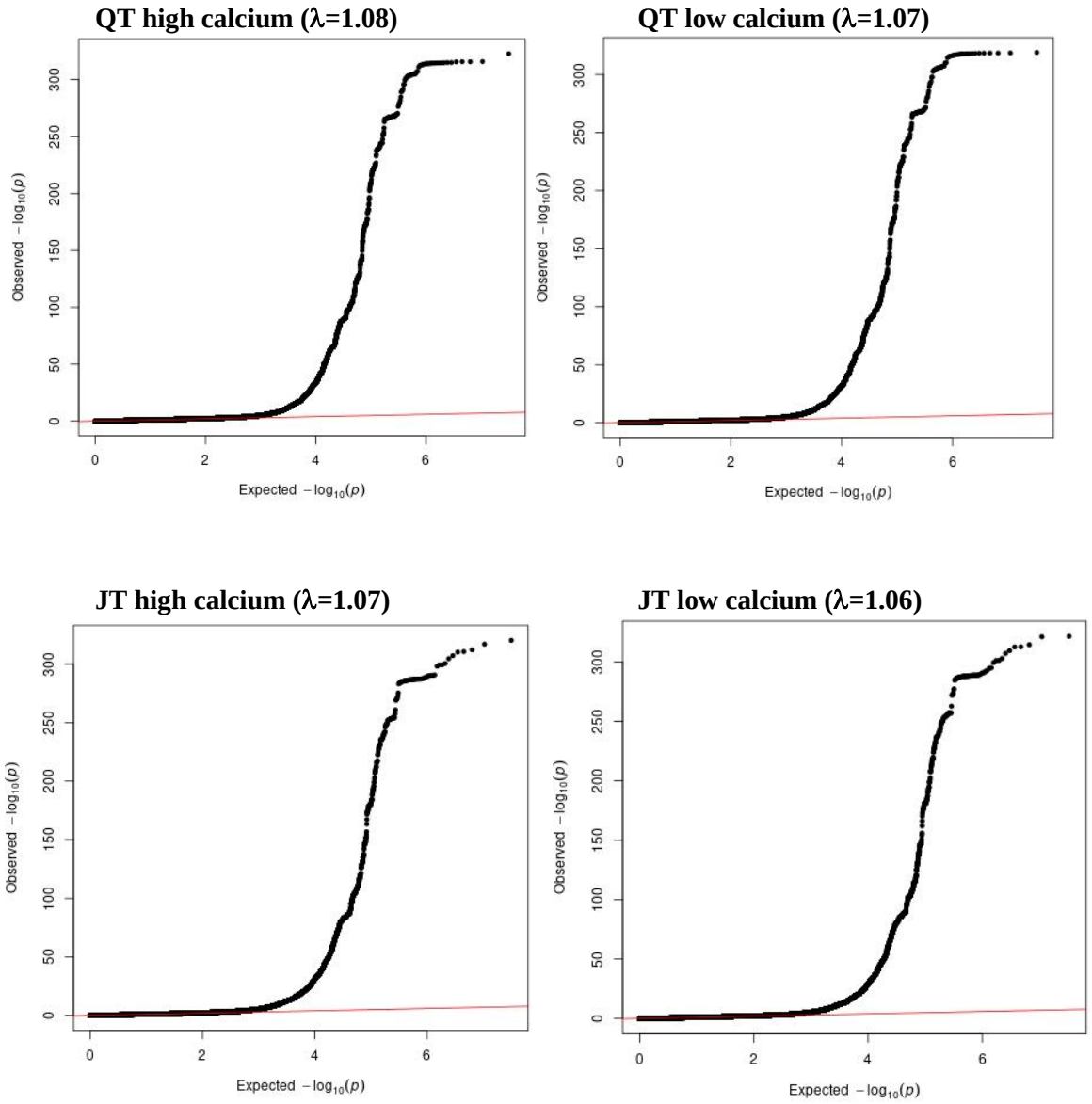

Figure S1: Joint (main and interaction) Quantile-Quantile plots and lambdas for the single-stage all cohorts SNP x calcium interaction analyses for QT and JT.
λ, Lambda

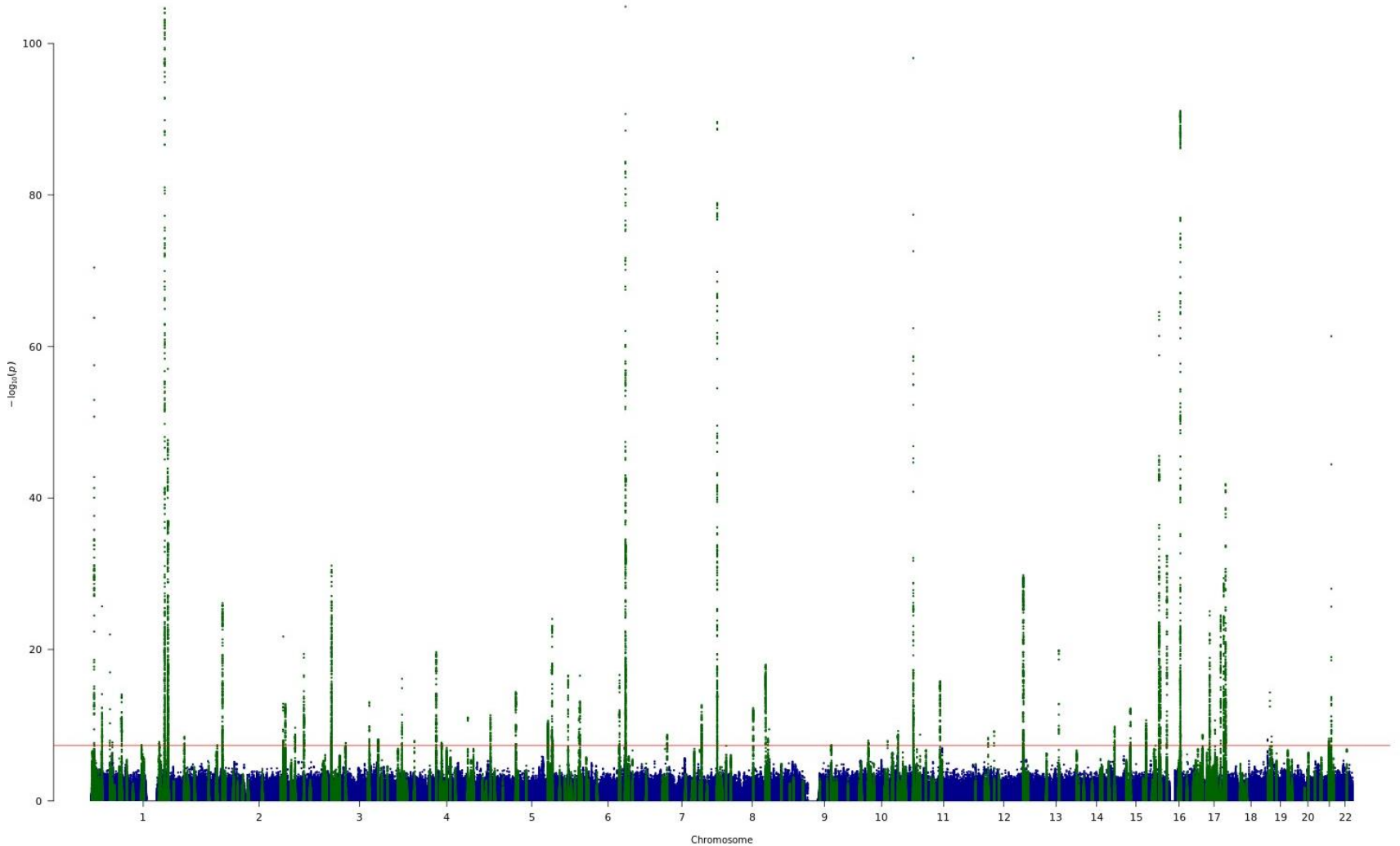

Figure S2: Manhattan plot for the single-stage all cohorts SNP x calcium interaction analyses for QT high calcium 2-df joint P-values

Joint 2-degree of freedom *P*-values from the single-stage all cohorts genome-wide high calcium-SNP interaction meta-analysis for the QT interval. Study level linear regression summary statistics for exposed (top 20% of serum calcium distribution) and unexposed (bottom 80% of serum calcium distribution) were meta-analysed separately before calculation of joint (main and interaction) effect *P*-values. Variants within the boundaries of previously reported loci for QT and JT are highlighted in green. *p* = *P*-value. Y-axis; log P-values, limited to > 1x10-110, X-axis; chromosome and base pair position (hg19).

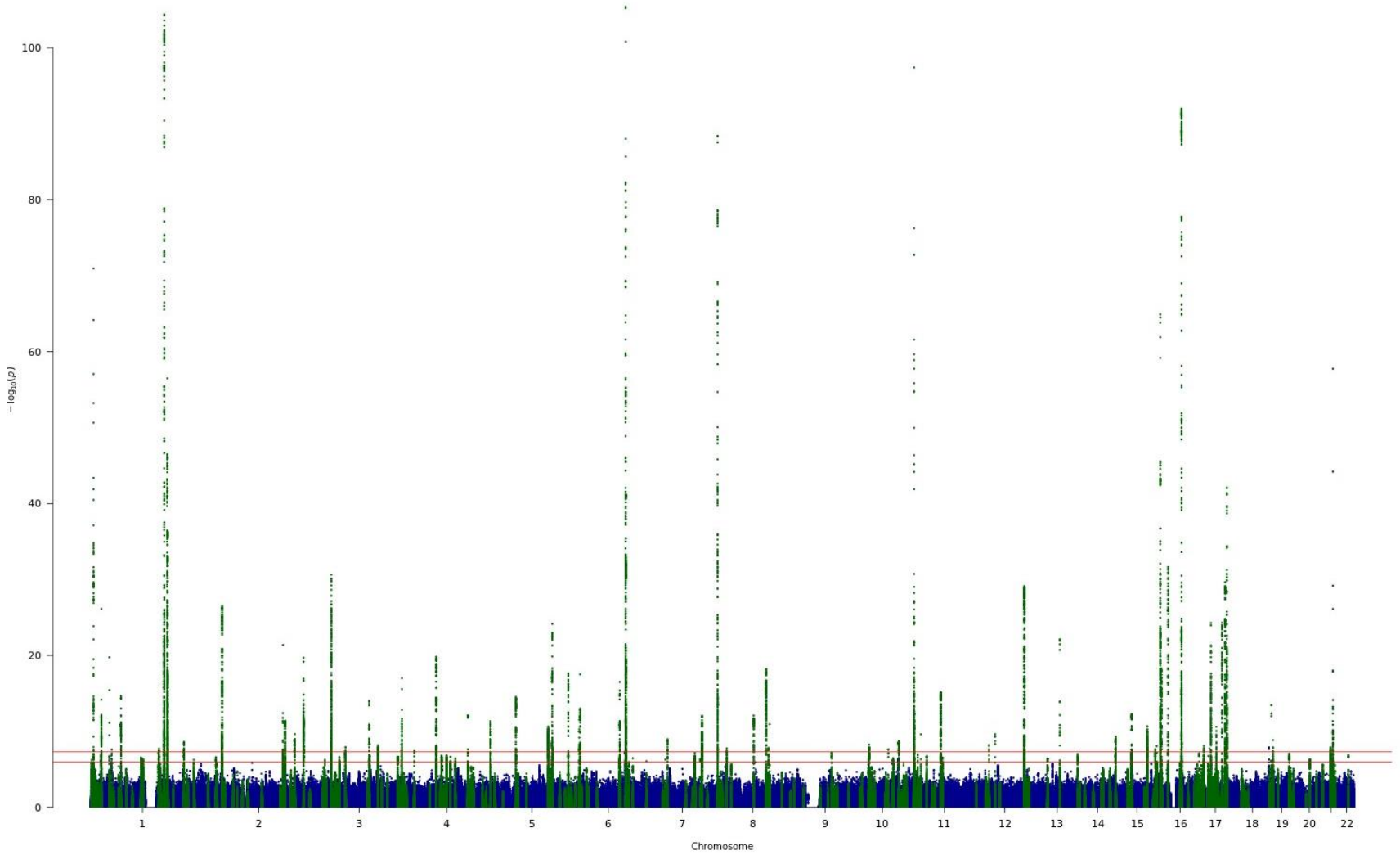

Figure S3: Manhattan plot for the single-stage all cohorts SNP x calcium interaction analyses for QT low calcium 2-df joint P-values

Joint 2-degree of freedom *P*-values from the single-stage all cohorts genome-wide low calcium-SNP interaction meta-analysis for the QT interval. Study level linear regression summary statistics for exposed (bottom 20% of serum calcium distribution) and unexposed (top 80% of serum calcium distribution) were meta-analysed separately before calculation of joint (main and interaction) effect *P*-values. Variants within the boundaries of previously reported loci for QT and JT are highlighted in green. *p* = *P*-value. Y-axis; log P-values, limited to > 1x10-110, X-axis; chromosome and base pair position (hg19).

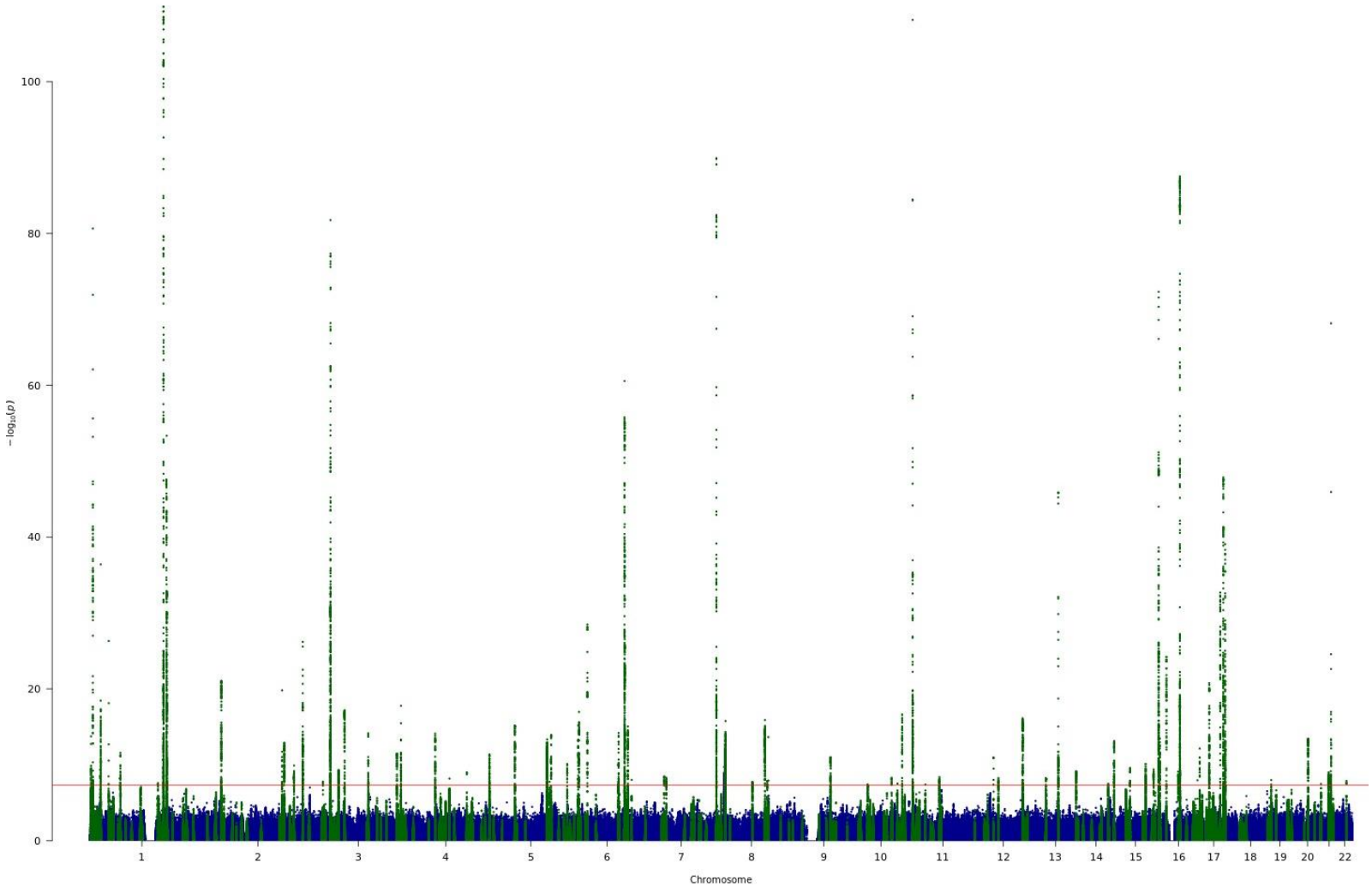

Figure S4: Manhattan plot for the single-stage all cohorts SNP x calcium interaction analyses for JT high calcium 2-df joint P-values

Joint 2-degree of freedom *P*-values from the single-stage all cohorts genome-wide low calcium-SNP interaction meta-analysis for the JT interval. Study level linear regression summary statistics for exposed (top 20% of serum calcium distribution) and unexposed (bottom 80% of serum calcium distribution) were meta-analysed separately before calculation of joint (main and interaction) effect *P*-values. Variants within the boundaries of previously reported loci for QT and JT are highlighted in green. *p* = *P*-value. Y-axis; log P-values, limited to > 1x10-110, X-axis; chromosome and base pair position (hg19).

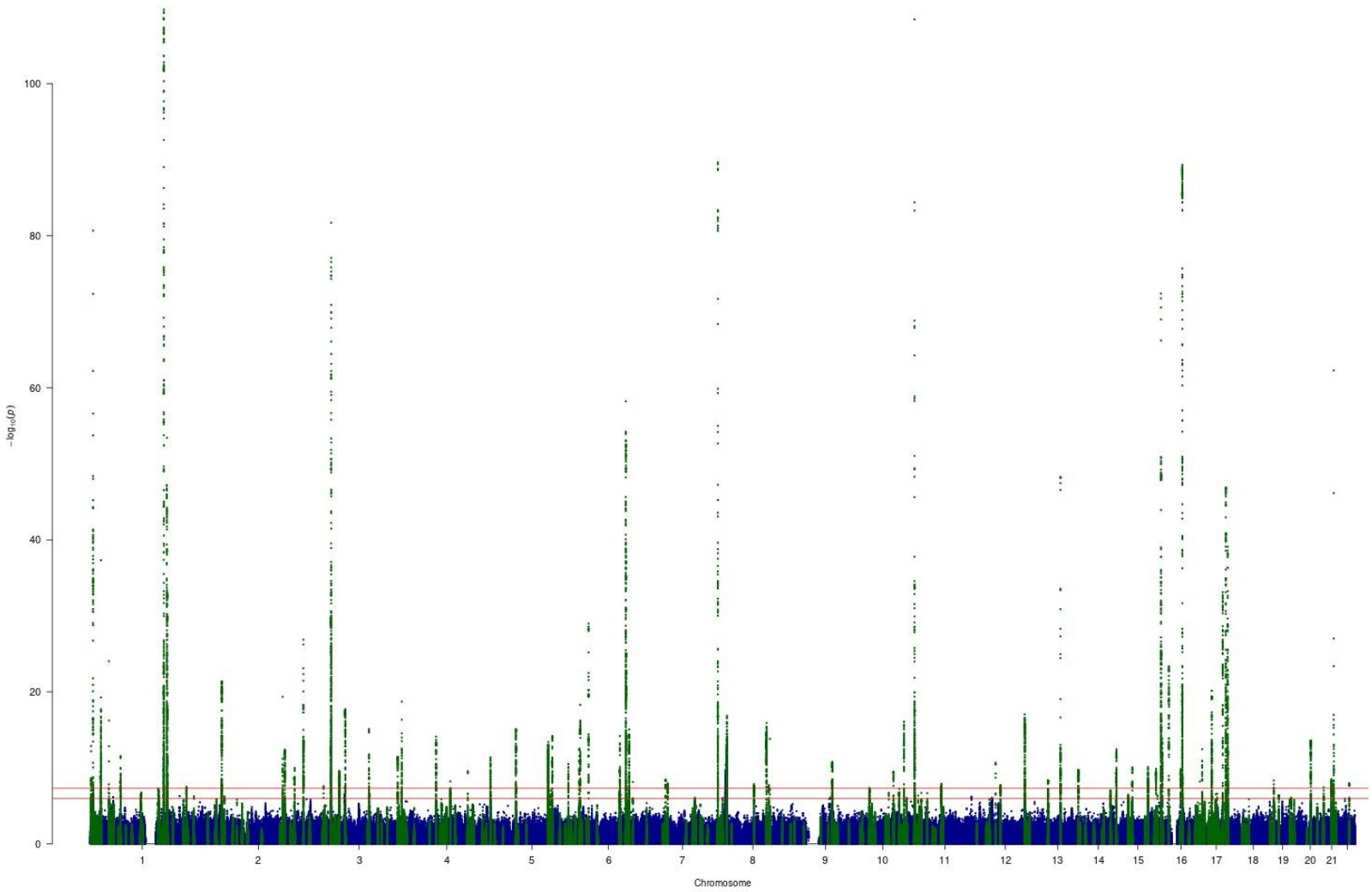

Figure S5: Manhattan plot for the single-stage all cohorts SNP x calcium interaction analyses for JT low calcium 2-df joint P-values

Joint 2-degree of freedom *P*-values from the single-stage all cohorts genome-wide low calcium-SNP interaction meta-analysis for the JT interval. Study level linear regression summary statistics for exposed (bottom 20% of serum calcium distribution) and unexposed (top 80% of serum calcium distribution) were meta-analysed separately before calculation of joint (main and interaction) effect *P*-values. Variants within the boundaries of previously reported loci for QT and JT are highlighted in green. *p* = *P*-value. Y-axis; log P-values, limited to > 1x10-110, X-axis; chromosome and base pair position (hg19).

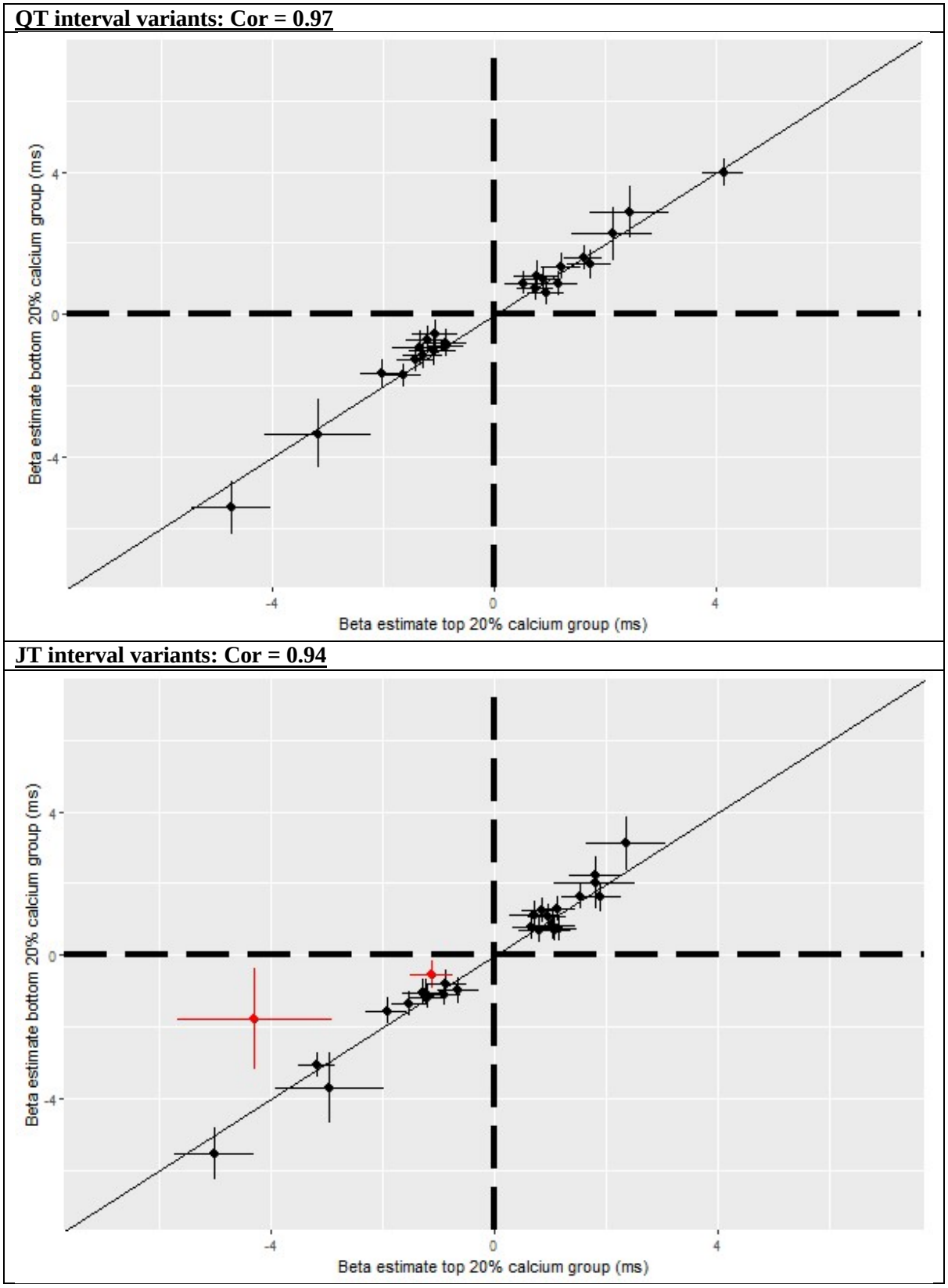

Figure S6: Comparison of lead variant main effect beta estimates between top and bottom 20% of individuals in the serum calcium concentration distribution

Correlation plots comparing lead variant main effect estimates between "top 20%" (X-axis) and "bottom 20%" (Y-axis) in each meta-analysis. Beta estimates for each strata are plotted in milliseconds (ms) along with 95% confidence intervals. Cor; Spearman correlation coefficient. Points in red have a 1-degree of freedom interaction P-value < 0.05.

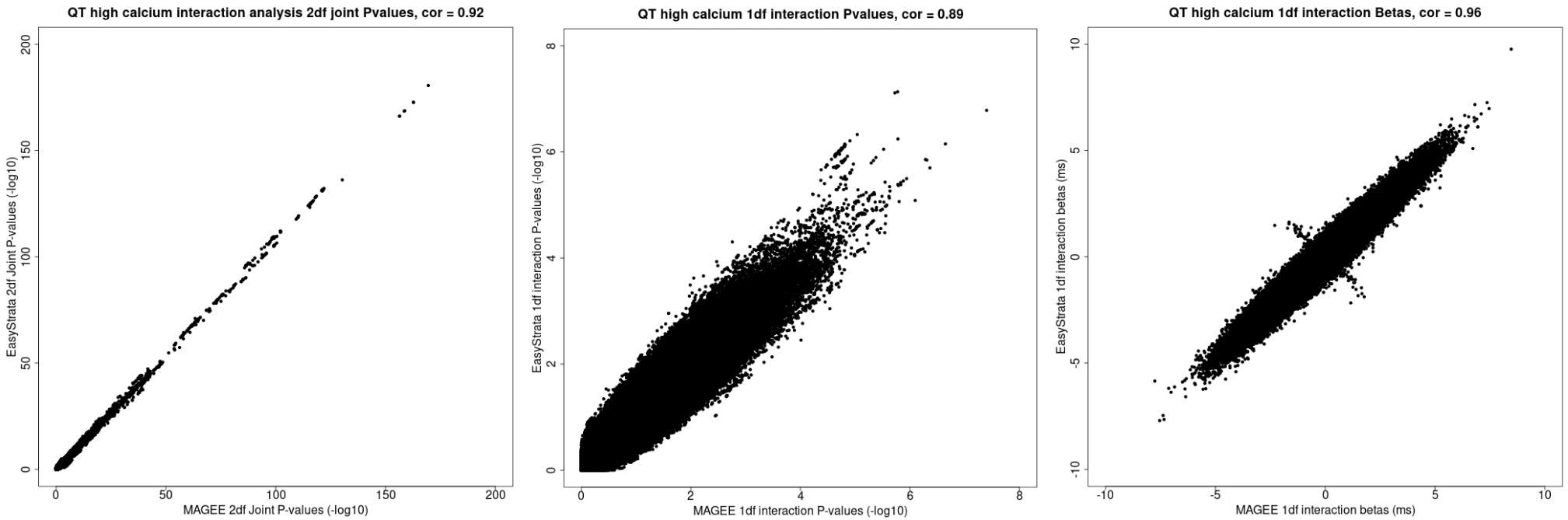

Figure S7A: Correlation plots comparing output from MAGEE and EasyStrata for the QT high-calcium interaction analysis
Analyses performed in UK Biobank using MAGEE (x-axis) and EasyStrata (Y-axis). Spearman's rank correlation coefficients were calculated and rounded to 2 decimal places. Plots display correlations for -log10 2df joint main and interaction P-values (left), -log10 interaction P-values (center) and interaction betas in milliseconds (right).

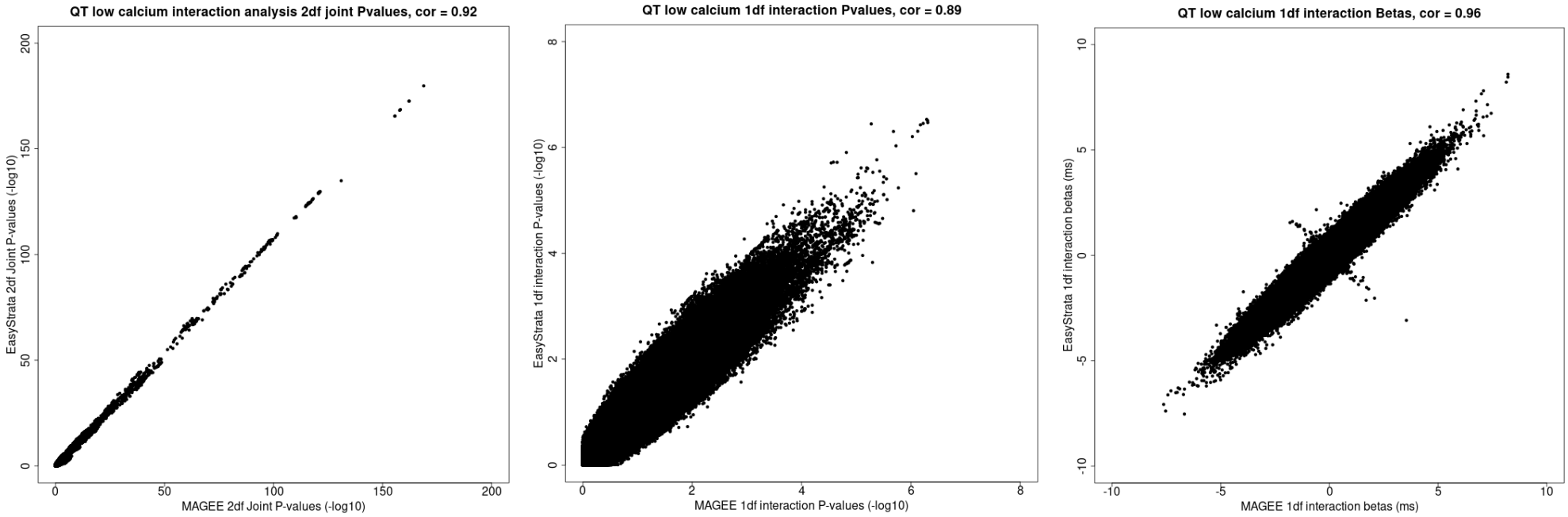

Figure S7B: Correlation plots comparing output from MAGEE and EasyStrata for the QT low-calcium interaction analysis
Analyses performed in UK Biobank using MAGEE (x-axis) and EasyStrata (Y-axis). Spearman's rank correlation coefficients were calculated and rounded to 2 decimal places. Plots display correlations for -log10 2df joint main and interaction P-values (left), -log10 interaction P-values (center) and interaction betas in milliseconds (right).

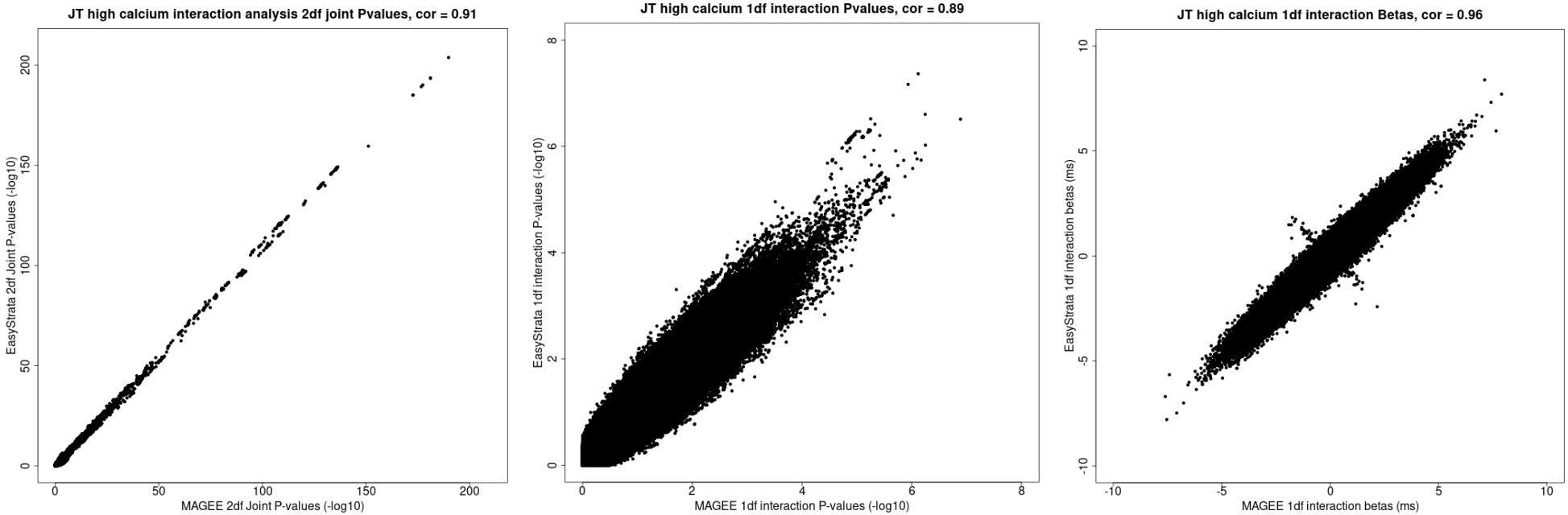

Figure S7C: Correlation plots comparing output from MAGEE and EasyStrata for the JT high-calcium interaction analysis
Analyses performed in UK Biobank using MAGEE (x-axis) and EasyStrata (Y-axis). Spearman's rank correlation coefficients were calculated and rounded to 2 decimal places. Plots display correlations for -log10 2df joint main and interaction P-values (left), -log10 interaction P-values (center) and interaction betas in milliseconds (right).

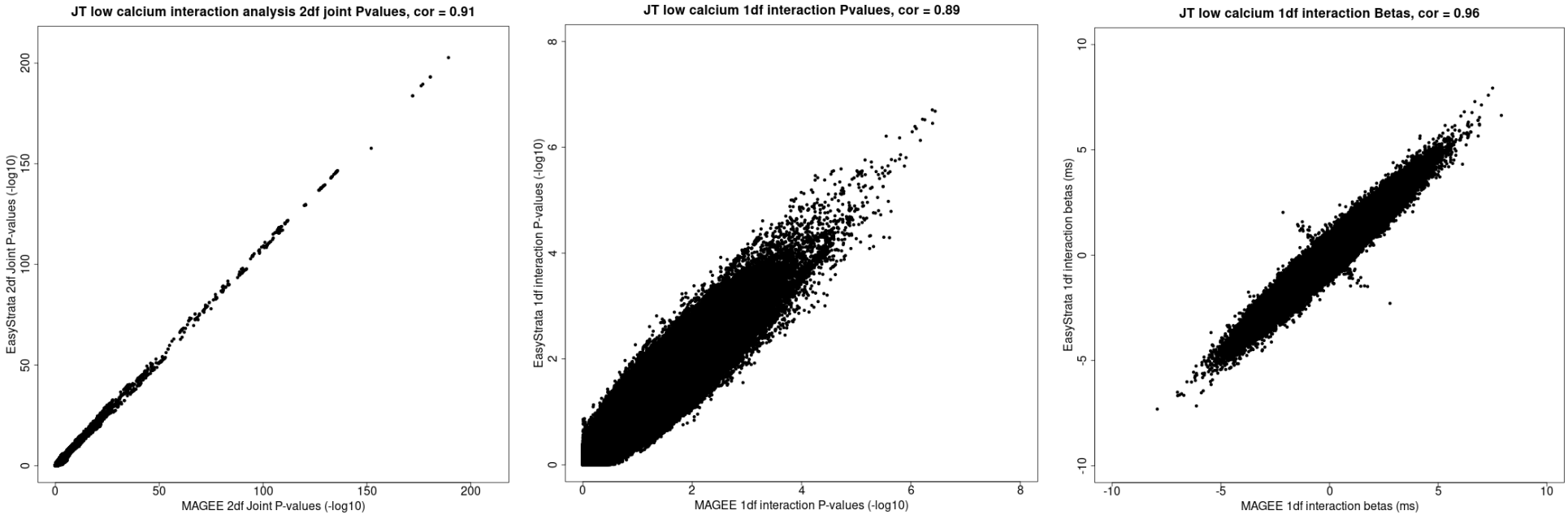

Figure S7D: Correlation plots comparing output from MAGEE and EasyStrata for the JT low-calcium interaction analysis
Analyses performed in UK Biobank using MAGEE (x-axis) and EasyStrata (Y-axis). Spearman's rank correlation coefficients were calculated and rounded to 2 decimal places. Plots display correlations for -log10 2df joint main and interaction P-values (left), -log10 interaction P-values (center) and interaction betas in milliseconds (right)