Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis

Supplement 1 Description of studies included

UK Biobank

UK Biobank is a large, population-based cohort study established to study the interrelationships between environment, lifestyle and genes. The UK Biobank (www.ukbiobank.ac.uk) recruited over 500,000 men and women between 2006 and 2010(1), aged between 37 and 73 years at baseline. For the analyses with CHD we could use 367,643 participants of which 25,352 CHD cases. The UK Biobank was approved by the North West Multi-Centre Research Ethics Committee and all participants provided written informed consent. Fatal or non-fatal CHD was defined as myocardial infarction (International Classification of diseases Tenth Edition [ICD-10] I21.0, I21.1, I21.2, I21.4, I21.9), coronary artery bypass grafting (Office of Population Censuses and Surveys four [OPCS-4] K40.1-40.4, K41.1-41.4, K45.1-45.5), and coronary angioplasty with or without stenting (OPCS-4 K49.1-49.2, K49-8-49.9, K50.2, K75.1-75.4, K75.8-75.9).

EPIC-CVD

EPIC-CVD is a case-cohort study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study(2). Briefly, EPIC consists of 519,978 participants (366,521 women and 153,457 men) recruited from 23 centers across 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) between 1992 and 2000, and aged between 35 and 70 years at baseline. For EPIC-CVD, a representative subcohort of 18,249 participants was selected by simple random sampling, stratified by center, from participants who had available stored blood and buffy coat samples(3,4). After exclusion of 615 participants with a prior history of myocardial infarction or

stroke at baseline, 17,634 subcohort members remained. Subsequently, incident CHD cases outside the subcohort were added to the study sample using the same exclusion criteria (N=17,821). Genetic data was available for 14,025 subcohort members and 9,029 CHD cases outside the subcohort, resulting in a study sample of 23,054 people. EPIC complies with the Declaration of Helsinki, and all participants gave written informed consent before participating in this study. The study was approved by the local ethics committees of the participating centers and the Institutional Review Board of the International Agency for Research on Cancer (IARC, Lyon). Fatal and non-fatal CHD was defined by codes 410-414 of International Classification of Disease Ninth Edition (ICD-9), and codes I20-I25 of ICD-10. Methods used in the recruitment centers to determine first non-fatal CHD events included self-report and linkage with morbidity or hospital registries. Non-fatal CHD events were further validated by a review of medical records and/or linkage with registries. Fatal CHD events were generally determined through mortality registries.

m-CARDIoGRAMplusC4D

Cardiogenics

Cases from Germany and England were under the age of 65 with a confirmed primary MI within the preceding 3-36 months. Exclusion criteria were 1) a history of diabetes mellitus based on plasma glucose >7.0 mmol/l or HbA1c >0.7, 2) renal insufficiency, 3) patients not on statin therapy, 4) current smokers. The Paris cohort comprised patients aged 33 to 87, recruited within the BAAAC (Banque d'ADN et d'ARN de patients présentant une Athérosclérose Coronarienne) study with symptoms of acute coronary syndrome who had one stenosis >50% diagnosed in at least one major coronary artery. Controls were healthy individuals (aged 32 to 65 years)

recruited in Cambridge who were blood donors recruited as part of the Cambridge Bioresource(5).

CCGB 2

Cases had at least one of myocardial infarction, coronary artery bypass graft, percutaneous intervention or a stenosis of at least 50% in at least one epicardial vessel. Diabetic cases and cases aged greater than 55 for men or 65 for women were excluded. Controls had a CTA or angiogram demonstrating no stenosis of greater than 50%. Controls were required to be at least 65 years old for men and 70 years old for women at the time of recruitment(5).

DUKE 2

The Duke Cathgen study recruited individuals though the cardiac catheterization laboratories at Duke University Medical Centre (Durham, NC, USA). Clinical data were provided by the Duke Database for Cardiovascular Disease (DDCD). Cases had at least one epicardial coronary vessel with at least 50% blockage. Age of onset was no older than 65 for women and 55 for men. Subjects (case and control), were excluded if they had severe pulmonary hypertension or congenital heart disease or were diabetic. Controls with a history of ICC/PCI, CABG, MI or transplant were excluded. Controls were required to be at least 50 years old(5).

GerMIFS I-IV

Cases from the GerMIFS I study had a strong positive family history for CAD and an early onset of disease, i.e. were enriched for a strong genetic component. Population-based subjects were entered as controls. GerMIFS II: patients had a validated MI with a strong genetic component as documented by an early age of onset (prior to the age of 60 years) and a positive family history

for CAD in 59.4% of patients. Patients were identified following their admission for acute treatment of MI or in cardiac rehabilitation clinics. Population-based controls were derived from the MONICA/KORA Augsburg survey F4 and the PopGen blood donor sample 2 (PopGen-BSP). GerMIFS III (KORA): cases of non-fatal MI were identified in the KORA registry with DNA available. Hospitalized survivors of MI who are 26-74 years of age are routinely entered into this registry. The diagnosis of a MI was made with the use of the algorithm of the World Health Organization's Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) projects. Controls were from the population based Augsburg KORA S4/F4 study and PopGen. The GerMIFS IV cases consist of consecutive patients referred for coronary angiography, classified as CAD or MI cases based on the coronary angiogram (at least a 50% stenosis in one major coronary vessel) and age of onset (<65 years in males, and <70 years in females). Control samples were recruited as part of the Berlin Aging Study II (BASE-II), a multidisciplinary study investigating factors related to human ageing. All subjects were recruited from the Berlin metropolitan area and underwent an extensive phenotypic assessment, including a two-day medical examination. None of the BASE-II subjects included here reported a history of CAD or MI, nor showed any current signs of acute cardiovascular disease(5).

LURIC

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study consists of 3,316 Caucasian patients hospitalized for coronary angiography between 1997 and 2000 at a tertiary care center in Southwestern Germany. Clinical indications for angiography were chest pain or a positive non-invasive stress test suggestive of myocardial ischemia. To limit clinical heterogeneity, individuals suffering from acture illnesses other than acute coronary syndrome, chronic non-cardiac diseases and a history of malignancy within the past 5 years were excluded. In LURIC,

CAD was defined as the presence of a visible luminal narrowing (>50% stenosis) in at least one of 15 coronary segments according to a classification of the American Heart Association. The study was approved by the ethics committee at the "Ärztekammer Rheinland-Pfalz" and was conducted in accordance with the "Declaration of Helsinki". Informed written consent was obtained from all participants(5).

OHGS A2, B2, C2

The Ottawa Heart Genomics study. Cases had at least one of myocardial infarction, coronary artery bypass graft, percutaneous intervention or a stenosis of at least 50% in at least one epicardial vessel. Diabetic cases and cases aged greater than 55 for men or 65 for women were excluded. Controls were either asymptomatic for cardiovascular disease or had had a CTA or angiogram demonstrating no stenosis of greater than 50%. Controls were required to be at least 65 years old for men and 70 years old for women at the time of recruitment(5).

THISEAS

The Hellenic Study of Interactions between SNPs and Eating in Atherosclerosis Susceptibility recruited from three hospitals in the area of Athens (Greece). Cases were subjects with a first-ever MI before age of 70 years presenting with either ACS or stable CAD defined as >50% stenosis in at least one of the three main coronary vessels assessed by coronary angiography. ACS was defined as acute MI or unstable angina corresponding to class III of the Braunwald classification. ACS patients have also undergone coronary angiography examination that verified the presence of significant stenosis. Controls were subjects age matched without MI/CAD history with negative coronary angiography findings (<30% stenosis), or negative stress test, or subjects without symptoms of disease that were admitted at the same hospitals as cases and were

free of any cardiovascular disease, cancer, or inflammatory diseases. Subjects with renal or hepatic disease were excluded from both study groups(5).

LIFE-Heart

LIFE-Heart is an observational study that recruits patients undergoing first-time diagnostic coronary angiography due to suspected stable CAD with previously untreated coronary arteries, patients with stable left main coronary artery disease and patients with acute myocardial infarction. For the present study, we defined CAD cases as luminal reduction of >50% in any vessel. Normal angiograms were considered as controls(6). The study meets the ethical standards or the Declaration of Helsinki. It has been approved by the Ethics Committee of the Medical Faculty of the University of Leipzig, Germany (Reg. No 276-2005) and is registered at ClinicalTrials.gov (NCT00497887). Written informed consent including agreement with genetic analyses was obtained from all participants. Details of genotyping and quality control can be found elsewhere(7).

ITH 2

The INTERHEART study uses worldwide cases and controls of European ethnicity. Incident acute MI, presenting to a hospital within 24 hours of symptom onset. Age and sex matched hospital and community based, with no previous diagnosis of heart disease or history of exertional chest pain(5).

Global Lipids Genetics Consortium

Low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and total cholesterol are heritable, modifiable, risk factors for coronary artery

disease. To identify new loci and refine known loci influencing these lipids, we examined 188,578 individuals using genome-wide and custom genotyping arrays. We identify and annotate 157 loci associated with lipid levels at $P < 5\times10-8$, including 62 loci not previously associated with lipid levels in humans. Using dense genotyping in individuals of European, East Asian, South Asian, and African ancestry, we narrow association signals in 12 loci. We find that loci associated with blood lipids are often associated with cardiovascular and metabolic traits including coronary artery disease, type 2 diabetes, blood pressure, waist-hip ratio, and body mass index. Our results illustrate the value of genetic data from individuals of diverse ancestries and provide insights into biological mechanisms regulating blood lipids to guide future genetic, biological, and therapeutic research(8).

MAGIC

We recently formed the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) to conduct large-scale meta-analyses of genome-wide data for continuous diabetes-related traits in non-diabetic participants. We aimed to identify additional loci that influence glycemic traits in persons free of diabetes, and investigate their impact on related metabolic phenotypes. We were also interested in understanding variation in the physiological range and evaluating the extent to which the same variants influence pathological FG variation and T2D risk. Here, we extend our previous approach by performing meta-analyses of ~2.5M directly genotyped or imputed autosomal SNPs from 21 genome-wide association studies (GWAS). These 21 cohorts include up to 46,186 non-diabetic participants of European descent informative for FG, and 20 GWAS including up to 38,238 non-diabetic individuals informative for fasting insulin (FI), as well as the surrogate estimates of β-cell function (HOMA-B) and insulin resistance (HOMA-IR) derived from fasting variables by homeostasis model assessment18.

Follow-up of 25 lead SNPs in up to 76,558 additional individuals of European ancestry identified nine novel genome-wide significant associations (empirically determined as $P<5\times10-8$)19 with FG and one with FI/HOMA-IR. Five of these novel loci also demonstrated genome-wide significant evidence for association between the glucose-raising allele and T2D risk in up to 40,655 cases and 87,022 non-diabetic controls(9,10).

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Supplement 2. Results of the association between age at natural menopause and cardiovascular risk factors.

Table S1. Estimates of the association between age at natural menopause and cardiovascular risk factors. Results for individual studies and the pooled cohorts.

	Women	**Men**	**Sex-combined**		
	EPIC-CVD	EPIC-CVD	EPIC-CVD	Publicly available data*	Pooled
			Total cholesterol (mmol/L)		
Simple median	-0.008 (-0.036;0.051)	0.026 (-0.028;0.080)	0.002 (-0.034;0.038)	0.000 (-0.014;0.013)	0.004 (-0.008;0.016)
Weighted median	-0.042 (-0.084;-0.001)	0.017 (-0.036;0.071)	-0.040 (-0.074;-0.005)	0.001 (-0.012;0.015)	-0.002 (-0.014;0.010)
IVW	-0.025 (-0.056;0.005)	0.024 (-0.011;0.059)	-0.010 (-0.035;0.014)	0.007 (-0.006;0.020)	0.005 (-0.007;0.017)
MR-Egger	-0.085 (-0.146;-0.024)	0.041 (-0.032;0.114)	-0.043 (-0.094;0.007)	0.008 (-0.019;0.036)	0.002 (-0.024;0.028)
			HDL cholesterol (mmol/L)		
Simple median	0.001 (-0.016;0.018)	0.001 (-0.018;0.019)	0.000 (-0.014;0.014)	-0.002 (-0.016;0.012)	-0.001 (-0.010;0.009)
Weighted median	-0.015 (-0.031;0.001)	0.002 (-0.016;0.020)	-0.002 (-0.015;0.012)	0.010 (-0.004;0.024)	0.001 (-0.008;0.010)

IVW	-0.006 (-0.018;0.006)	0.001 (-0.012;0.014)	-0.003 (-0.014;0.008)	0.008 (-0.006;0.022)	0.003 (-0.007;0.013)
MR-Egger	-0.026 (-0.050;-0.001)	-0.001 (-0.029;0.027)	-0.015 (-0.038;0.007)	0.008 (-0.023;0.039)	-0.003 (-0.025;0.018)
Triglycerides (mmol/L)					
Simple median	-0.002 (-0.031;0.027)	0.038 (-0.016;0.092)	0.021 (-0.007;0.049)	0.000 (-0.014;0.013)	0.006 (-0.005;0.018)
Weighted median	0.004 (-0.025;0.033)	0.047 (-0.008;0.103)	0.021 (-0.008;0.049)	0.001 (-0.012;0.015)	0.004 (-0.007;0.015)
IVW	0.006 (-0.014;0.025)	0.045 (0.010;0.080)	0.020 (0.000;0.040)	0.007 (-0.006;0.020)	0.001 (-0.016;0.018)
MR-Egger	0.006 (-0.035;0.047)	0.069 (-0.004;0.143)	0.021 (-0.014;0.070)	0.008 (-0.019;0.036)	0.006 (-0.080;0.043)
Apolipoprotein A1 (g/L)					
Simple median	-0.001 (-0.012;0.010)	0.005 (-0.008;0.018)	0.001 (-0.009;0.010)	/	/
Weighted median	-0.009 (-0.019;0.002)	0.006 (-0.007;0.018)	-0.001 (-0.010;0.008)	/	/
IVW	-0.005 (-0.013;0.003)	0.006 (-0.002;0.014)	-0.001 (-0.008;0.006)	/	/
MR-Egger	-0.019 (-0.036;-0.003)	0.008 (-0.009;0.024)	-0.009 (-0.023;0.005)	/	/

Apolipoprotein B (g/L)					
Simple median	0.002 (-0.008;0.011)	0.000 (-0.013;0.013)	0.001 (-0.008;0.009)	/	/
Weighted median	-0.008 (-0.018;0.001)	0.001 (-0.011;0.014)	-0.005 (-0.013;0.003)	/	/
IVW	-0.002 (-0.009;0.005)	0.003 (-0.006;0.012)	-0.001 (-0.006;0.005)	/	/
MR-Egger	-0.013 (-0.026;0.001)	0.010 (-0.009;0.028)	-0.005 (-0.017;0.007)	/	/
C-reactive protein (mg/L)					
Simple median	0.028 (-0.136;0.193)	0.044 (-0.167;0.255)	0.076 (-0.118;0.271)	/	/
Weighted median	0.057 (-0.104;0.219)	-0.024 (-0.232;0.183)	0.062 (-0.135;0.259)	/	/
IVW	0.023 (-0.089;0.135)	0.075 (-0.060;0.211)	0.072 (-0.035;0.259)	/	/
MR-Egger	0.050 (-0.186;0.286)	-0.012 (-0.295;0.271)	0.099 (-0.233;0.431)	/	/
Glucose (mmol/L)					
Simple median	0.035 (-0.021;0.090)	0.016 (-0.071;0.102)	0.029 (-0.020;0.079)	-0.002 (-0.011;0.007)	-0.001 (-0.011;0.008)
Weighted median	-0.004	0.008	-0.002	-0.003	-0.003

	(-0.059;0.052)	(-0.076;0.092)	(-0.050;0.046)	(-0.011;0.005)	(-0.010;0.005)
IVW	0.011	0.020	0.015	0.002	0.003
	(-0.024;0.047)	(-0.036;0.076)	(-0.018;0.048)	(-0.004;0.008)	(-0.003;0.008)
MR-Egger	-0.028	-0.016	-0.021	-0.001	-0.002
	(-0.101;0.045)	(-0.131;0.098)	(-0.089;0.046)	(-0.012;0.010)	(-0.012;0.009)
			HbA1c (%)		
Simple median	-0.006	0.018	0.005	-0.001	0.004
	(-0.028;0.015)	(-0.013;0.049)	(-0.013;0.022)	(-0.011;0.009)	(-0.005;0.013)
Weighted median	-0.004	0.006	0.000	-0.003	0.000
	(-0.025;0.017)	(-0.023;0.036)	(-0.018;0.017)	(-0.012;0.007)	(-0.008;0.009)
IVW	-0.004	0.015	0.003	0.000	0.000
	(-0.018;0.011)	(-0.005;0.035)	(-0.008;0.015)	(-0.007;0.007)	(-0.006;0.007)
MR-Egger	-0.002	-0.001	-0.002	0.004	0.004
	(-0.032;0.028)	(-0.042;0.040)	(-0.026;0.023)	(-0.012;0.020)	(-0.012;0.017)